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Research article

PILOT STUDY OF INVASIVE MOSQUITO SPECIES CONTROL IN SELECTED SLOVENIAN MUNICIPALITIES

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To reduce the population of the Asian tiger mosquito Aedes albopictus (Skuse, 1894) (Diptera: Culicidae), a mosquito control campaign was organized by five Slovenian municipalities in 2020. The campaign focused on the application of Aquatain AMFTM in public water containers. The main objective of our study was to test the efficacy of the agent by directly testing the water drains for mosquito larvae and monitoring the density of the mosquito population. The drains were inspected before and after the application of the product from May to September. A water sample was taken from the drains using a dipper. For mosquito monitoring, the number of mosquito eggs and adult mosquitoes was counted from June to October.

Up to two weeks after the application of the product, we observed the lethal effect of Aquatain on mosquito larvae, pupae and adult mosquitoes. After rainfall, the product was washed away and the live mosquitoes were sampled in the inspected water drains. The average number of eggs collected per site was twice as high in the area without mosquito control as in the threated locations. Nevertheless, we assessed the mosquito control campaign as partially successful, as mosquito abundance was still high in the locations with mosquito control. However, we believe that this is a good starting point and that mosquito control should be continued in the future together with the citizen education campaign.

Keywords: *Aedes albopictus*, Aquatain AMF^{TM}, invasive mosquito species, mosquito control.

INTRODUCTION

Invasive mosquito species represent an increasing global threat to public health. With increasing human mobility and international traffic, non-native mosquito species are passively migrating into new areas, posing a new threat to public health [1]. Three alien mosquito species have been recorded in Slovenia so far, the Asian tiger mosquito (*Aedes albopictus* (hereafter ATM)), the Asian bush or rock pool mosquito (*Aedes* (*Finlaya*) *japonicus japonicus* (Theobald, 1901) (syn. *Hulecoeteomyia japonica*)) and *Aedes koreicus*

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(Edwards, 1917) (Diptera, Culicidae) [2,3]. ATM was first observed in the Municipality of Nova Gorica in 2002 [4]. Over the years, its population has been increasing and expanding. To date, this species has established stable populations, especially in the Primorska region, the Vipava Valley, Nova Gorica and Ljubljana and its surroundings. ATM has also been observed in other parts of the country (Notranjska, Dolenjska, Štajerska) [2]. The Asian bush mosquito has been present in Slovenia since 2011, when it was observed near Maribor [5], and in just a few years it has spread throughout the country [2]. The first findings of *Ae. koreicus* were in 2013 in Lovrenc on Dravsko polje [3], and the next time the species was recorded in 2019 in the Municipality of Nova Gorica (unpublished data).

In addition to mosquitoes, pathogens and parasites are also transmitted passively to new areas. Infected individuals coming from endemic areas of a disease can be a source of local transmission of pathogens in the new area, such as dengue and chikungunya, if an appropriate vector is present in the area. For this reason, mosquito control is crucial to reduce the incidence of mosquito-borne diseases [6]. To reduce the burden that tiger (and other) mosquitoes pose to public health, four municipalities in Slovenian Istria and the Municipality of Nova Gorica conducted an alien mosquito species control campaign focusing on ATM in selected areas. This was the first attempt to reduce the population of invasive mosquitoes through mosquito control in the mentioned municipalities. The campaign focused on the application of Aquatain AMF™ in public water drains and raising awareness of mosquito control measures. Aquatain is a silicone-based liquid (polydimethylsiloxane - PDMS) that spreads rapidly over the water surface and prevents the respiration of larvae and pupae of all mosquito species that need to come to the water surface for oxygen. At the same time, it prevents adult females from landing on the water surface when laying eggs [7]. The aim of our study was to evaluate the effectiveness of the first invasive mosquito control in selected areas in southwestern Slovenia. The results obtained will be very valuable not only for Slovenia but also for the international community.

