Effects of Larval Density and Habitat Drying on Developmental Success of *Ochlerotatus albifasciatus* (Diptera: Culicidae) in Urban Rain Pools: Evidence From Field and Experimental Studies

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ABSTRACT *Ochlerotatus albifasciatus* (Macquart) (Diptera: Culicidae) larvae develop synchronously after rainfall events in ephemeral or temporary pools, where they occasionally attain very high abundance. The aims of the current study were to analyze the response of life history parameters such as daily larval mortality, time to pupation, and adult size of *Oc. albifasciatus* to increasing larval density under controlled conditions, and to analyze the relationships of daily larval mortality with density and environmental variables (drying rate, temperature, and season) in urban rain pools in Buenos Aires, Argentina. An exponential increase in mortality was observed at high larval densities under controlled conditions. Development times and adult size (wing length) differed between males and females, and were also affected by density. Development times extended for 0.36 d for each order of magnitude of increase in larval density, and wing length decreased 0.0021 mm per additional larva in 600 cm^2. Larval density in the field varied from <1 larva per square meter to nearly 1100 larvae per square meter. Daily larval mortality values in the field were variable (0.02–0.91), positively related to the drying rate, and exhibited seasonal differences. No significant relation with larval density or temperature was found in the field. It remains to be established whether the density-independent mortality observed in this study is a generalized pattern of *Oc. albifasciatus* populations in Buenos Aires Province or a pattern restricted only to urban habitats.

KEY WORDS floodwater mosquito, larval mortality, population regulation, habitat drying, density dependence

Ochlerotatus albifasciatus (=*Aedes albifasciatus* (Macquart), see Reinert et al. 2005, 2009) is a floodwater mosquito species whose distribution range extends from southern Brazil and Bolivia to Tierra del Fuego in the southern limit of South America (Prosen et al. 1960, Forattini 1965). This species is considered one of the most annoying species in temperate South America because of its persistent attacks of humans and domestic mammals (Prosen et al. 1960, Forattini 1965). This mosquito is also of public health importance, as it has been involved in the transmission of the western equine encephalitis virus in Argentina (Mitchell et al. 1987, Avilés et al. 1990).

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The larval habitats of *Oc. albifasciatus* in Argentina are ephemeral or temporary bodies of water, which are mainly filled by rain (Fontanarrosa et al. 2000, García and Micieli 2000, Gleiser et al. 2000a, Fischer et al. 2002, Campos and Sy 2003, Quiroga et al. 2013), and in some cases by river overflow (Ludueña Almeida and Gorla 1995) or artificial flooding of land for agricultural purposes (Garzón et al. 2013). The drought-resistant eggs are laid on the humid soils that surround temporary water bodies and hatch when they are covered by water and environmental conditions are favorable (Campos and Sy 2006). After hatching, immature stages develop synchronously, and *Oc. albifasciatus* is often the only mosquito species present in the larval habitats (Fischer and Schweigmann 2008).

Larval mortality is an important factor that influences adult mosquito populations. Among the main causes of larval mortality are adverse climatic or environmental conditions, competition for food or space, and the action of natural enemies such as pathogens, parasites, and predators (Silver 2008). Because of their high abundances, mosquito species developing in temporary pools are believed to be subjected to intraspecific competition, which in turn leads to density-dependent effects with increased larval mortality,

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prolonged development times, or smaller adults with lower fecundity (Renshaw et al. 1993, Juliano 2007).

Negative effects of high larval densities have been demonstrated for *Oc. albifasciatus* under laboratory conditions with constant food supply per larva (Gleiser et al. 2000b), showing that the interference among larvae competing for space (i.e., at the surface) might be the most important mechanism of intraspecific competition in this species. On the contrary, competition for food does not seem to have a similar importance, as shown by another study under controlled conditions, where the lowest mortality occurred at low (10 larvae per 600 square centimeter) and high (100 larvae per 600 square centimeter) larval densities within a narrow range of food availability (32–64 mg of organic matter per 600 square centimeter; Sy 2008). In addition, it has been suggested that food would not be limiting in temporary groundwater habitats where *Oc. albifasciatus* larvae develop (Gleiser et al. 2000b).

