Field Trial To Evaluate Two Different Procedures For Monitoring the Efficacy of Aquatain® Against *Culex pipiens* and *Aedes albopictus* IN CATCH BASINS

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FIELD TRIAL TO EVALUATE TWO DIFFERENT PROCEDURES FOR MONITORING THE EFFICACY OF AQUATAIN® AGAINST *CULEX PIPIENS* AND *AEDES ALBOPICTUS* IN CATCH BASINS

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ABSTRACT. *Aedes albopictus* and *Culex pipiens* are commonly distributed in Italy and represent the main species found in catch basins. The application of a silicone-based film (e.g., Aquatain®) is a new tool recently introduced for treating catch basins. While the efficacy of Aquatain has been experimentally demonstrated, its use is still lacking an appropriate monitoring procedure. The present study compared the differences in the efficacy of treatment assessment between a newly developed floating system (FS), which was designed to collect emerging adults, with the standard dipper procedure, used for estimating the abundance of mosquito larvae. Forty catch basins, half treated with Aquatain and half untreated (control basins), were monitored weekly using dipper (10 treated + 10 control basins) or FS (10 + 10) 5 times after 2 subsequent treatments. Both monitoring procedures recorded high percentages of larvae and adult reduction for the 1st 1–3 wk after treatments, confirming the simultaneous activity of Aquatain against all stages of mosquitoes. Differences in adult emergence were recorded also when monitoring of larvae was ineffective, suggesting that the newly developed FS is a promising method for assessing the efficacy of monomolecular films in catch basins.

KEY WORDS Aquatain, dipper, efficacy, floating system, mosquitoes

INTRODUCTION

The 2 species of mosquitoes commonly found in urban catch basins in Italy are *Aedes albopictus* (Skuse) and *Culex pipiens* L. (Bellini et al. 2009, Caputo et al. 2015). *Aedes albopictus* is an invasive species and a growing problem in Italy and other European countries, both for its nuisance activity and its vectorial capacity (Baldacchino et al. 2015). *Culex pipiens* is a ubiquitous mosquito in southern Europe, where it is believed to act as one of the primary amplifying vectors of West Nile virus (Mughini-Gras et al. 2014). Municipalities and local health authorities are engaged in reducing the mosquito burden and decreasing the pathogen transmission, promoting and monitoring control programs, usually by means of different strategies, as per suggestion of the Italian National Health Institute (Istituto Superiore di Sanità) (Romi et al. 2009). Control activities in the field are usually implemented by both public and private companies, and include treatment of nonremovable larval sites such as catch basins by bacterial insecticides (e.g., *Bacillus thuringiensis* Berliner), chitin synthesis inhibitor (e.g., diflubenzuron or novaluron), and juvenile hormone analogues (juvenoids; e.g., methoprene or piryproxifen). The assessment of treatment efficacy is generally performed by estimating the abundance of the different larval instars collected using a dipper (Silver 2008). Because of the mode of action juvenoids, which affect mosquito aquatic stages at the metamorphosis phase, the right evidence of larval and pupal lethality is a long process that can last for several days. Consequently, when catch basins are treated with these products, it is recommended that mosquito aquatic instars would be brought to the laboratory to observe adult emergence (WHO 2005). However, innovative mosquito control agents, such as monomolecular surface films, are increasingly used for mosquito control and an adequate system for monitoring their efficacy is still lacking.

Aquatain® is a silicone-based film that has demonstrated ovicidal, larvicidal, pupicidal, and adulticidal activity against many species of mosquitoes, both in laboratory trials (Bukhari and Knols 2009, Webb and Russell 2009, Bukhari et al. 2011) and in field tests (Bukhari et al. 2011, Webb and Russell 2012, Mbare et al. 2014). The insecticidal property of Aquatain is a physical, rather than chemical mode of action. The effect is obtained by lowering the water surface tension, which affects all stages of the mosquito life cycle. In fact, the reduced surface tension wets and drowns eggs, suffocates larvae and pupae, and kills emerging and ovipositing females by drowning (Mbare et al. 2014).

