

The Potential of Cladocerans as Controphic Competitors of the Mosquito *Culex pipiens*

SABINE DUQUESNE,^{1,2} IRIS KROEGER,¹ MAGDALENE KUTYNIOK,¹ AND MATTHIAS LIESS¹

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ABSTRACT This study assesses the potential of cladocerans as competitors for controlling the oviposition and larval abundances of mosquitoes. Control of mosquito larvae involving the use of antagonists has focused mostly on predators. We hypothesized that cladoceran competitors have a strong potential to control larval populations of some species of mosquitoes that can be early colonizers of newly-filled waterbodies, and should be less efficient competitors. To test this hypothesis, the establishment and development of larval populations of wild *Culex pipiens* were investigated in outdoor microcosms varying in terms of *Daphnia magna* populations. When the population was well established (i.e., high densities of *D. magna*), oviposition was fully inhibited and there was consequently no mosquito larvae. When the population was more recently established (i.e., lower densities of *D. magna*), oviposition and larval development of *Cx. pipiens* occurred. In the absence of *D. magna*, oviposition, larval biomass, and abundance of *Cx. pipiens* reached high values. In this situation, conspecifics were inhibiting further oviposition of *Cx. pipiens*. Based on these results, we suggest that competing zooplankton species, such as *D. magna*, could be used for the control of mosquito species such as *Cx. pipiens*. This approach could be beneficial for the management of wetlands.

KEY WORDS mosquito larvae, zooplankton, oviposition, competition, development

Concern about mosquitoes posing a threat as pests and vectors of disease is growing. This is related to the increase in global trade (Lounibos 2002) as well as to global warming and associated changes in weather patterns (Epstein et al. 1998). The problem is more severe in tropical regions, but is also increasing in temperate climates. For example, *Aedes albopictus* has become more abundant in Italy since 1990 and has recently spread northward toward Germany (Knudsen et al. 1996). The 2007 outbreak of chikungunya fever in northeastern Italy (Enserink 2008) and the number of cases of West Nile virus reported recently in the United States and Europe (Balenghien et al. 2007) illustrate the threat to humans. It is thus evident that there is an increasing need for controlling mosquito populations.

Biological methods involving antagonistic species (i.e., predators or competitors) have the advantage of being less associated with environmental problems compared with pesticide use. Many studies have shown the deterrent effects of predators on mosquito populations, either by altering the oviposition process (a trait-mediated effect related to behavioral change) or by direct feeding on larvae (a density-mediated

effect related to decreased abundance) (i.e., some genera of cyclopoid copepods and aquatic insects) (Marten et al. 1994, Blaustein et al. 1995, Chansang et al. 2004, Kumar and Hwang 2006). For example, *Culiseta longiareolata*, *Culex pipiens*, and *Anopheles gambiae* preferentially oviposit in the absence of aquatic insects such as *Notonecta sp.* and *Anax imperator* or tadpoles such as *Bufo viridis* (Blaustein and Kotler 1993; Stav et al. 2000; Kiflawi et al. 2003a, 2003b; Blaustein et al. 2004; Munga et al. 2006; Beketov and Liess 2007). However, the use of predators as an ecological method to control mosquito larvae was not always satisfactory. One reason is that some species of predator are typically not found in temporary waterbodies or do not feed specifically on mosquito larvae. In the latter case, predators can even favor the development of mosquitoes by feeding on alternative prey species, which decreases the negative competitive effect of controphic species on mosquito larvae (so-called apparent mutualism) (Bence 1988, Juliano 2007, Stav et al. 2005). Furthermore, interactions between multiple predator species can result in complex effects on prey populations and community structure that are not predictable from the direct effects of each species alone (Griswold and Lounibos 2006).

An alternative approach is the use of competitors (i.e., controphic species) to regulate larval populations of mosquitoes. Many mosquito species are early colonizers and may have poor competitive abilities. Therefore, their reduction or even elimination by competitors is likely to occur. However, despite the

¹Department of System Ecotoxicology, Umwelt Forschung Zentrum, Helmholtz Centre for Environmental Research, Permoserstrasse 15, D-04318 Leipzig, Germany.