MATERIALS AND METHODS

An important part of mosquito control actions is the simultaneous evaluation of their effectiveness [8,9]. To monitor the effectiveness of Aquatain AMF™ applications, we worked with public utilities to regularly survey public drains in selected locations for the presence of mosquito larvae and pupae. The project area included the municipalities of Nova Gorica (the settlements of Kromberk, Nova Gorica, Solkan, Pristava and Rožna dolina) and four municipalities in Slovenian Istria (Koper, Izola, Piran, Ankaran) (Figure 1). The drains were inspected before and after the application of the product during the period from May to September 2020. The exact dates of the inspections are listed in Table 1. A water sample from the drains was collected using a dipper and the collected larvae were kept in 70% ethanol for species identification. Species identification was based on morphology using available keys [6,10].

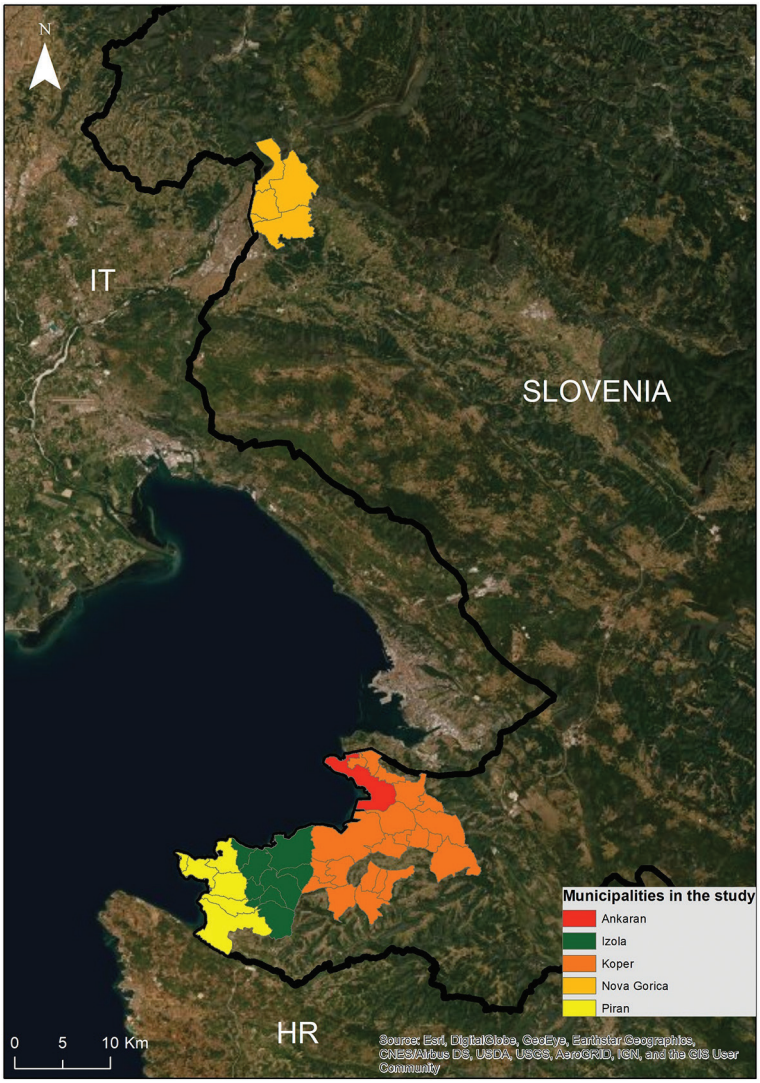

Figure 1. Area of the pilot Asian tiger mosquito control program in 2020. (Source: Google Maps, Edited in ArcMap by: Katja Kalan)

The efficacy of the product was also monitored by counting mosquito eggs in ovitraps and adult specimens at selected sites throughout the treatment area from June to October. A total of 136 ovitraps were placed and eggs were collected every fortnight. In addition, 5 ovitraps were set up in a village in the Vipava Valley, where no mosquito control was carried out in 2020. Ten BG SENTINEL adult mosquito traps were set once per month of the study. To determine whether the time after application of Aquatain affects the frequency of drains with dead/live larvae and pupae, the data of drains with dead or live larvae/pupae in weeks after application were presented (Table 2). Likelihood ratio statistics was used and a significant difference between the drains

with dead or live individuals in a time scale was accepted at $p < 0.05$. Standardized residuals were used to assess the relative contributions of cells to the overall Chi-square value. Significance at $p < 0.05$ was accepted when the value standardized residual of a cell was higher than |2.0| [11].

