Consequences of the introduction of the planarian *Girardia anceps* (Tricladida: Dugesiidae) in artificial containers with larvae of the mosquitoes *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae) from Argentina

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HIGHLIGHTS

- *Girardia anceps* was tested against mosquito larvae of two vector species.
- The predator reduced significantly the number of larvae in artificial containers.
- This reduction was sustainable along several months.
- Reduction was higher for *Aedes aegypti* than for *Culex pipiens* colonizing containers in the field.
- *Girardia anceps* is potentially an effective control agent against immature mosquitoes.

GRAPHICAL ABSTRACT
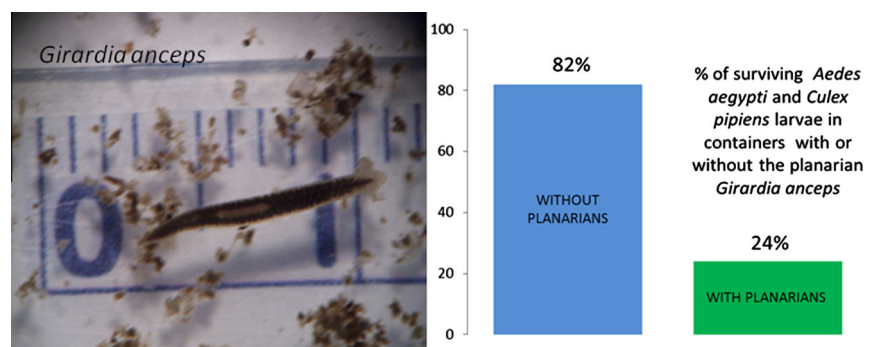
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ABSTRACT

Aedes aegypti and *Culex pipiens* are container-dwelling mosquito species that are vectors of important diseases to man, such as dengue and lymphatic filariasis, respectively. Predators of these pests are an interesting alternative to be incorporated to biological control measures. We tested the consequences of introducing individuals of *Girardia anceps*, a native freshwater flatworm species, within artificial water containers where larvae of these mosquitoes thrive. Our goals were to ascertain if mosquito species, density of larvae (high or low), type of water container (tires or ovitraps), and presence or absence of planarians affected mosquito survivorship (measured as number of individuals reaching the pupa stage) in manipulated artificial containers. Furthermore, we monitored ovitraps in the field along several months in order to explore the long-term effect of the presence of planarian on the colonization of these containers by feral mosquitoes under natural conditions. We found that the presence of planarians reduced the number of mosquitoes reaching pupation and that such reduction depends on the initial density of larvae. Reduction of populations of *A. aegypti* was high along the breeding season of this mosquito, being the effect less evident in *C. pipiens*. *G. anceps* could be an agent of control against container-breeding mosquitoes if its release in small water containers is complemented with other suitable management strategies.

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1. Introduction

The consumption of mosquito larvae by planarian worms was observed by Lischetti (1912) for the first time. Since then, studies were performed on several species of predatory flatworms in order

to evaluate them as an agent against culicids (Meyer and Learned, 1981; Ali and Mulla, 1983; George et al., 1983; Kolasa, 1984; Blaustein, 1990; Loh et al., 1992; Melo and Andrade, 2001; see also reviews by Legner 1995 and Mogi 2007). Some features of the biology of planaria enables to place them among several candidates for biological control, such as high predatory rates (Tranchida et al., 2009a), the possibility of population recycling in the habitat (Case and Washino, 1979), potential use in several kinds of artificial (Melo et al., 1996) or natural freshwater environments such as rice fields (Yu and Legner, 1976), catch basins (George et al., 1983) and shallow ponds (Legner, 1977), easy mass rearing (Callahan and Morris, 1989) and the possibility of asexual reproduction through artificially-induced fission (Legner and Tsai, 1978). Besides lack of risk for the environment and tolerance to pesticides by flatworms was demonstrated (Levy and Miller, 1978; Mogi, 2007). Field evaluations of predation by planarian worms on specific target populations were performed in large aquatic environments, but studies concerning the capacity of planarians to predate on larval mosquito populations from small containers are scarce. One of the reasons for the lack of such studies could be related to the difficulty of introducing worms in a high number of water containers where immature of some important vectors thrive, such as tree-holes and man-made recipients. One possible approach is to seed those small aquatic habitats with fragments of planarian using sprayers (Darby et al., 1988), taking advantage of the capacity of worms to develop a whole individual from each fragment. Another method to make possible man-induced colonization of containers by planarians is the release by hand, in a similar way to the method used with predatory copepods (Marten, 1990; Marten et al., 1994a, b). Kay and Nam (2005) reported an outstanding example of biological control with copepods in a sustainable, community-based approach of vector reduction in Vietnam.