²Corresponding author: Department of Conservation Biology, UFZ, Helmholtz Centre for Environmental Research, Permoserstrasse 15, D-04318 Leipzig, Germany (e-mail: Sabine.Duquesne@uba.de).

potential of using competitors to regulate larval populations of mosquitoes, knowledge in this area has mainly focused on the use of other mosquito species (Lounibos 2007). For mosquito control, this approach implies the acceptance of a second species of mosquito in the area of concern. Surprisingly, the role of non-mosquito species as competitors remains largely unexplored, even though they may be an effective tool for controlling mosquito populations, as hypothesized by Blaustein and Chase (2007). Studies on the competitive effects of nonmosquito species on mosquito larvae focus on either oviposition or larval development, as processes on which competition could act, but not both simultaneously, although they are tightly linked (e.g., Knight et al. 2004, Stav et al. 2005, Mokany and Shine 2003a). In addition, the outcomes of most existing studies have limited relevance to natural field conditions because relevant processes were interrupted by experimental manipulations (e.g., the removal of egg clutches or the artificial introduction of larvae).

We investigated the hypothesis that larval populations of mosquitoes can be reduced successfully over time through competition with species other than mosquitoes. Zooplankton species such as cladocerans are potential competitors that could fulfill the requirements for the biological control of mosquitoes (e.g., widespread occurrence in the field, adaptation to the breeding habitats of mosquitoes, and close interactions with larval populations of mosquitoes) (Kumar and Hwang 2006). Furthermore, they are neither medically important, nor nuisances, which is an advantage compared with the method using mosquito-mosquito competition. Our study is performed under realistic outdoor conditions with introduced populations of cladocerans *Daphnia magna* and naturally developing populations of *Cx. pipiens*, which can be an early colonist of newly-filled water bodies, and has been shown to be a poor competitor relative to other mosquitoes (Costanzo et al. 2005, Carrieri et al. 2003). Furthermore, it is a dominant species in the study area and a vector of West Nile virus. The aim of the investigation was to test whether *D. magna* and *Cx. pipiens* compete successfully, and to determine whether the cladoceran could be an effective biocontrol agent for the reduction of this vector species. *D. magna* was chosen because daphnids are typical inhabitants of temporary waterbodies, and therefore, potential competitors of *Cx. pipiens* in the wild. Outcomes of this study could be also relevant to other mosquito species. The knowledge gained on competitive processes and mechanisms of biological interactions could be also applied either on its own or in combination with other methods (e.g., pesticide application) to the management and reduction of mosquito populations.

Materials and Methods

Experimental Conditions. We performed pond experiments in outdoor plastic microcosms of 20 liters each (≈ 38 cm height and 25 cm diameter), which contained a layer of natural sediment (< 1 cm thick-

ness) that was collected from a pond in a forested area (Abtnaundorfer pond $51^{\circ}22'29.66''N$, $12^{\circ}25'40.39''E$, Leipzig, Germany) and 1 liter of filtered water ($55 \mu\text{m}$ mesh size) from the same pond. The sediment was a mixture of 70% sandy sediment (previously kept at 100°C for 10 h to eliminate autochthonous organisms) and 30% dried organic sediment (leaves and degraded particles). They were topped up with tap water to the final volume. As water evaporated, it was replaced with tap water. Limited replacement was needed; no increase of conductivity was observed. The experiment consisted of three treatments (three replicates for the no treatment and low cladoceran treatment; four replicates for the high cladoceran treatment). The treatments differed in the density of *D. magna* that was introduced. The microcosms of the high and low cladoceran treatments were provided with 20 *D. magna* (10 individuals > 3 wk old and 10 individuals $< 1-2$ wk old) obtained from laboratory cultures (clone B, Bayer CropScience, Monheim, Germany), but introduced on different dates (01 August 2006 in high cladoceran, and 14 August 2006 in low cladoceran treatments). The population of high cladoceran could therefore grow to carrying capacity before the microcosms' possible colonization by mosquitoes. During these 2 wk, all units from the three treatments filled with water were covered with a mesh to avoid unwanted oviposition by mosquitoes. The experiment began on 14 August 2006. On day 9 (23 August), the means (and standard deviations) were 489 ± 308 , 42 ± 28 , and $0 \text{ mg } Daphnia/L$, corresponding to 695 ± 143 , 48 ± 19 , and $0 \text{ Daphnia}/L^{-1}$, in the high, low, and no cladoceran treatments, respectively. These different biomass or densities would represent the different levels of competitive pressure on the mosquitoes. The microcosms were placed randomly ≈ 2 m apart in a grassland plot partially surrounded by bushes in the experimental outdoor area of the Umwelt Forschung Zentrum (Leipzig, Germany). The egg clutches deposited by *Culex* and resultant larvae were left to develop in the ponds without further handling or removal.