In Córdoba province, strong density-dependent effects have been inferred for *Oc. albifasciatus*, based on the analysis of size distribution of adults emerged from natural sites where densities vary seasonally (Gleiser et al. 2000c). However, in the Pereyra Iraola Provincial Park, located 30 km from Ciudad Autónoma de Buenos Aires (CABA), the analysis of life tables of *Oc. albifasciatus* failed to show a relationship between larval mortality and density, and the main cause of mortality was found to be associated with the parasitism by *Strelkovimermis spiculatus* Poinar and Camino (Campos and Sy 2003). The daily larval mortality observed in that study ranged from 0 to 0.53, and no consistent differences among larval instars were detected.

Pool drying has also been recognized as a major cause of mortality in mosquitoes that develop in temporary waters (Casanova and Do Prado 2002, Schäfer and Lundström 2006, Silver 2008). In three rain pools of La Plata city (Buenos Aires Province, Argentina), the drying out of the pools led 20% of the cohorts to fail to complete their development (Micieli et al. 2012). In addition, a study of 91 pools in CABA showed that pools dried out in 68% of the occasions when *Oc. albifasciatus* cohorts were developing. In these pools, complete drying accounted for the mortality of 21 and 36% of larvae in winter and summer, respectively, and only 13 and 4% of larvae reached the fourth larval instar (Fischer et al. 2002). Although the remaining mortality was suggested to be related to a drastic decrease in the water volume in the pools, the relationship between partial drying and larval mortality was not assessed quantitatively.

The understanding of population dynamics of pest species depends strongly on the knowledge of the regulatory processes to which populations are subjected, including the biotic or abiotic factors related to the mortality of the different stages of the life cycle. Estimating daily mortality rates has the advantage that the estimated values are comparable between different situations, as they are independent of the duration of the instar analyzed or the time interval of measurements (Neal 2004). Estimating daily mortality rates is also necessary for modeling purposes, as many population models include such parameters (Renshaw 1991).

The aims of the current study were to analyze the response of life history parameters such as daily larval mortality, time to pupation, and adult size of *Oc. albifasciatus* to increasing larval density under controlled conditions, and to analyze the relationships of daily larval mortality with density and environmental variables (drying rate, temperature, and season) in urban rain pools in Buenos Aires, Argentina.

Materials and Methods

Laboratory Study. *Experimental Design.* The individuals used in the experiment were obtained from eggs laid by females captured in the field in Buenos Aires Province in December 2004 and April 2005. After oviposition, eggs were maintained at room temperature for 7–10 d, and then stored at low temperatures (range, 4–10°C) until the beginning of the experiment. Larvae were obtained by immersion of the eggs in a solution of 10 mg of dry yeast in 70 ml of water to induce hatching. Recently hatched larvae were transferred to plastic pans (20 cm in width by 30 cm in length by 6 cm in height) containing 700 ml of distilled water. Natural food, consisting of 64 g of fine and coarse particulate organic matter (Sy and Campos 2008) collected from the dry substrate at local larval habitats, was added to each pan. The amount of food represented levels that did not cause density-dependent effects across the larval densities tested (Sy 2008). Five larval densities were analyzed: 10, 25, 50, 100, and 200 first instars per pan, and six replicates for each density. Larvae were raised at 22 ± 2°C, under a photoperiod of 14:10 (L:D) h. The pans were examined daily, and the time of pupation was recorded for each individual. Pupae were transferred to individual tubes conditioned for adult emergence. After emergence, the right wing of each individual was removed and measured from the alular notch to the distal margin excluding the fringe scales to the nearest 0.01 mm using a dissecting microscope fitted with a graduated eye-piece.