In Buenos Aires province, Argentina, two important mosquitoes are common in urban areas: *Aedes aegypti* (L), and *Culex pipiens* (L.). Their medical relevance is evident since they act as vectors of important diseases to man, mainly arbovirosis such as yellow fever and dengue, and lymphatic filariasis (Kettle, 1984). They are usually found breeding in tanks, cemetery vases, water accumulations in discarded recipients, automobile tires stored outdoors, and swimming pools, although *A. aegypti* explodes this last habitat in low numbers. In some situations, both species coexist in the same container (Stein et al., 2002; Rossi and Almirón, 2004; Micieli et al., 2006; Vezzani, 2007).

In a previous work, Tranchida et al. (2009a) conducted a survey of free-living flatworms in mosquito breeding places near La Plata city (34°51′7″S, 58°57′30″W). They found *Bothromesostoma* cf. *evelinae* Marcus, *Mesostoma ehrenbergii* (Focke) Örsted (Typhloplanidae), and *G. anceps* (Kenk) Ball (Dugesiidae) in transient and permanent freshwater pools where immature culicids occurred. In complementary laboratory experiments, these authors evaluated the daily predation rate, differential predation on each mosquito larval instar, selective consume on either *A. aegypti* or *C. pipiens*, and tolerance to water quality and survival after a dry period within containers. As a result, Tranchida et al. (2009a) established that, among the three field-collected native species, *G. anceps* was the most promising candidate for reducing numbers of immature mosquitoes in small water containers. The outcome of their bioassays showed that *G. anceps* can prey on all instars of both mosquito species, maintain a steady predation rate over time, was able to be easily reared in large numbers, and resist environmental variations within micro-habitats filled with water. Large size relative to larvae was another benefit of *G. anceps* as well. These features can be viewed as advantages for biological control of vectors, because *A. aegypti* and *C. pipiens* frequently are associated to low-volume recipients for their larval development, thus colonizing cemeteries in urban environments.

The aim of this work was to evaluate the potential of *G. anceps*, a common flatworm from Argentina and Paraguay (Cazzaniga and Curino, 1987), to control mosquito larvae in small water containers. Specifically, we artificially introduced *G. anceps* and analyzed its predation capacity in two kinds of containers on both mosquito species at two manipulated prey densities. Furthermore, we studied the consequences of the presence of the predator on mosquito colonization of containers along a single mosquito breeding season. Both studies were carried out in semi-field experiments.

2. Materials and methods

2.1. Experiment 1

This experiment was performed in the Zoological Garden from La Plata city, Buenos Aires province. We evaluated if the kind of microhabitat, prey species and its density influences on the predator capacity of planarian in field conditions. We tested predation in two artificial aquatic habitats: small containers resembling cemetery flowerpots, and automobile tires. Both were chosen because they are frequent mosquito breeding sites in urban environments.

One to two days-old larvae of *A. aegypti* and *C. pipiens* were obtained from colonies at the Centro de Estudios Parasitológicos y de Vectores, La Plata. A description of the procedure of maintenance of colonies can be found in Tranchida et al. (2009a). Briefly, two separate colonies of adult *A. aegypti* and *C. pipiens* were kept in cages inside a bioterium under controlled environmental conditions (L:D 16:8 photoperiod, 80% relative humidity and 26 ± 2 °C). Mosquitoes had free access to raisins and females were fed with blood from a restrained chicken. A black jar lined with absorbent paper and a plastic bowl, both half filled with water, were offered to *A. aegypti* and *C. pipiens* gravid females, respectively, in order to collect eggs. After hatching, larvae of both species were raised in the same bioterium in 3-L pans and fed with powdered rabbit chow. *G. anceps* individuals were obtained from previous field-collected samples and maintained for several generations in the laboratory to form a cultured stock. Planarians were placed in plastic containers with ≈500 ml of dechlorinated tap water and a 10–20 mm in depth layer of mud coming from the same places where *G. anceps* was collected. Young mosquito larvae were added as food. As cocoons and young flatworms appeared, they were transferred to new containers to increase the number of individuals in cultures. Planarians were selected at random and deprived of food for three days prior to start the experiment.