The appropriateness of larval estimation by dipper to assess Aquatain efficacy is questionable, since larvae may escape collection because they lie at the bottom of the catch basin under hypoxic conditions, but still alive. Besides, collecting pupae or larvae after Aquatain treatment and bringing them to the laboratory is introducing a bias due to the change in environmental conditions that is interrupting siliconic film action. In fact, collected larvae may not be able

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to develop into adults if left on-site with Aquatain. The monitoring of adult emergence to verify monomolecular film effectiveness has already been attempted in different kinds of environments, such as rice paddies (Bukhari et al. 2011) and artificial ponds or tubs (Webb and Russell 2012, Mbare et al. 2014). However, no standard method for monitoring of adult emergence in catch basins has been developed, considering that wild mosquitoes would be allowed to continuously lay eggs after treatment. In order to overcome this problem, a new tool developed by Entostudio (Padova, Italy) consists of a floating system (FS) designed to collect part of emerging mosquitoes and to allow wild mosquitoes continuing oviposition. The FS permits water level fluctuation due to rain or evaporation.

The aim of this study was to conduct an evaluation of the use of this new floating tool for monitoring adult emergence in comparison with the standard dipper sampling used for estimating larval abundance as well as to assess the efficacy of Aquatain against *Ae. albopictus* and *Cx. pipiens* found in urban catch basins in Italy.

MATERIALS AND METHODS

Study site

The test was carried out in catch basins located in the town of Spinea, Venezia District (45°29′36″N, 12°08′06″E), northeastern Italy, in a residential area with many single houses and public parks. The summer climate is usually hot and humid with very little rain, with a high risk of intensive showers. The area is infested with mosquitoes usually reaching very high densities. The city is under chemical control but in the test area no applications were carried out during the whole year 2016. Precipitation data were obtained from the nearby weather station of Mira, identified as the station number 167 by the Regional Environmental Agency (http://www.arpa.veneto.it/bollettini/storico/Mappa_2017_TEMP.htm).

The presence of 2 new invasive species of the genus *Aedes* was recently recorded (i.e., *Ae. koreicus* Edwards and *Ae. japonicus japonicus* Theobald) in northeastern Italy; their distribution is carefully monitored and their presence in the study site was excluded (Montarsi et al. 2015, Seidel et al. 2016). *Culiseta longiareolata* Macquart, which can also be confused with *Aedes* spp. at the larval stage, is seldom reported in the broader area of northeastern Italy (Mughini-Gras et al. 2014) and has never been found during the monitoring activities conducted for several years in the study area previously. As a consequence, we can assume that all specimens identified at the genus level as *Aedes* can be assigned to the species *Ae. albopictus*. Similarly, all individuals belonging to the genus *Culex* can be reasonably assigned to the species *Cx. pipiens*.

Test design

The test was performed by applying the product Aquatain AMF™ (Bleuline, Italy; http://www.bleuline.it/), as per manufacturer instructions, in 20 catch basins never treated with any larvicide during 2016. Ten treated catch basins were monitored using a 500-ml dipper, while other 10 treated catch basins were monitored using the newly developed FS. Twenty other catch basins, located nearby to treated ones, acted as control basins and were monitored: 10 by dipper and 10 by FS. The capacity of the catch basins was very variable and could range from almost dry to those with >50 liters of water. Catch basins with low number or absence of larvae at pretreatment sampling were excluded from the test.

All 40 catch basins selected for the test were monitored weekly after treatment. The 1st treatment was performed on July 4, 2016, for each of the 20 treated catch basins, and a 2nd treatment was done on August 8, 2016. In total, 11 samplings were performed, starting on June 30, 2016, before the initial treatment and 5 weekly samplings after the treatments. The same operator performed all assessments for the whole test.

Sampling methodology

Monitoring of larvae with dipper methodology: Samples of water were taken from each catch basin, using a 500-ml-capacity dipper, to estimate density of larval population. Before collecting the samples, the catch basin's grid was removed carefully not to hit it, to avoid scaring the larval population. Once the grid was removed, 3 samples were taken from 3 different corners, at approximately 30-sec intervals. The 3 samples were transferred to a white bucket for observation after which both the water and larvae were added back to the same catch basin. The total number of 1st and 2nd larval instars present in the white bucket were counted (number of larvae per 1,500 ml) without identification of the genus, whereas 3rd and 4th instars were identified to the genus level according to morphological characteristics (in particular the shape of the syphon) and counted separately.

Monitoring of adults with FS: The capability of the larvae to emerge as adults was assessed using the newly developed FS. The system consisted of a plastic cylinder (25-cm diam, 6-cm height), closed on the top by a mosquito net (mesh size: 1 mm). Three polystyrene pieces were glued on the external side to allow flotation (Fig. 1). The inside of the cylinder was completely covered with an adhesive polyvinylchloride (PVC) tape, and kept in position using binder clips. Adult mosquitoes emerging into the FS were captured on the sticky internal surface. The PVC tape was replaced at each sampling. Adults were identified on-site according to specific morphological characters.