Data Analyses. For each pan, the number of individuals that reached the pupal stage was recorded and the average time from hatching to pupation was calculated. Daily larval mortality (M_{day}) during development was assumed to be constant, and was calculated for each pan using the following equation (Service 1977):

$$M_{day} = 1 - (N_t/N_0)^{1/t} \quad [1]$$

where M_{day} is the proportion of individuals dying each day, t is the average time from hatching to pupation, N_t is the number of individuals that reached the pupal stage, and N_0 is the number of individuals initially present in the pan.

The relationship of M_{day} with initial larval density was analyzed with least squares regression analysis. Two density variables were compared: *Dens* (larvae per pan) and *Logdens* (logarithm of larvae per pan),

and three models were obtained for each independent variable: a simple linear regression model ($M_{day} = a + b$ density), an exponential growth model ($M_{day} = a$ exp (b density)), and a nonlinear piecewise regression model ($M_{day} = a + b_1$ density (density$<d$) + b_2 density (density$>d$)), where d corresponds to the breakpoint density (threshold) estimated by the model. Because in the piecewise models the parameters b_1 were nonsignificant, the simpler models ($M_{day} = a + b$ density (density$>d$)) were estimated. The obtained models were compared for their Akaike Information Criterion (AIC). These analyses were performed with Infostat software (Di Rienzo et al. 2014).

The relationship between development times from hatching to pupation (T_{pu}) and density was analyzed with a General Linear Mixed Model (GLMM) using R software, Version 3.1.0 (R Core Team 2014), accessed through a user friendly interface in Infostat software (Di Rienzo et al. 2014). Models were fitted using the lme function from the nlme library, and parameters were estimated using the restricted maximum likelihood (REML) method (Pinheiro and Bates 2004). Initial larval density (*Dens*) and log-transformed initial larval density (*Logdens*), *sex*, and the interaction of density × *sex* were included as fixed effects. Pan identity was included as a random effect to account for the lack of independence between individuals from the same pan, and the heteroscedasticity between densities and sexes was accounted for with the varident procedure. The selection of the best model was performed using the AIC. The same analysis was conducted to assess the relationship between wing length (*Wing*) and density.

For each pan, the number of emerged females was divided by the number of emerged males. These relations of female/male were compared between densities with the Kruskal–Wallis test (StafSoft, Inc. 2005).

Field Study. *Study Area.* CABA is located in a humid temperate region, with an annual mean temperature of 17.6°C, annual mean relative humidity of 71%, and 1,089 mm of annual accumulated rainfall. It is a densely urbanized area covering 200 km^2, with 3,000,000 inhabitants (Atlas Ambiental de Buenos Aires 2010). Within CABA, the main larval habitats of *Oc. albifasciatus* are located in green areas, mainly recreational parklands. The field study was performed in Saavedra Park (34° 33′ S, 58° 29′ W) located near the northern boundary of the city. The irregular relief of the park, which extends over ≈13 ha, favors the formation of a great number of ephemeral pools after rain events. These water bodies range from 0.1 to 600 m^2 in surface area and from 1 to 24 cm in depth, and last from 1 wk to several months depending on the climatic conditions. The substrate vegetation of the pools is mainly represented by grass (Gramineae), which is periodically cut. Because the park is located in a densely populated urban area, these pools were often disturbed by human activities.

Data Collection. Rain pools were sampled from June 1998 through May 1999. During this period, sampling was started 2–4 d after every time that the pools were filled by rainfall, and continued at 3–8-d intervals in winter and at 2–4-d intervals in summer and fall until the first pupae were detected. The sampling frequency was adjusted in relation to temperature, taking into account that this is the main factor that determines the velocity of physical (pool drying) and biological (larval development) processes. This sampling schedule allowed at least three sampling events for each *Oc. albifasciatus* cohort. In each sampling event, the flooded area of each pool (F) was assessed by multiplying maximum length, width, and proportion of the resulting rectangle occupied by water. Samples of mosquito larvae were taken by dragging a hand net (350 μm mesh size, 10 cm in width by 8 cm in depth) along a known distance within the pool. When pools were too shallow to use the hand net, several 80-ml subsamples were taken with a dipper. The larval density of *Oc. albifasciatus* (L_{dens}) for each pool was calculated for each date as: number of collected individuals/ area covered by the net or dipper. The abundance of *Oc. albifasciatus* larvae in each pool (N) was calculated for each date as: $F \times L_{dens}$.