Ethical approval

The research conducted is not related to the use of animals. No ethical approval was obtained as no laboratory animals were used in this study and only non-invasive procedures were used (e.g. collection of tissue waste after surgery, stool samples, urine samples, etc.).

RESULTS

Aquatain was applied to public water tanks (mostly water drains) by public utilities between May and October 2020. The product was applied five times in Nova Gorica and Izola, eight times in Piran and three times in Ankaran and Koper. In the spring and summer of 2020, rainfall was frequent and occasionally abundant. Almost all treatments with Aquatain were followed by rainfall in up to one week, which washed the agent away from the water drains. Periods with at least one dry week were very rare. When we inspected the drains about a week after the rain, they were already full of live mosquito larvae. Although the agent should be stable for about 28 days [7], it was washed away with the drain water. A faster leakage of the agent was observed in larger drains with a larger drain pipe. In such shafts, the agent was washed away very quickly, while in smaller drains some agent remained on the water surface despite the rain.

When inspecting the drains up to 2 weeks after the application of the product and before rainfall, we observed the desired effect of Aquatain on mosquito larvae, pupae and adult mosquitoes (Table 1). Immediately after the application of the product, we found dead mosquitoes of all stages in the drains. The mosquitoes collected were identified as *Ae. albopictus*, *Culex* sp., *Aedes* sp. and *Culiseta longireolata*. Species identification was not possible in all cases, as the dead larvae were already in a process of decomposition. In addition to the dead mosquito larvae, live aquatic isopods (Asellidae), water mites (Acarina) and larvae of chironomids (Chironomidae) were also observed in the drains. The product therefore had no noticeable effect on these animals and was only effective on mosquitoes, as stated in the product specification.

Statistical analysis showed that there was a statistically significant deviance of frequency of drains with dead and live mosquito individuals on a weekly basis after the application of Aquatain ($LR = 22.8$, $df = 4$, $p < 0.001$). The effect of Aquatain was shown by a high frequency of drains with dead individuals up to the second week after application, while the frequency of drains with live individuals gradually increases over time (Fig. 2).

Table 1. Observations from water drains inspections after Aquatain AMF™ applications

Municipality	Date of sampling	No of days after the Aquatain appli-cation	ID of inspected drain	Dead or live larvae/ pupae / no larvae/ pupupae)	Nº of larvae	Nº of pupae	Mosquito species	Observations
Ankaran	11.9.2020	0	An_11_9_2020/2	live larvae	50		*Culex* sp.	covered, untreated drain
Ankaran	21.7.2020	7	An_21_7_2020/1	live larvae	15		*Culex* sp., *Culiseta longiareolata*, *Aedes* sp.	
Ankaran	21.7.2020	7	An_21_7_2020/2-7	no larvae/pupae	0			
Ankaran	11.9.2020	16	An_11_9_2020/1	dead larvae	5		*Aedes albopictus*	deep drain
Ankaran	11.9.2020	16	An_11_9_2020/3-7	no larvae/pupae	0			
Ankaran	24.8.2020	42	An_24_8_2020/1	live larvae	3		*Culex* sp.	deep drain
Ankaran	24.8.2020	42	An_24_8_2020/2	live larvae	9		*Culex* sp.	drain with accumulated tree leaves
Ankaran	24.8.2020	42	An_24_8_2020/3-7	no larvae/pupae	0			
Izola	20.5.2020	0	Iz_20_5_2020/1	live larvae	8		*Culex pipiens*	
Izola	20.5.2020	0	Iz_20_5_2020/2	live larvae	3		*Culex pipiens*	
Izola	20.5.2020	0	Iz_20_5_2020/3-10	no larvae/pupae	0			
Izola	21.7.2020	3	Iz_21_7_2020/1	dead larvae	15		species not identified to species level due to degradation	live Chironomidae larvae
Izola	21.7.2020	3	Iz_21_7_2020/2-10	no larvae/pupae	0			
Izola	8.7.2020	17	Iz_8_7_2020/1	live larvae	3		*Culex pipiens*	
Izola	8.7.2020	17	Iz_8_7_2020/2-10	no larvae/pupae	0			
Koper	21.7.2020	0	Kp_21_7_2020/1	live larvae	5		*Culex* sp.	non-treated drain, live larvae
Koper	21.7.2020	1	Kp_21_7_2020/2	dead larvae	2		*Culex* sp.	
Koper	21.7.2020	1	Kp_21_7_2020/3	dead larvae	1		*Culex* sp.	
Koper	21.7.2020	1	Kp_21_7_2020/4-10	no larvae/pupae	0			
Koper	29.9.2020	7	KP_29_9_2020/1	live larvae	1		*Culex* sp.	larger drain, Aquatain absent
Koper	29.9.2020	7	KP_29_9_2020/2	live larvae	1		*Culex* sp.	larger drain, Aquatain absent