On February, 2006, 16 halves of automobile tires (diametrically cut) and 16 ovitraps (plastic pots, diameter: 10 cm, high: 11 cm, volume: 0.75 L) were placed on the ground of the Zoological Garden, filled with water, and leave there without any further manipulation. On April 16, 2006, we collected all material (liquid and solid detritus) from tires and ovitraps. This procedure allowed us to gather organic matter to be added later to experimental containers as food for mosquitoes. Tires and ovitraps contents were examined for insect larvae; all living material was eliminated, and the rest of the material was mixed in a big container. Solid material, consisting mainly of leaves fallen from trees after abscission, was dried in an oven at 40 °C for two days, and cut in fragments <1 cm in size. Then, resulting material was assorted among cleaned tires and ovitraps. Four or 24 g of organic matter and 0.5 or 3 L of dechlorinated water were added to each ovitrap and tire, respectively.

On April 18, 2006, 100 first instar larvae (high density) of *A. aegypti* (less than one day old) were added to four tires and 20 first instar larvae (low density) of the same species to another four tires. The same procedure was repeated for another eight tires, but instead of *A. aegypti*, 100 or 20 larvae of *C. pipiens* were added. In

eight tires (two in each treatment) 10 individuals of *G. anceps* were introduced by hand. At the same time, 16 ovitraps received the same treatments (two densities of larvae and two species of mosquitoes). For each combination eight tires and eight ovitraps were used as controls (without planarians). A total of 32 containers were used, with two replicates for each combination of main effects. Once all experimental containers were set with mosquitoes and planarians, they were covered with a fine mesh cloth fixed with rubber bands in order to avoid oviposition by gravid mosquito females and addition of new organic material (Fig. 1).

Every two days, tires and ovitraps were emptied in white, plastic trays and checked for presence of mosquito pupae. We assumed that all larvae were either consumed by planarians or reached metamorphosis. All pupae were withdrawn and counted. Larvae and planarians were not counted during the course of the experiment with the aim of reduce the disturbance generated by manipulation. At the end of the experiment, all remaining planarians were counted.

Response variable was the accumulated number of pupae per treatment. We did not attempt to discriminate between male and female surviving pupae, because we assumed that intensity of predation is not dependent on sex of prey, based on our previous experience (Tranchida et al., unpublished). Variance homogeneity of raw data was checked with residual analysis. Data normality was verified calculating symmetry and kurtosis. To achieve homoscedasticity, data were transformed to $\log_{10}(n + 1)$. Results of this experiment were subjected to multifactorial ANOVA, with the following fixed effects: type of containers (ovitraps and tires), prey species (*C. pipiens* and *A. aegypti*), density of prey (high and low), presence of predator (with or without planarians), and the interactions between them (first to fourth degree).

2.2. Experiment 2

We run this experiment in order to explore the long-term effect of the presence of planarians within the containers on the colonization of such habitats units by feral mosquitoes. Twenty ovitraps were set in the Zoological Garden, filled with 0.5 L of dechlorinated water and 4 g of organic matter, obtained as described above, to act as a food source for larvae. Ovitraps were covered with a fine mesh, and the containers were left during two days to stabilize. In the third day, 10 individuals of *G. anceps* from the colony, selected by size (all >1 cm length) and not previously starved were released in 10 ovitraps, while other 10 remained as controls. All mesh covers were removed in order to allow mosquitoes to oviposit freely. Starting on September 12, 2006, containers were checked weekly. Number of larvae per stage and per species was recorded. The presence of planarians was registered, although their numbers were not recorded to avoid excessive manipulation. At the end of the experiment (June 7, 2007), all containers were removed and planarians were counted in the laboratory.

We analyzed number of larvae of *A. aegypti* and *C. pipiens* (species pooled) in containers with and without *G. anceps*. Residual analysis rendered heterogeneity of variance; therefore data were logarithmically transformed. A test of repeated measures ANOVA was performed, with presence of planarians as the main effect and time as the within-subject factor, followed by a Duncan multiple range test to detect differences among means.