Intervals between successive sampling dates included in the analysis were those when 1) no rainfall occurred (to allow for the calculation of the daily drying rate), and 2) *Oc. albifasciatus* larvae were collected both at the beginning and at the end of the interval (to avoid the underestimation of final density when adults emerged during the interval). Data were collected for two cohorts in winter, three cohorts in summer, and four cohorts in fall.

For each interval between successive sampling dates, the average air temperature ($T°_{interval}$) was calculated from data provided by the Servicio Meteorológico Nacional.

Data Analyses. M_{day} between successive sampling dates was estimated using equation 1, where t is the time interval considered, N_t is the larval abundance at the end of the time interval, and N_0 is the number of

Table 1. Mean (range) of *Oc. albifasciatus* life history variables in pans at different initial larval densities

Life history variable	Initial densities (larvae per pan)				
	10 (n = 6)	25 (n = 6)	50 (n = 6)	100 (n = 6)	200 (n = 6)
Time to pupation (d)	6.33 (6.00–6.89)	6.54 (5.67–7.10)	6.66 (6.31–6.90)	6.64 (6.32–6.91)	6.86 (6.63–7.00)
No. of pupae	6.8 (4–9)	16.5 (9–24)	32.7 (20–46)	40.7 (11–84)	33.5 (9–95)
Daily larval mortality	0.07 (0.02–0.14)	0.06 (0.007–0.13)	0.06 (0.01–0.12)	0.15 (0.03–0.27)	0.27 (0.10–0.36)
Wing length (female; mm)	4.32 (4.25–4.40)	4.34 (4.28–4.43)	4.29 (4.24–4.36)	4.08 (3.82–4.20)	4.00 (3.63–4.20)
Wing length (male; mm)	4.33 (4.25–4.51)	4.36 (4.27–4.48)	4.31 (4.25–4.35)	4.11 (3.92–4.23)	4.08 (3.73–4.23)
Female/male	0.95 (0.33–2)	1.35 (0.6–3.2)	1.03 (0.82–1.2)	1.58 (0.7–4.5)	0.68 (0.2–1.1)

Table 2. Comparison of AIC and estimated values (confidence intervals) of parameters *a*, *b*, and *d* for different models of the relationship of M_{day} with density

Model	Variable	AIC	*a* (CI)	*b* (CI)	*d* (CI)
Linear	*Dens*	−69.30	0.035 (−0.0034; 0.0734)	0.0011 (0.0007; 0.0014)	
Exponential	*Dens*	−69.41	0.058 (0.0308; 0.0852)	0.0077 (0.0049; 0.0104)	
Piecewise	*Dens*	−68.59	0.0651 (0.0249; 0.1053)	0.0013 (0.0008; 0.0018)	44.98 (−3.88; 93.84)
Linear	*Logdens*	−60.65	−0.1232 (−0.2363; −0.0101)	0.1458 (0.0807; 0.2109)	
Exponential	*Logdens*	−67.73	0.0051 (−0.0029; 0.01314)	1.7064 (0.9812; 2.4316)	
Piecewise	*Logdens*	−68.88	0.0647 (0.0320; 0.0974)	0.3844 (0.1182; 0.6506)	1.7797 (1.4898; 2.0696)

Dens, initial number of larvae per pan; *Logdens*, logarithm of *Dens*.

individuals present at the beginning of the time interval.

An analogous equation was used to estimate the daily drying rate (D_{day}):

$$D_{day} = 1 - (F_t/F_0)^{1/t} \quad [2]$$

where D_{day} is the proportion of flooded area drying each day, t is the time interval considered, F_t is the flooded area at the end of the time interval, and F_0 is the flooded area at the beginning of the time interval.