cont. Table 1.

Koper	29.9.2020	7	KP_29_9_2020/3	live larvae	2		*Culex* sp.	larger drain, Aquatain absent
Koper	29.9.2020	7	KP_29_9_2020/4	live larvae	3		*Culex* sp.	larger drain, Aquatain absent
Koper	29.9.2020	7	KP_29_9_2020/5-15	no larvae/pupae	0			smaller drain, Aquatain present
Koper	24.8.2020	34	Kp_24_8_2020/1	live larvae	2		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/2	live larvae	1		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/3	live larvae	1		*Aedes* sp.	
Koper	24.8.2020	34	Kp_24_8_2020/4	live larvae	4		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/5	live larvae	2		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/6	live larvae	2		*Aedes* sp.	
Koper	24.8.2020	34	Kp_24_8_2020/7	live larvae	2		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/8	live larvae	1		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/9	live larvae	1		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/10	live larvae	2		*Aedes albopictus*	
Koper	24.8.2020	34	Kp_24_8_2020/11	live pupae	0	2	?	
Koper	24.8.2020	34	Kp_24_8_2020/12-15	no larvae/pupae	0			
Lucija	9.9.2020	27	Lu_9_9_2020/1	live larvae	2		*Aedes albopictus*	rain after previous application
Lucija	9.9.2020	27	Lu_9_9_2020/2	live larvae	1		*Culex* sp.	rain after previous application
Lucija	9.9.2020	27	Lu_9_9_2020/3	live larvae	1		*Aedes albopictus*	rain after previous application
Lucija	9.9.2020	27	Lu_9_9_2020/4-5	no larvae/pupae	0			
Nova Gorica	17.9.2020	3	NG_17_9_2020/1	live larvae	40		*Culex sp.*	
Nova Gorica	17.9.2020	3	NG_17_9_2020/2	dead larvae	1		*Culex sp.*	one dead adult, Culex sp.
Nova Gorica	17.9.2020	3	NG_17_9_2020/3-20	no larvae/pupae	0			
Nova Gorica	20.8.2020	13	NG_20_8_2020/1	live larvae	4		*Aedes albopictus*	
Nova Gorica	20.8.2020	13	NG_20_8_2020/2	live larvae	1		*Aedes albopictus*	
Nova Gorica	20.8.2020	13	NG_20_8_2020/3	live larvae	18		*Culex* sp., *Aedes albopictus*	
Nova Gorica	20.8.2020	13	NG_20_8_2020/4-25	no larvae/pupae	0			

cont. Table 1.