3. Results

3.1. Experiment 1

Approximately one month after setting all containers, the experiment was finished when tires and ovitraps produced no longer pupae. Between 7 and 10 individuals of *G. anceps* were recorded in treatment containers after a destructive sampling in the laboratory. Planarian cocoons were present in 10 containers with *G. anceps*.

There was a significant effect of presence of planarians ($F = 26.39$; df = 1, 16; $p < 0.01$) and larval density ($F = 12.84$; df = 1, 16; $p < 0.01$) on larval mortality. The effects of type of container and mosquito species were non-significant ($p > 0.05$). The first to fourth degree interactions among main effects were non-significant ($p > 0.05$).

Fig. 1. (a) Ovitrap covered with fine mesh. (b) Ovitrap without fine mesh cover. (c) Automobile tire covered with fine mesh. (d) One individual of *Girardia anceps*. Scale: 1 cm.

G. anceps reduced nearly 70% of the larval population of *A. aegypti* in tires when initial density was high (Fig. 2a). In ovitraps, this percentage reached 50%. The control elicited by planarians at low initial densities was almost complete because very few pupae were recovered from tires and ovitraps. A high dispersion in treatments was recorded.

A 43% of reduction in population numbers of *C. pipiens* was registered in tires when initial larval density was high, whereas nearly 20% of mosquito larvae were eliminated at low initial densities (Fig. 2b). In ovitraps, very low number of survivors was recorded in recipients where *G. anceps* were introduced.
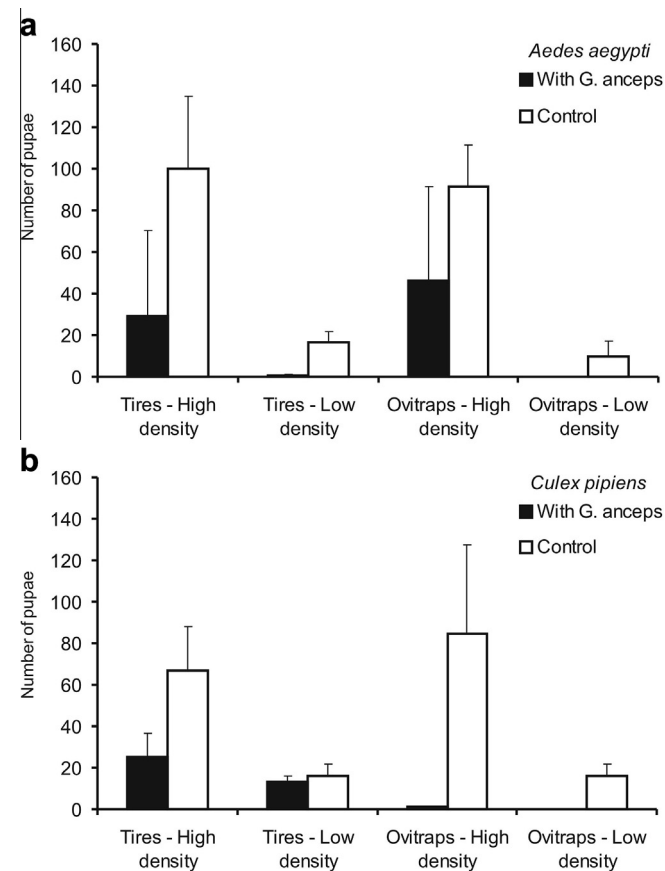
3.2. Experiment 2

All recipients were colonized by mosquitoes during this assay. The first larvae were detected on the eighth sampling week (November 24) (Fig. 3). In six ovitraps immature of *A. aegypti* and *C. pipiens* were sharing the microhabitat in eight dates between December and February, out of a total of 25 sampling weeks. Three of them contained planarians and the remaining were controls.

A. aegypti seasonality spanned between November and May, with a population peak on March of 1499 individuals (mean: 115 individuals/ovitrap). *C. pipiens* was present only during December, with a maximum of 825 individuals (mean: 137.5 individuals/ovitraps), except for five individuals recorded in a single ovitrap on April 18.

At the end of the experiments, all treated containers contained 6–15 planarians. In eight out of 10, cocoons were recorded.

The result of the repeated measures ANOVA showed significant effects of presence of planarians within containers ($F = 361.1$;

Fig. 3. Seasonal changes in number of larvae of (a) *Aedes aegypti* and (b) *Culex pipiens* in ovitraps with *Girardia anceps* and in controls (without *Girardia anceps*) from December, 2006 to June, 2007, in La Plata city during the Experiment 2.