The time intervals were classified according to the season (fall, winter, and summer), and M_{day} was compared between seasons with the Kruskal–Wallis test. Post hoc comparisons of mean ranks of all groups were performed with Infostat software (Di Rienzo et al. 2014). The same procedure was used to compare D_{day}, $T°_{interval}$, and L_{dens0} between seasons.

The relationship between M_{day} and environmental variables was analyzed with GLMM using R software, as described in the analysis of the experimental data. Initial larval density (*Dens* and *Logdens*), daily drying rate (D_{day}), season, temperature of each interval ($T°_{interval}$), and a season × $T°_{interval}$ interaction term were included as explanatory variables. Pool identity was included as a random effect to account for the lack of independence between time intervals within each pool, and the heteroscedasticity between seasons was accounted for with the varident procedure. The selection of the best model was performed using the AIC.

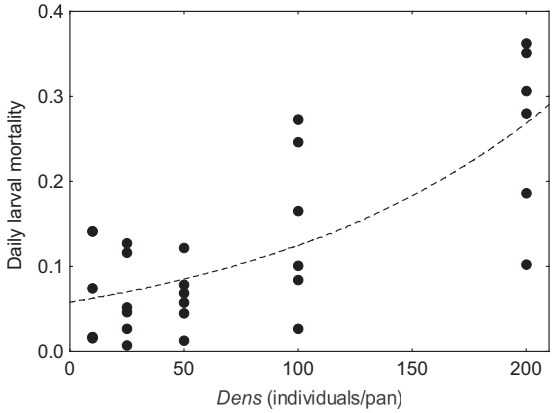

Fig. 1. Relationship of daily larval mortality with density (*Dens*). Dotted line indicates the prediction of the model with the best fit.

Results

Laboratory Study. Daily larval mortality was relatively low at the lowest densities and increased for the highest densities. Consequently, the mean number of individuals per pan that reached the pupal stage remained relatively constant at the three highest density treatments (Table 1). The comparison of the regression models of daily larval mortality showed the lowest AIC value for the exponential model with *Dens* as independent variable, followed by the linear model with *Dens* as independent variable, and in third place the piecewise model with *Logdens* as independent variable (Table 2). It should be noticed that in the piecewise model with *Dens* as independent variable, the parameter *d* (breakpoint) was not significant, indicating an inadequate model for the data regardless of the obtained AIC value. According to the best model, daily larval mortality is around 0.058 at densities close to zero, and increases exponentially in relation to larval density with the function: $M_{day} = 0.058 \exp(0.0077\ Dens)$ (Fig. 1).

The mean development time from egg hatching to pupation increased throughout the whole range of densities (Table 1). According to the obtained model (adjusted $R^2 = 0.41$, residual mean-square error = 0.46), T_{pu} showed a dependence on *sex*, with shorter development times for males than for females. *Logdens* exhibited also a significant effect on T_{pu}, which extends 0.36 d with an increase in density of one order of magnitude (Table 3).

With the exception of the lowest density, both male and female wing length decreased at higher densities (Table 1). According to the obtained model (adjusted $R^2 = 0.81$, residual mean-square error = 0.10), *Wing* showed a dependence on *Dens*, and although no differences between sexes were detected at low densities, the negative effect of density was larger for females than for males as shown by the significant interaction term (Table 4).

The ratio females/males exhibited high variability within densities (Table 1). No significant differences

Table 3. Coefficients and statistics for the relationship of time to pupation with density (log transformed) and sex

Parameter	Value	SE	df	*t*-value	*P* value
Intercept	6.28	0.22	744	28.65	<0.0001
Logdens	0.36	0.13	28	2.89	0.0074
Sex (male)	−0.55	0.03	744	−16.14	<0.0001

Table 4. Coefficients and statistics for the relationship of wing length with density and sex × density interaction

Parameter	Value	SE	df	*t*-value	*P* value
Intercept	4.36	0.03	712	137.05	<0.0001
Dens	−0.0021	0.00031	28	−6.59	<0.0001
Sex (male) × *Dens*	0.00049	0.000072	712	6.75	<0.0001

between densities were detected (Kruskal–Wallis test: $H = 5.84$, df = 4, $N = 30$, $P = 0.21$).