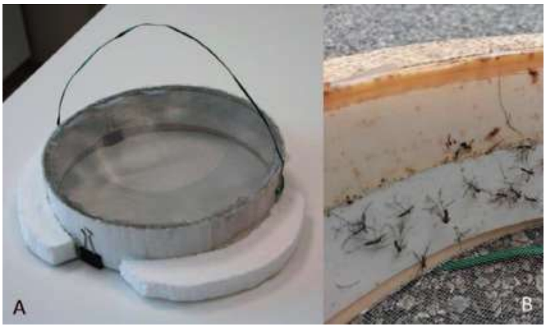

Fig. 1. Image of the (A) floating system and (B) detail of the sticky surface with adult mosquito.

Statistical analysis

The mean number of adult and 3rd–4th larval instars of *Ae. albopictus* and *Cx. pipiens* and of 1st–2nd larval instars for each sampling was calculated for treatment and control catch basins. A descriptive statistics showing the trend of the different mosquitoes' stages monitored was performed using Excel® 14.7.1 (Microsoft, Redmond, WA), and the difference in the number of larvae or adults between treatment and control was evaluated by the Mann–Whitney *U*-test, using the IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY). The overall level of statistical significance was set to $P < 0.05$.

As far the dipper-monitored basins, the effectiveness of the product was calculated at the 1st sampling after the 1st application, with respect to the pretreatment density, according to Mulla's formula (Mulla et al. 2003). For all samplings in terms of larval reduction with respect to the control, we used the percentage reduction formula:

$$\%\,\text{reduction} = 100 - [(\text{T}/\text{C}) \times 100],$$

where T = mean number of larvae in treated basins and C = mean number of larvae in control basins.

Concerning the adults, product efficacy was estimated by using the inhibition of adult emergence, indicating the reduction of emerging adults from the treated catch basins with respect to the untreated ones (WHO 2005).

RESULTS

Twenty catch basins were monitored for 11 times, 1 pretreatment and 10 posttreatment dipper samplings. Two times, one catch basin was not assessed because of a car parked on the top of it. An equal amount of 20 catch basins was monitored for 10 times (only posttreatment) by FS methodology; 2 basins (in 2 different samplings) were found with the FS overturned and were not considered in data elaboration.

The efficacy in reduction of larvae estimated by Mulla's formula was equal to 100% for all instars at the 1st posttreatment sampling. The mean numbers of larvae collected for each sampling, including the level of significance of the differences between treatment and control basins (Mann–Whitney *U*-test), are shown in Fig. 2, and the percentage of reduction for each sampling is reported in Table 1. The mean numbers of emerging adults at each sampling, and the level of significant differences between treatment and control basins, are shown in Fig. 3; the percentages of emergence inhibition are reported in Table 1. Catch basins monitored by FS and assessed for the presence of larvae by dipper sampling before the 1st treatment showed no significant differences in larval densities between the treated and control basins (Mann–Whitney *U*-test; $P > 0.05$).

The efficacy of Aquatain in reducing the number of 1st–2nd larval instars is shown in Fig. 2A. The data show that the percentage of reduction was 50–80% or higher for 5 wk posttreatment. The differences in mean numbers of larvae between treated and control catch basins were significant in all weekly samplings but one. This result suggests that, in spite of high variability and high densities of 1st–2nd instars in control basins (ranging from about 10 to 225 larvae per 1,500 ml of collected water), Aquatain-treated basins were constantly kept at low density, under 25 larvae.

The efficacy of Aquatain against 3rd–4th instars of *Ae. albopictus* was clear only for the 1st sampling after both treatments, when reduction percentage was very close or equal to 100% (Table 1). Besides, no significant difference in the number of larvae was found between treated and control basins, except for the 1st sampling after the 2nd treatment (Fig. 2B). This is also partly due to the low-density values encountered in control basins, which were constantly below an average amount of 10 larvae. Instead, the monitoring of adult *Ae. albopictus* emergence using the FS method recorded a 100% of emergence inhibition for 2 wk after both treatments (Table 1) and showed significant differences between treated and control basins in 7 out of 10 samplings. The mean number of adults emerging was variable in control basins, ranging from 2 to about 50 adults, whereas it was constantly fewer than 10 in treated ones (Fig. 3A).