$df = 1, 18$; $p < 0.01$), time ($F = 9.98$; $df = 21, 378$; $p < 0.01$), and the interaction between them ($F = 9.09.1$; $df = 21, 378$; $p < 0.01$). Average numbers of larvae per container (cumulative number of larvae per ovitrap divided by number of sampling dates) were compared with Duncan's test (Table 1). Post hoc comparisons showed eight homogenous groups of means (G1 to G8 of Table 1), i.e. the differences among means within groups G1 to G8 were non-significant ($p < 0.01$). Means integrating exclusively the first group of means (G1) corresponded to containers with *G. anceps*, with only one exception in a single container without planarians. Time effect was mostly evident for average number of immature stages recorded in containers where *G. anceps* was not introduced. Therefore, numbers of larvae in ovitraps were significantly affected by planarians along time.

Population curves for *A. aegypti* clearly showed that the presence of planarians in ovitraps significantly reduced the number of larvae, and that such decrease was sustained along the mosquito breeding season (Fig. 3). The difference between population levels of *C. pipiens* in containers with planarians and in control containers was low. The number of larvae in ovitraps without *G. anceps* reached its maximum with a slight delay compared to ovitraps with planarians (Fig. 3).

4. Discussion

Herein we showed that planarians were efficacious predators of larvae of two vector mosquito species within artificial containers in semi-field conditions. Although suppression of larvae was not total, the control of target populations was satisfactory. The main outcomes of these experiments were: *G. anceps* can eliminate the majority of mosquitoes before metamorphosis, there was a depen-

Fig. 2. Number of pupae of (a) *Aedes aegypti* and (b) *Culex pipiens* retrieved from tires and ovitraps with *Girardia anceps* and from controls (without *Girardia anceps*) at two initial larval densities (High: 100 1st instar larvae per container, and Low: 20 1st instar larvae per container) in the Experiment 1.

Table 1

Results of the Experiment 2 showing comparisons with Duncan's a posteriori test after a repeated measures analysis in a two-way ANOVA, on data transformed as $Y = \log$ (number of larvae) to meet ANOVA's assumptions. Columns show: *G. anceps*: presence/absence of *G. anceps* in ovitraps; Time: number of sampling date; G1 to G8: homogeneous groups of contrasted means with $p \leqslant 0.05$.

G. anceps	Time	G1	G2	G3	G4	G5	G6	G7	G8
Present	4	*							
Present	22	*							
Present	3	*							
Present	11	*							
Present	19	*							
Present	9	*							
Present	8	*							
Present	10	*							
Present	14	*							
Present	2	*							
Present	20	*							
Present	1	*							
Present	7	*							
Present	15	*							
Present	18	*							
Present	5	*							
Present	6	*							
Present	21	*							
Present	16	*							
Present	17	*							
Present	13	*							
Absent	22	*							
Absent	6	*	*						
Absent	4	*	*						
Absent	9	*	*						
Present	12	*	*						
Absent	2	*	*						
Absent	7	*	*						
Absent	10	*	*	*					
Absent	1	*	*	*					
Absent	5	*	*	*	*				
Absent	3	*	*	*	*	*			
Absent	11	*	*	*	*	*			
Absent	8		*	*	*	*	*		
Absent	14		*	*	*	*	*	*	
Absent	18			*	*	*	*	*	
Absent	21			*	*	*	*	*	
Absent	13					*	*	*	
Absent	19						*	*	
Absent	20						*	*	
Absent	12							*	
Absent	17								*
Absent	15								*
Absent	16								*

dence of predatory efficiency on abundance of prey, mortality caused by planarians was sustained at the long term (several weeks), and the reduction along time was more efficient on *A. aegypti* than on *C. pipiens*.