Sampling and Monitoring. Oviposition of the wild mosquitoes was monitored by counting the number of egg clutches in each microcosm, five times per week. The average lifetime of a freshly deposited egg until hatching is estimated as 3–4 d under these experimental conditions. For an appropriate estimation of the correct numbers of egg clutches, the numbers were therefore divided by 3 to account for repeated counting of the same clutch on successive days. The abundances of mosquito larvae and cladocerans were monitored twice per week from day 7. The larvae were collected by dipping a plastic tray ($22 \times 17 \times 4$ cm) below the surface of the water. They were then counted directly and returned to the unit. Numbers were adjusted for the entire surface of the microcosm. Given that most mosquito larvae were close to the water's surface, the calculated numbers are estimates of the total abundances per microcosm; this was then expressed as abundance per liter. The larvae were classified according to body size as small, medium, and

large, corresponding to first, second/third, and fourth instars, respectively. Larval abundances were converted into biomass by applying mean fresh weights per individual of 0.28, 0.68, and 2.43 mg for the different size classes, respectively. These weights were obtained by weighing 20 replicate samples per size class. The mosquito larvae were identified to the level of species using the determination key of Becker et al. (2003). Individuals of *Cx. pipiens* were the most prevalent (<98%), with <2% being *Anopheles maculipennis* and <0.1% being *Culex territans*.

D. magna in water samples were collected after gentle manual mixing of the water within the unit. Water samples were collected with a plastic tube of ≈ 0.3 liters (25 cm length \times 4 cm diameter), inserted vertically in the whole water column, and then sealed at the bottom. Samples were sieved through a 180- μ m mesh and subsequently preserved in 70% ethanol. Individuals were counted and classified into three groups according to their approximate body size (<1.5 mm, 1.5–2 mm, >2 mm). Cladoceran abundance was converted into biomass by applying mean fresh weights of 0.57, 1.09, and 5.31 mg for individuals of body size <1.5 mm, 1.5–2 mm, and >2 mm, respectively. These weights were obtained by weighing 20 replicate samples per size class.

Physicochemical Parameters. Turbidity and the concentration of chlorophyll were measured twice per week. Turbidity was measured with a turbidimeter (TurbiQuant 1100IR, Merck, Darmstadt, Germany) and chlorophyll with a spectrofluorometer (Gemini EM Spectramax [Sunnyvale, CA], wavelengths of 400 nm for excitation, 700 nm for emission, and 690 nm as cutoff), in relative fluorescence units as an indication of the concentration of algal food in the water (Bern and Dahl 1999).

Weather parameters (i.e., soil and air temperature, precipitation, air moisture, wind speed, and global radiation) were recorded at 2-min intervals; data were obtained from the Department of Bioremediation, UFZ (Leipzig, Germany).

Data Analysis. Data on oviposition and larval biomass or abundance were calculated as moving averages of measurements obtained at three consecutive sampling times. The differences of oviposition (or biomass and abundance of larvae) between the three different treatments were tested at each monitoring time using the nonparametric Kruskal-Wallis test, followed by the post hoc Dunn's test. The Kruskal-Wallis test was used because high density of cladocerans inhibited oviposition, and thus, larval biomass and abundance were reduced to values close or equal to 0; normality could therefore not be assumed.

The relationships between oviposition data and physicochemical variables were analyzed by computing either the nonparametric correlations (Spearman's rank correlation) when data of the three treatments were considered or the parametric Pearson test when only data of the no cladoceran treatment were considered.