Field Study. The temperature of intervals ($T°_{interval}$) ranged from 11.5°C to 22.6°C, and significant differences were detected between seasons (Kruskal–Wallis test: $H = 23.14$, df = 2, $N = 35$, $P < 0.005$). $T°_{interval}$ values were higher in summer than in fall, and in fall than in winter (Table 5). The daily drying rate (D_{day}) of the pools ranged from 0.08 to 0.87 d^{-1}, and significant differences were detected between seasons (Kruskal–Wallis test: $H = 12.34$, df = 2, $N = 35$, $P < 0.005$). The initial larval density (L_{dens}) ranged from 1.5 to 1080 larvae per square meter, and no significant differences between seasons were detected (Kruskal–Wallis test: $H = 3.20$, df = 2, $N = 35$, $P = 0.2$).

The M_{day} of *Oc. albifasciatus* ranged from 0.02 to 0.91, and significant differences were detected between seasons (Kruskal–Wallis test: H = 17.71, df = 2, $N = 35$, $P < 0.001$). M_{day} values were highest in summer, intermediate in fall, and lowest in winter (Table 5).

Larval density in the pools studied varied widely, from <1 individual per square meter to near 1100 individuals per square meter. M_{day} exhibited a large variability in a wide range of densities (Fig. 2).

GLMM analysis showed a significant relationship of M_{day} with D_{day} ($P < 0.001$) and season ($P < 0.02$). The obtained model did not include *Dens*, *Logdens*, $T°_{interval}$, or a season × $T°_{interval}$ interaction, which showed no significant effect on M_{day}.

Based on this model (adjusted $R^2 = 0.69$, residual mean-square error = 0.18), M_{day} of *Oc. albifasciatus* increases positively with pool drying, and is lower in the winter and higher in the summer, although summer showed only marginally significant differences to the reference season (fall; Table 6).

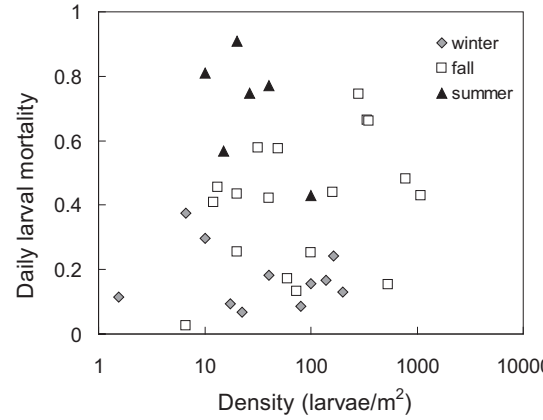

Fig. 2. Relationship of daily larval mortality with larval density in field conditions for different seasons.

Discussion

A key feature of any population is whether density-dependent processes regulate its size and growth rate, and at what stage of the life cycle these processes act (Juliano 2007). It is generally accepted that, for mosquito species, density-dependent effects act on the larval stage, although the relative importance of density dependent and independent processes may depend on the characteristics of the larval habitats (Washburn 1995, Juliano 2007).

Our results obtained in laboratory conditions show that larval mortality of *Oc. albifasciatus* depends on density. Although density-independent mortality at low densities may be inferred from the graphical analysis, according to the AIC criterion the evidence is not enough to support the existence of a breakpoint in the relationship of mortality with density. Moreover, our data fitted better to a model with an exponential increase of mortality with density, showing proportionally larger effects at high densities. Considering that the main mechanism causing density dependent effects in this species is probably its competence for space (interference) and not for food resources (exploitation), it seems likely that the obtained model might be representative for this species, at least within certain range of food supply. Nevertheless, extrapolations of results obtained in the lab to the field should be made with caution, because although the type of food resources used in the experiment is similar to that present in natural environment, the exact amount available in the larval habitats is not known.