The present work deepens the results obtained by Tranchida et al. (2009a), who proposed *G. anceps* as a species novel to be used against immature *A. aegypti* and *C. pipiens*. They concluded that this planarian could be applied in small water holding receptacles to diminish the number of mosquitoes developing in them, but their approach was based solely on laboratory tests. Our experiments were designed to resemble an urban environment, because halves of tires and pots were used as habitats mimicking those of tire piles and cemetery vases. As they are discrete units, some important variables could be surveyed as in a small biological control trial. In general, we confirmed that *G. anceps* predatory performance was good. The kind of habitat in which *G. anceps* can be applied as a control agent was not a major determinant of the predatory capacity of this species, as there was a non-significant effect of tires or ovitraps as microhabitats on larval mortality. An impact on the efficiency of planarians consuming larvae may be important if other habitat features as size are to be taken into consideration. For instance, big containers such as drums or tanks are important

breeding sites to production of mosquito vectors (Maciel-de-Freitas et al., 2007; Harrington et al., 2008). It is very likely that mosquito larvae could be out of the range of planarians in these large-sized containers, rendering a less efficient control. Although we were not able to statistically validate this, it seems that predation on *C. pipiens* was interfered in tires at low larval density. It is suggested that mobility of this species and/or a greater time spent near surface of water can impinge the predation capacity of *G. anceps* in this scenario. Further investigation is worth to be conducted in this sense, but we choose automobile tires and vases because its ubiquity as breeding microhabitats for the species analyzed here.

Habitat complexity can alter the predator–prey interaction too (Gause, 1934). The addition of plant debris to our experimental containers rendered a more natural milieu for larvae, and eventually a refuge from predation. However, these debris were easily reached by planarians as well, as we could observe in ovitraps. We think that a complex structure of the habitat may be profitable for planarians, because it would not impede the contact with their prey. Furthermore an extra source of nutrients from the microbiota attached to detritus would be available for them besides mosquitoes. Fischer et al. (2013) prove that the habitat structure only slightly affected the consumption rates of a notonectid on mosquito larvae. Likewise, Alto et al. (2005) conclude that habitat complexity is relatively unimportant in the organization of the interactions between predators and preys within tree holes.

The identity of prey (*A. aegypti* or *C. pipiens*) did not have a significant effect in Experiment 1. Planarians could prey preferentially on one of two alternative preys, owing to their different behavior. *Aedes* species were characterized as bottom feeders, while *Culex* species usually spent more time near the water–air interface, and feed mostly on the water column (Merritt et al., 1992). A higher number of encounters between planarian and *A. aegypti*, and consequently more chances to feed on this mosquito, could occur than between planarian and *C. pipiens*, owing to the benthic habits of the predator, which would be spatially separated from *C. pipiens*. We did not detect a differential reaction of *A. aegypti* and *C. pipiens* to the presence of *G. anceps*. Melo and Andrade (2001) observed that the larvae of *Culex quinquefasciatus* Say had a more efficient escape from the flatworm *Dugesia dorotocephala* (Woodsworth) than *Aedes albopictus* Skuse in automobile tires. In this case, predation rate on *C. quinquefasciatus* was lower than on *A. albopictus*, which low escape response permitted a better manipulation of prey and a longer period for secretion of mucus for capture. Kar and Aditya (2003) observed a preference of larvae of *Anopheles* over *Culex* by *D. dorotocephala*. In the particular case of *G. anceps*, Tranchida et al. (2009a) showed that this flatworm did not exhibited a preference for either *A. aegypti* or *C. pipiens*, a fact that is expected, as flatworms generally do not coexist with mosquito larvae in artificial containers. Thus, there was no opportunity for planarians to develop a differential attack upon prey species.

The presence of alternative prey is another factor to be taken into account during the release of planarians in mosquito breeding sources. Other preys may be selected by predators, thus in field conditions biocontrol can be less effective. Several *Culex* species and *Toxorhynchites theobaldi* Dyar and Knab (a mosquito predator of mosquitoes) are possible non target organisms living in urban containers from the area under study (Rubio et al., 2011). Blaustein and Dumont (1990) presented evidence on the impact of flatworms on invertebrate community structure from rice fields, and showed that this effect change seasonally. On the other hand, the combination of *G. anceps* with other selected predators can be a more effective strategy to reduce abundance of vectors, but as far as we know this subject was not studied yet.