The ratios of the maximum larval biomass (or abundances) to the total number of egg clutches (i.e.,

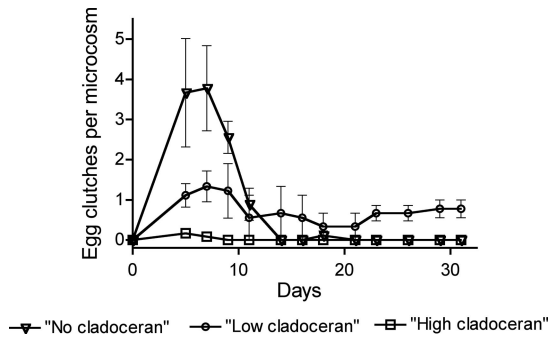


Fig. 1. Changes over time of oviposition of the mosquito *Cx. pipiens*. Mean values and standard deviations in the no, low, and high cladoceran treatments.

larvae to eggs ratio), between no treatment and low cladoceran treatment, were computed using unpaired *t* tests, because normality of data was assumed. The larvae to eggs ratio for the high cladoceran treatment was not compared with the others as both larvae and eggs were absent in these ponds. For changes in proportions of larvae of the large-size class over time, the data were compared using analysis of variance at each sampling time. Statistical analyses were performed using Prism, version 4.0a, GraphPad (San Diego, CA).

Results

Oviposition of *Cx. pipiens*. In the first 10 d of the experiment, although biomass and abundances of cladocerans and *Culex* larvae were still low in the no treatment and low cladoceran treatment, oviposition was at its maximum (Fig. 1). In contrast, oviposition did not occur in the high cladoceran treatment throughout the experiment, i.e., in presence of cladocerans biomass varying between 180 and 780 mg/L⁻¹. Only the differences in oviposition between high and no cladoceran (absence of cladocerans) treatments until day 10 were significant (i.e., Kruskal-Wallis test, $P < 0.05$; post hoc test Dunn's, $P < 0.05$) (Table 1).

Data of Figs. 1 and 2 also indicate that larvae themselves seem to influence oviposition. Indeed, in absence of cladocerans (i.e., in the no cladoceran treatment), oviposition was the highest during the first days, and afterward it was fully inhibited when larval population developed (i.e., after ≈ 10 d).

Larval Populations of *Cx. pipiens*. The biomass of mosquito larvae was generally higher in the no treatment than in the low cladoceran treatment (Fig. 2) and negligible in the high cladoceran treatment. Similar patterns were observed for abundances of mosquito larvae (data not shown). The differences in larval biomass were only significant between the no treatment and high cladoceran treatment for each monitoring time over the period of 9–34 d (i.e., Kruskal-Wallis test, $P < 0.05$; post hoc test Dunn's, $P < 0.05$ for biomass) (Table 1). Larval populations were thus reduced when cladoceran population was better established.

Table 1. Differences in mosquito oviposition and biomass of *Culex* larvae between no, low, and high cladoceran treatments at different sampling times (represented as moving averages), using Kruskal-Wallis and followed by post hoc Dunn's test

Time (days)	Kruskal-Wallis test		Dunn's test	
	P value	Kruskal-Wallis statistics	P value	Difference in rank sum
Oviposition				
5	0.027	7.25	<0.05	6.05
7	0.022	7.62	<0.05	6.17
9	0.038	6.54	<0.05	5.5
Larval biomass				
9.3	0.024	7.45	<0.05	6.0
11.6	0.024	7.45	<0.05	6.0
14	0.035	6.71	<0.05	6.0
16.3	0.035	6.71	<0.05	6.0
18.6 until 32.3	0.024	7.45	<0.05	6.0

Only the results with significant differences were reported; this is only about comparisons between no and low cladoceran treatments, as differences between no and low and between low and high cladoceran were never significant ($n = 4$ for high, and $n = 3$ for no and low cladoceran treatments).