For *Cx. pipiens*, the trend of 3rd–4th instars was not clearly different between control and treatment basins, and only for the 1st 2 wk after both treatments was there a reduction in percentage close or equal to 100% (Table 1). The absence of clear reduction of larvae in treated basins (Fig. 2C) depends in part due to the larval density, which was generally low in treated basins and also in control basins (never exceeding 15 larvae). Moreover, the mean numbers were also low also for emerging adults. The percentage of emergence inhibition was >90% for 3 samplings after both treatments, but it dropped dramatically starting from the 4th week, with no significant differences between control and treated basins (Fig. 3B).

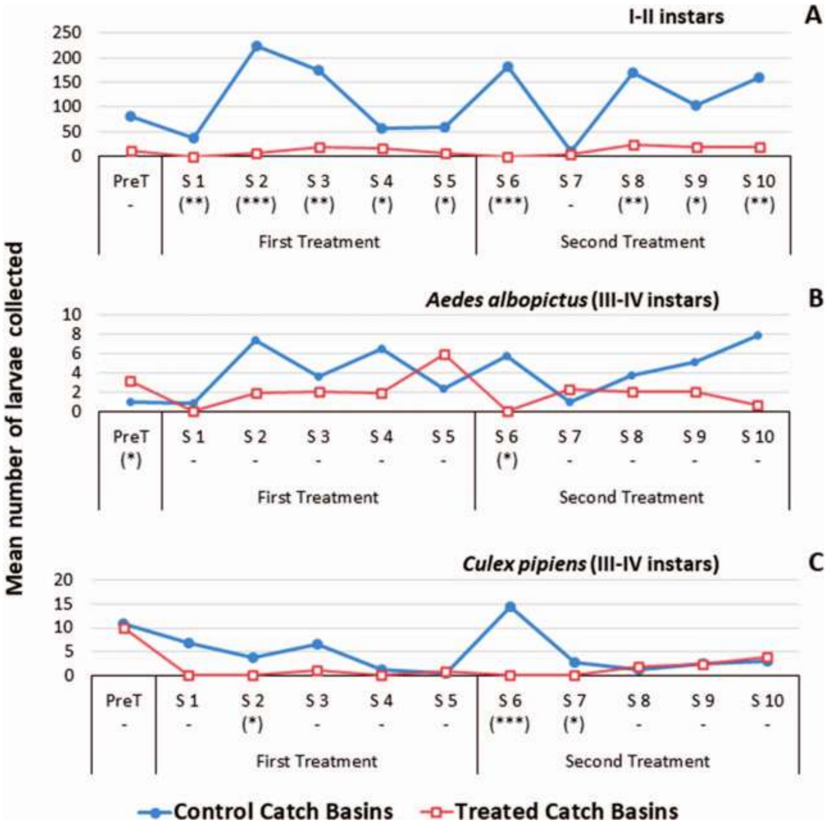

Fig. 2. Mean number of (A) 1st–2nd larval instars, (B) 3rd–4th larval instars of *Aedes albopictus*, and (C) *Culex pipiens* in treated and control basins at pretreatment (preT) and subsequent samplings (S1–S10). The level of significance of the difference within each sampling (control versus treated) is indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

This study represents the first attempt to apply a new methodology for monitoring the efficacy of Aquatain application in catch basins. The monitoring activity is a fundamental component of mosquito control program and therefore it is important to provide health operators and local municipalities with appropriate tools for implementing their supervision. Aquatain differs from other mosquito control operations (e.g., chemical adulticides or larvicides, or insect growth regulators) in its mode of action and therefore the methodologies commonly used may lack appropriateness.

Table 1. Percent reduction (% R) of mosquito larvae and inhibition of emergence (% IE) of adult mosquitoes at each sampling (S1–S10).

	First treatment (Jul. 4, 2016)					Second treatment (Aug. 8, 2016)				
Pretreatment, Jun. 30	S1 1 wk, Jul. 11	S2 2 wk, Jul. 18	S3 3 wk, Jul. 25	S4 4 wk, Aug. 1	S5 5 wk, Aug. 8	S6 1 wk, Aug. 16	S7 2 wk, Aug. 22	S8 3 wk, Aug. 29	S9 4 wk, Sep. 5	S10 5 wk, Sep. 12
% R of 1st–2nd instars	100.0	97.5	89.0	72.2	90.8	99.9	51.3	86.7	81.7	87.8
% R of 3rd–4th instars—*Ae. albopictus*	100.0	74.3	45.5	70.8	−150.0	98.3	−130.0	44.7	61.5	91.1
% IE of adults—*Ae. albopictus*	100.0	100.0	76.5	87.0	72.6	100.0	100.0	99.1	80.6	79.8
% R of 3rd–4th instars—*Cx. pipiens*	100.0	100.0	81.7	91.7	−50.0	100.0	92.8	−66.7	4.0	−33.3
% IE of adults—*Cx. pipiens*	100.0	100.0	95.6	−433.3	−700.0	100.0	100.0	99.3	49.7	−248.1