Nova Gorica	24.7.2020	16	NG_24_7_2020/1	live larvae	4		*Culex* sp.	
Nova Gorica	24.7.2020	16	NG_24_7_2020/2	live pupae	0	1	?	
Nova Gorica	24.7.2020	16	NG_24_7_2020/3	live larvae	2		*Aedes albopictus*	
Nova Gorica	24.7.2020	16	NG_24_7_2020/4	live pupae	0	1	?	
Nova Gorica	24.7.2020	16	NG_24_7_2020/5	live larvae	14		*Culex* sp.	
Nova Gorica	24.7.2020	16	NG_24_7_2020/6	live larvae	7		*Aedes albopictus*	
Nova Gorica	24.7.2020	16	NG_24_7_2020/7	live larvae	2		*Culex* sp.	
Piran	29.5.2020	0	Pi_29_5_2020/3	live larvae	1		*Culiseta longiareolata*	
Piran	29.5.2020	4	Pi_29_5_2020/1	dead pupae		2	?	live Asellus sp. and Chironomidae larvae
Piran	29.5.2020	4	Pi_29_5_2020/2	dead larvae	4		*Culiseta longiareolata*	
Piran	29.5.2020	4	Pi_29_5_2020/4-13	no larvae/pupae	0			
Piran	9.9.2020	12	Pi_9_9_2020/1	dead larvae	2		*Aedes albopictus*	
Piran	9.9.2020	12	Pi_9_9_2020/2	dead larvae	4		*Culex* sp.	
Piran	9.9.2020	12	Pi_9_9_2020/3	dead larvae	6		*Culex* sp.	
Piran	9.9.2020	12	Pi_9_9_2020/4	dead pupae	0	1	?	
Piran	9.9.2020	12	Pi_9_9_2020/5	dead larvae	3		*Aedes* sp. and *Culex* sp.	
Piran	21.7.2020	38	Pi_21_7_2020/1	live larvae	2		*Culex* sp.	
Piran	21.7.2020	38	Pi_21_7_2020/2	live larvae	1		*Culex* sp.	
Piran	21.7.2020	38	Pi_21_7_2020/3-8	no larvae/pupae	0			
Portorož	21.7.2020	1	Po_21_7_2020/1-5	no larvae/pupae	0			

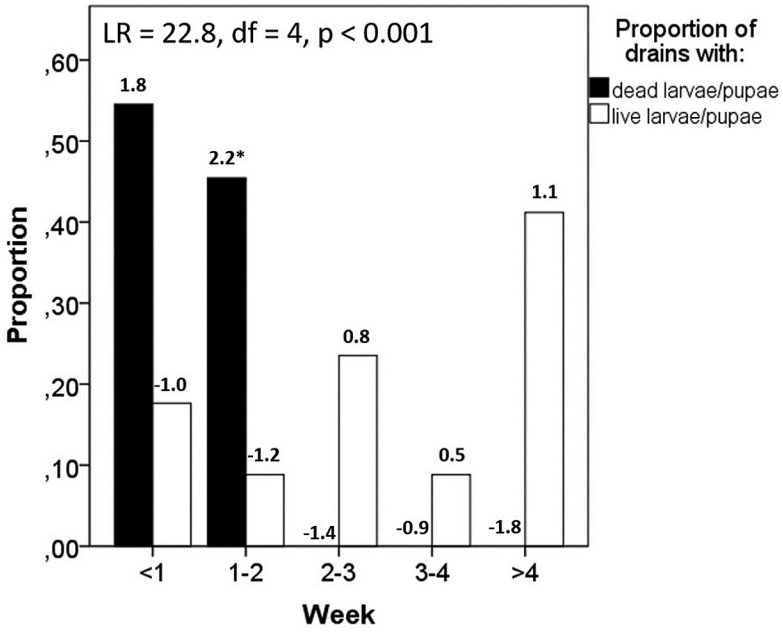

Figure 2. Proportion of drains with dead (black columns) and alive mosquito individuals (white columns) for five time periods (in weeks) after the application of Aquatain. Overall LR test is shown above the histogram. Residual values ≥ |2.0| that contribute most to the overall significant LR test equals $p < 0.05$ (designated by "*").