The proportion of egg clutches developing to surviving larvae (the larvae to eggs ratio) was estimated as the ratios between the maximum larval biomass (i.e., biomass of $\approx 218 \text{ mg/L}^{-1}$, equivalent to 113 daphnids L^{-1} , reached on day 23) and the total number of egg clutches laid before day 20. This ratio was not defined in the high cladoceran treatment as a result of absence of oviposition. It was significantly higher in the no treatment than in the low cladoceran treatment (i.e., ratios of 24.6 ± 2.28 and 13.6 ± 1.39 , respectively; unpaired t test, $P = 0.002$, equal variances). The larvae to eggs ratio was thus reduced by $\approx 45\%$ in the presence of $\approx 218 \text{ mg cladocerans/L}^{-1}$. This result shows that cladocerans (controphics) have a strong adverse effect on the biomass or abundances of mosquito larvae.

The changes over time in the proportions of the different size classes of mosquito larvae provide an insight into the development of larval populations. In the no cladoceran treatment, the abundance of larvae in the large-size class gradually increased over time (Fig. 3). The increase in proportion of larvae in the

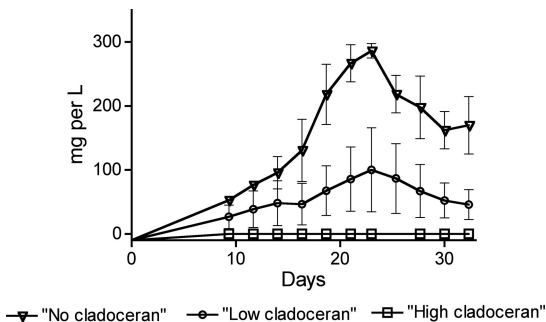


Fig. 2. Changes over time of the biomass of *Cx. pipiens* larvae. Mean values and standard deviations in the no, low, and high cladoceran treatments.

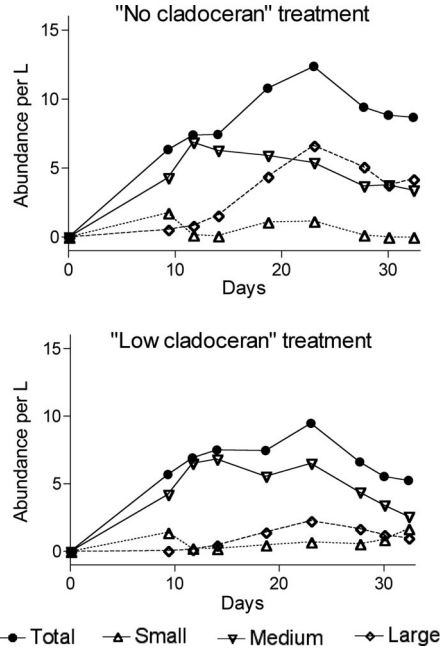


Fig. 3. Changes over time of the size-class composition of the *Cx. pipiens* larval population in the no and low cladoceran treatments (mean abundances [L^{-1}] of total, small-, medium-, and large-size class). High cladoceran treatment is not represented as a result of absence of larvae.

large-size class compared with those in the small-size class was significant from day 19 (analysis of variance, $P < 0.01$). This ratio was correlated negatively with the proportions of large larvae present earlier in the experiment (e.g., at day 14, $r = -0.97$, $P = 0.03$), which indicates that mosquito larvae (conspecifics) themselves would slow down the larval development. In the low cladoceran treatment, the abundance of large larvae did not distinctly increase over time (Fig. 3B). It was also noted that the growth of the cladocerans during the first 20 d of the experiment was slower in the low cladoceran than in the no cladoceran treatments (Fig. 4).

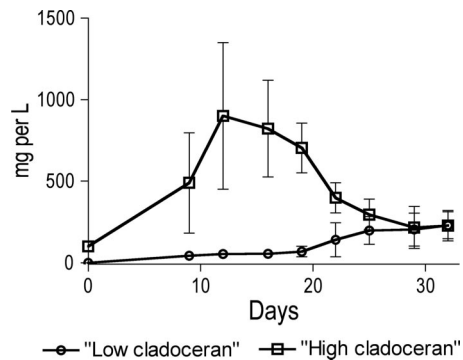


Fig. 4. Changes over time of the biomass of *D. magna*. Mean values and standard deviations in the low and high cladoceran treatments.