Density of prey, expressed in the Experiment 1 as the initial number of larvae available for planarians, had a significant effect on mortality, as seen in ovitraps either at low or high densities. A

functional response is elicited when density of prey affects predator efficiency (Solomon, 1949). If prey does exist in high numbers, a higher number of encounters between prey and predator can occur, enhancing mortality of preys, but also more prey individuals would be able to survive. If prey does exist in low numbers, its population could be suppressed by the predator. Our results showed an important effect of density of prey on predation rate, although it should be taken into account the observations made by George et al. (1983), whom stated that at densities over 100 larvae per liter, a possible mechanism of overcompensation can be triggered. These authors claim that consume of *C. pipiens* by *Dugesia tigrina* (Girard) can reduce the consequences of intraspecific competition of the mosquito, so the final effect could be a promotion of its survivorship. At high larval densities, George et al. (1983) found that depredation by *D. tigrina* was minimal; therefore the number of adults was similar to the population in absence of planarians. In contrast to the present work, George et al. (1983) registered no control at low prey density and attributed this to a low frequency of encounters between prey and predator.

In field conditions (Experiment 2), we observed a significant reduction of larvae in containers left for free colonization of feral mosquitoes. This effect was mostly seen on *A. aegypti*, if ovitraps with *G. anceps* were compared with controls. This signifies a contradiction in results from Experiments 1 (no effect of prey species) and 2 (as seen in abundance curves of Fig. 3). Some possible explanations for a lower impact of the presence of *G. anceps* on colonization by *C. pipiens* can be argued. First, predation could have different dynamics depending on the larval age structure, a condition that was not tested by us. Females of the genus *Culex* deposits egg rafts on surface of water, and accordingly to this, many first-stage larvae share a low volume of water in small containers. On the other hand, females of *A. aegypti* deposits eggs in the walls of the container, near or just above the water line, so a lower number of first-stage larvae can be expected if this species is compared to *C. pipiens*. In fact, the maximum number of first-instar larvae of *A. aegypti* in a single container was 97, while more than 300 *C. pipiens* first-instar larvae were recorded for a single container. Thus, in a given moment, there would be more larvae of *C. pipiens* able to survive than of *A. aegypti*. A second explanation could arise from local conditions during our field work, such as abundance of adult mosquitoes in the place where samples were obtained, or weather conditions, which could affect colonization events as well.

The effect of seasonality (time as a variation source in Experiment 2) was evident in containers without planarians. This effect can be mainly attributable to seasonality of abundance of *A. aegypti* because abundance curves for larvae in containers with or without planarians behaved differently. Both curves varied with some synchronicity in the case of *C. pipiens*. We suggest that the population of this species sampled in Experiment 2 could be not representative of the seasonality of *C. pipiens* in the latitude of La Plata, in terms of presence along time or density of immature. In other studies performed in this locality (Campos et al., 1993; García et al., 2002) and in Buenos Aires city (Fischer and Schweigmann, 2010) (60 km apart), *C. pipiens* was abundant year-round. On the other hand, the significant interaction term in our analysis of variance suggest that a proper evaluation of the impact of the presence of planarians in water containers on mosquito larvae would be only valid if there is a continuous monitoring of the results, instead of only pre- and post-treatment evaluations.

A comparison of the potential use of planarian worms and copepods deserves a mention. As both are ubiquitous and can share several kinds of freshwater habitats with culicids, similar methods could be employed for their augmentative release. Tranchida et al. (2009b) arrive to similar conclusions to the present work based on laboratory trials about larval mosquito consumption by copepods from the same locality. Their main findings (no predatory preference for mosquito species, capacity of survive in small water bodies, predation activity sustained along several days) are consistent with the results about planarians, but their application together should be made with caution. Unplanned ecological interactions are to be considered, for instance, some species of planarians feed on copepods (Trochine et al., 2005). In a broader sense, a high scale control programme based solely in release of planarians can be difficult to achieve due to the constraints generated by artificial introduction of worms in multiple size containers. In specific situations such as cemeteries, this difficulty can be avoided.

The presence of cocoons within pots revealed the potential of self-sustaining of planarian populations in our experimental habitats. An increase in population size within ovitraps along several weeks was also recorded by in Brazil. Multiplication is a desirable attribute for mass rearing and reproduction after inoculation (Legner, 1995).

In conclusion, *G. anceps* can be an effective control agent if this organism is incorporated into a biorational program against mosquito vectors. Although its use is constrained to small water containers, its advantages as natural enemies of mosquitoes can be complemented with other measures in order to enhance the reduction of noxious populations. Comparative bioassays in the laboratory and in the field would clarify possible discrepancies between the response of laboratory-reared and field-collected specimens.

Acknowledgments

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