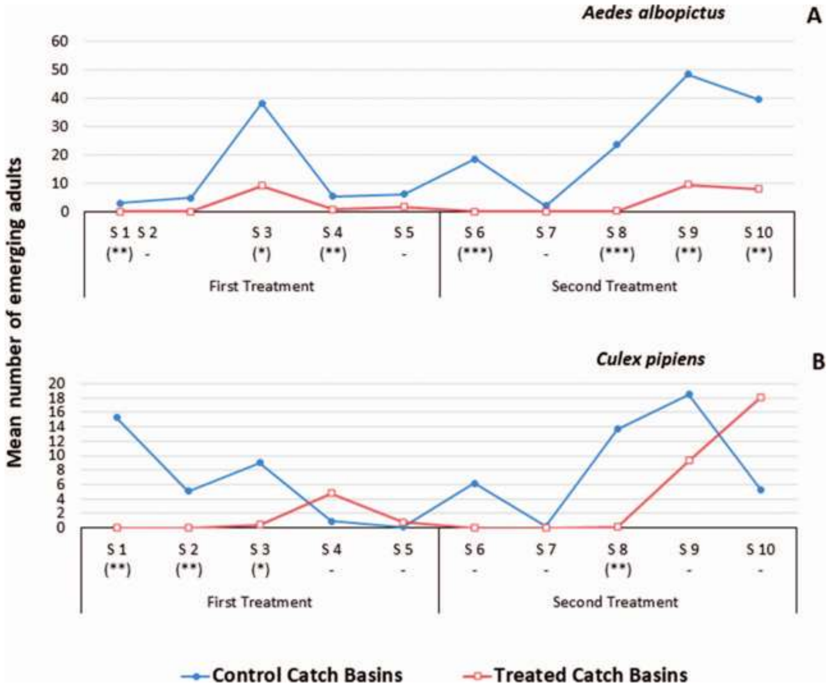

Fig. 3. Mean number of emerging adults of (A) *Aedes albopictus* and (B) *Culex pipiens* in treated and control basins at each sampling after treatment (S1–S10). The level of significance of the difference within each sampling (control versus treated) is indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The efficacy of Aquatain in reducing 1st–2nd instars is in agreement with previous field tests (Bukhari et al. 2011, Webb and Russell 2012). However, due to the difficulty to identify specifically larvae at these early stages, the indication provided by collection of larvae (dipper methodology) is of reduced value. On the contrary, both assessing the 3rd–4th instars by dipper and estimating adult emergence rate allow for a more specific identification of collected specimens.

The new FS developed in this study appeared to be more reliable as a monitoring system compared with dipper methodology. In fact, dipper results were not showing any difference in trends between control and treated basins for both species, mostly because of the low numbers of larvae recorded. On the contrary, particularly in the case of *Ae. albopictus*, the monitoring of adult emergence showed to be able to highlight such differences.

In general, both dipper and FS recorded very high percentages of reduction (or emergence inhibition) for the 1st 1–3 wk after treatments, confirming the simultaneous activity of Aquatain against all stages of mosquitoes (eggs, larvae, pupae, and adults), as previously demonstrated (Bukhari and Knols 2009, Bukhari et al. 2011). Specifically, in the case of *Ae. albopictus*, 3rd–4th instars were highly reduced for only 1 wk and adult emergence for 2 wk. Instead, *Cx. pipiens* showed a high percentage of reduction of larvae for 2 wk and adults for 3 wk. This is in agreement with the biology of mosquito life cycle and suggests that after 1 or 2 wk, the effect of the product may reduce its capacity to prevent larval presence, whereas its effect on preventing adult emergence can last for 1 more week. Data collected during the test suggest that the efficacy of the product lasts for at least 5 wk in reducing 1st–2nd instars. However, other factors may have influenced the treatment efficacy, such as the rainfall pattern. During the test, many showers were recorded in the study site and this aspect probably contributed to a decrease in larval presence in control catch basins, thus complicating data interpretation.

In conclusion, the newly developed FS seems to be a useful tool for monitoring the efficacy of catch basins treated with Aquatain or other monomolecular films. Further investigations are necessary to study in details the efficacy of this product and consequently to establish the time lapse between 2 consecutive treatments.

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