Correlations Between Abiotic and Biotic Parameters. Oviposition was correlated neither with turbidity nor with chlorophyll levels (used as an indicator of food quantity) even in the absence of daphnids (i.e., no cladoceran treatment) (data not shown). It was also not correlated with weather parameters (soil and air temperature, precipitation, air moisture, wind speed, and global radiation; data not shown). Daphnid abundances were also correlated neither with chlorophyll levels nor with turbidity. Both parameters chlorophyll and turbidity were significantly correlated (Pearson: $n = 252$, $R^2 = 0.86$, $P < 0.0001$) (data not shown).

Comparison of Effects of Controphics on Oviposition and Larval Development. The effects of cladocerans were more pronounced on mosquito oviposition than on larval development. The oviposition was reduced by $\approx 65\%$ when daphnid biomass was ≈ 50 mg/L⁻¹ (Figs. 1 and 4), but the larval biomass was reduced only by $\approx 45\%$ when daphnid biomass was ≈ 218 mg/L⁻¹ (see above larval to egg ratios).

Discussion

The aim of the study was to determine the efficacy of using competing species of zooplankton to reduce larval populations of mosquitoes by inhibiting the following: 1) oviposition and 2) larval development and survival. Despite its potential for the control of mosquito populations, this strategy has received little attention, and previous work was mostly focused either on the use of predators or on other mosquito species used as competitors for the species to control. The experimental conditions used in this study closely simulated the natural dynamic of an aquatic community dominated by mosquito larvae of *Cx. pipiens* and the cladoceran *D. magna*.

Influence of Competition on Oviposition. Competitive interactions can induce changes in oviposition site selection. Such trait-mediated effects occur when effects of stress on individuals are sublethal (e.g., impaired behavior, reduced body size, delayed growth). Our results showed that oviposition of wild *Cx. pipiens* was strongly reduced in ponds with well-developed controphic populations (i.e., cladocerans *D. magna*). Reduced oviposition based on avoidance behavior in the presence of controphics has been reported for anuran tadpoles (Blaustein and Kotler 1993; Mokany and Shine 2003a, 2003b; Munga et al. 2006). But the only study dealing with species of zooplankton (*D. magna*) showed no oviposition avoidance in *C. longiareolata* (Stav et al. 1999). The discrepancy between those results and ours may be related to the following: 1) the larval ecology of the two species of mosquito: *Culiseta* larvae are primarily feeders on periphyton, whereas *Culex* larvae are filter feeders and thus may be in closer competition with *D. magna*, or 2) the fact that in our experiment oviposition avoidance of *Culex* was also influenced by the conditions linked to the presence of *D. magna* (e.g., microbial community).

Our results showed that oviposition of *Cx. pipiens* was also reduced by conspecifics. Such a reduction has

also been found for *An. gambiae* in western Kenya under experimental conditions (Munga et al. 2006) and for *Culex restuans* in Michigan in a semifield experiment (Reiskind and Wilson 2004). However, other species (e.g., *Culex stigmatosoma* and *Culex quinquefasciatus*) preferentially oviposit when conspecifics are present (Bentley and Day 1989, Beehler and Mulla 1995), whereas others (e.g., *C. longiareolata*) seem uninfluenced (Blaustein and Kotler 1993). However, it should be noted that site selection for oviposition is density dependent, as shown by Munga et al. (2006), and the investigations cited may not have been always performed at relevant densities. Oviposition site selection may necessitate a trade-off between the risk of choosing an unsuitable habitat (i.e., avoidance of sites previously selected, and thus most likely suitable) and the cost of competition (i.e., avoidance of sites with competing conspecifics), as previously suggested (Spencer et al. 2002, Kiflawi et al. 2003b, Blaustein et al. 2004).

We showed that mosquito larvae significantly inhibit oviposition. This is understandable because conspecifics compete for the same ecological niche. Although this inhibition of oviposition by conspecifics density is ecologically interesting, it is irrelevant for mosquito control. By contrast, the performances of the candidate cladoceran *D. magna* are satisfying, as they significantly inhibit mosquito oviposition (i.e., by $\approx 65\%$ of maximum oviposition observed in absence of cladocerans, when daphnid biomass was ≈ 50 mg/L⁻¹).