In addition to the lethal effects, the consequences of density on populations may also include nonlethal effects (Juliano 2007). Previous experimental studies on *Oc. albifasciatus* have demonstrated extended development times at high densities (Gleiser et al. 2000b). In our study, extended development times and reduced adult size were observed at increasing den-

Table 5. Seasonal means (range) of environmental variables and daily larval mortality in the studied rain pools

Variable	Fall ($n = 18$)	Winter ($n = 11$)	Summer ($n = 6$)	Significant differences between seasons ($P < 0.05$)
Temperature	16.8 (11.8–20.9)	14.07 (11.5–15.6)	21.9 (21.2–22.6)	Winter<fall<summer
Daily drying rate	0.39 (011–0.76)	0.27 (0.08–0.65)	0.73 (0.58–0.87)	Winter = fall<summer
Larval density (ind/m^2)	219.2 (6.7–1080)	105.4 (1.5–200)	35.3 (10–100)	Winter = fall = season
Daily larval mortality	0.40 (0.02–0.75)	0.17 (0.07–0.38)	0.71 (0.43–0.91)	Winter<fall < summer

Table 6. Coefficients and statistics for the variables that were included in the GLMM model

Parameter	Value	SE	df	*t*-value	*P* value
Intercept	0.19	0.08	25	2.51	0.0187
D_{day}	0.43	0.13	25	3.39	0.0023
Winter	−0.13	0.06	25	−2.31	0.0292
Summer	0.15	0.08	25	1.83	0.0800

sities, and these effects were evidenced throughout the whole range of studied densities.

These results suggest that at low densities nonlethal effects may be more important, while at high densities lethal and nonlethal effects could act additively, thus increasing the potential of the regulatory effect of density on population dynamics. This might be increased by the differential effects of density on females, causing a larger reduction of body size, together with the associated reduction in fecundity (Sy 2008).

In contrast with the experiment under controlled conditions, no density-dependent mortality was detected in the urban pools analyzed. It is not clear whether this is related to the fact that larval densities observed in the field almost never exceeded the lower end of the range of densities assessed in the lab. A study of the amount of food resources available to larvae in the field would improve our understanding on this subject.

Unfortunately, nonlethal effects were not measured in our field study, but it is likely that both development times and adult size are at least to some extent influenced by the densities that occur in the studied rain pools. Nevertheless, such effects could be extremely difficult to prove, as development time and adult size of *Oc. albifasciatus* are sensitive to environmental variables such as temperature (Fontanarrosa et al. 2000, Sy 2008, Garzón and Schweigmann 2014).

Although it has been suggested that mosquitoes are controlled by intra- and interspecific competition in temporary bodies of water (Chase and Knight 2003, Juliano 2007), most studies considered sites where the duration of water exceeded several times the development time of mosquito immature stages (e.g., Renshaw et al. 1993, Gleiser et al. 2000c, Chase and Knight 2003). On the contrary, in habitats where the duration of water is approximately as long as the development time of mosquitoes, pool drying has been documented as a main cause of mortality for mosquitoes (Casanova and Do Prado 2002, Silver 2008), including *Oc. albifasciatus* (Fischer et al. 2002, Campos and Sy 2003). In our study, we found a direct relationship between larval mortality and pool drying. The effect of this partial drying on larval survival may act directly, by trapping a proportion of the larvae at the borders of the drying pool in small collections of water, which later dry out completely (Becker 1989).

Besides the density-independent effect discussed above, the gradual drying of the pool might increase competition effects when larvae are concentrated in a reduced flooded area (Renshaw et al. 1993). In addition, pool drying may also impose density-dependent mortality, taking into account that increasing density causes a slower development, resulting in a greater fraction of individuals potentially being unable to complete development before drying.

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