We did not observe any link between food availability (represented by chlorophyll levels as in Bern and Dahl 1999) and oviposition, whereas such links that illustrate the influence of competition for food were previously demonstrated (Blaustein and Kotler 1993, Beehler and Mulla 1995, Rodcharoen et al. 1997). However, this does not exclude that food availability may influence mosquito oviposition via preferences of components in the food web other than chlorophyll [e.g., bacteria, protozoans, or organic matter that are also important components in the diet of mosquito larvae, as reported by Mokany and Shine (2003a)].

There was also no link between chlorophyll levels and daphnids. This is in agreement with the study of Olsen et al. (1986), who attributed these findings to the fact that daphnid fecal matter also contains high chlorophyll levels, which interfere with the measurements as food items.

Influence of Competition on Larval Populations of Mosquitoes. Competitive interactions can induce changes in larval survival and reduction in emergence success (Liess 2002). Such density-mediated effects occur when the limited availability of food resources leads to mortality of individuals. Our results showed that the development of larval populations of *Cx. pipiens* was strongly reduced by interspecific competition with individuals of the same trophic level (i.e., cladocerans, controphics) and intraspecific competition with *Culex* larvae (conspecifics).

Controphics exerted a strong adverse effect on the larval development. Indeed, the proportion of surviv-

ing larvae was significantly reduced in presence of controphics (reduction of 45% by a biomass of cladocerans of $\approx 218 \text{ mg/L}^{-1}$). These results confirm our initial hypothesis that *Culex* larvae, frequently encountered as early colonizers, are relatively poor competitors and therefore may be controlled by competing species. It should be noted that reciprocally conspecifics seem to also have adverse effects on the growth of daphnid populations, although this is secondary in terms of importance. Furthermore, we showed that, in the presence of controphics, the proportion of mosquito larvae of large-size class did not increase over time by contrast to the observation in absence of controphics. Hence, controphics reduced abundance and thus biomass by hindering larval development.

Our findings support the hypothesis of Blaustein and Karban (1990), who suggested that competition with *Daphnia* affects larvae of *Culex tarsalis* in rice fields. This was revealed in a study that focused primarily on the effects of predation by mosquitofish. Other investigations also found indications that controphics may reduce the abundance of mosquito larvae. For example, Stav et al. (1999) showed a slight negative effect of *D. magna* on the survival of larvae of *C. longiareolata*, but no effect on the number of egg clutches. Knight et al. (2004) showed that competition with mixed species of zooplankton reduced the survival of *Anopheles quadrimaculatus* larvae that had been introduced artificially into outdoor microcosms.

Our results also suggest that conspecifics influence mosquito larval populations by slowing down development, as the proportions of large mosquito larvae present later were correlated negatively with the proportions of large larvae present earlier in the experiment. Furthermore, it is likely that larval competition leads to density-dependent effects on adult traits (e.g., body size and longevity), as reported previously (Juliano 1998, Lounibos 2001, Reiskind et al. 2004, Yee et al. 2007, Reiskind and Lounibos 2009). Such competitive interactions are highly relevant in population dynamics because traits as body size (usually linked to fertility) and longevity will possibly induce changes at the population level. Competitive interactions are also important as a driving factor for the succession of species that establish in small and temporary habitats (e.g., mosquito breeding sites) where predators are rare.

Conclusion

We found that populations of *Cx. pipiens* were reduced in the presence of competing cladoceran *D. magna*, and that this reduction increased with densities of competitors. This deterrent effect on mosquito populations results primarily from competitors inhibiting oviposition, and secondarily from competitors inhibiting development and survival of mosquito larvae. Thus, we have confirmation that nonmosquito organisms that can compete with mosquitoes are an effective strategy for controlling larval populations of mosquitoes. Solutions such as the active introduction

or passive facilitation of competitive cladoceran species should be explored in practices of landscape management. This novel approach could add substantially to the traditional ecological approach of using predators in mosquito management and could be used on its own or in conjunction with more conventional methods such as the application of pesticides.

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