Table 2. Drains with dead or live larvae/pupae after Aquatain applications per week

Week after Aquatain application	Number of drains with live larvae/pupae	Number of drains with live larvae/pupae
1 day - to 1 week	6	6
1st to 2nd week	3	5
2nd to 3rd week	8	0
3rd to 4th week	3	0
more than 4	15	0
Sum	35	11

The effectiveness of mosquito control was also observed from the eggs and adult mosquitoes caught. The average number of eggs collected per site was higher in the area without mosquito control than in the other locations. In general, the abundance of eggs and adults at all sites started to increase in early July and peaked in August and early September. The number of eggs collected was highest in peri-urban areas with more greenery. In adult mosquito samples, the ATM predominated. The detailed data on adult mosquito catches and number of eggs in ovitraps data have previously been published [12].

DISCUSSION

Although Aquatain showed a lethal effect on mosquito larvae, pupae and adults, we assessed the mosquito control campaign as partially successful, as the number of mosquitoes in the locations with mosquito control was still very high, probably due to frequent rains that quickly washed the agent out of the drains. Moreover, in some municipalities (Koper, Ankaran) the periods between two applications were too long. In Koper, for example, almost two months passed between the second and third application. From the data on the seasonality of ATM we know that the mosquito population grows very fast during the period from July to the end of September and control before this time is very important. If we skip this period and do not apply the larvicides, all the larvae hatch into adult mosquitoes and (after mating) there are more females laying eggs. The number of mosquitoes increases more under such conditions without control than when at least some of the larvae are suppressed and prevented from developing into adult mosquitoes and laying eggs. When inspecting the drains in August in Koper before the last control, we found live larvae in almost all drains, but 7 days after the application of the product there were no larvae left. Further applications were carried out in the municipality of Izola, but these were to start at a specific date each month, regardless of the weather conditions. Looking at the results from 2020, we can say that Aquatain can also be used in subsequent years, with some modifications regarding the timing and frequency of application. We believe that the application of Aquatain should not only be based on a monthly work schedule, but also with regard to rainfall. After heavy rain, it is essential to reapply the product. In addition to control in public areas, the use of Aquatain in private areas should be strongly encouraged. For the first month of application, we recommend April, and during the peak mosquito season (July to September), we recommend using the product twice a month instead of once a month. As this additional effort places a heavy burden on utilities, we recommend combining the application of mosquito repellent with the application of rodent and cockroach repellent in drains where possible.

Any current plans to control mosquito populations should aim to minimize toxicological impacts on the environment in order to maintain biodiversity [13,14]. The use of pesticides is recommended as a last resort and emphasis should be placed on environmental and biological control [6]. In this sense, we believe that mosquito control plan which is presented here was properly prepared and implemented, and the campaign was limited by the recommendations and restrictions that apply to the use of biocides in Slovenia. Although we did not use biocides, we managed to slightly reduce the burden of mosquitoes in 2020. We know from comparisons with studies abroad (e.g. [15-17]) that the number of mosquitoes can be significantly reduced. To achieve even better success, we advise following the above recommendations in future campaigns. We need to be aware that we cannot eradicate mosquitoes completely, but we will be able to reduce their abundance to a tolerable level, which will increase the quality of life and reduce the threat to human health in the treated areas.

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Author's contributions

KK conceived and designed the analysis, collected the data, performed the analysis and interpreted the results, wrote the paper, approved the final version to be submitted, JŠ collected the data, performed the analysis, wrote the paper, approved the final version to be submitted, VI collected the data, performed the analysis, critically reviewed the paper, approved the final version to be submitted, JJ performed statistical analyzes, critically reviewed the paper, approved the final version to be submitted.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PILOT STUDIJA KONTROLE INVAZIVNIH VRSTA KOMARACA U ODABRANIM OBLASTIMA U SLOVENIJI

Katja KALAN, Jana ŠUŠNJAR, Jure JUGOVIC, Vladimir IVOVIĆ

U cilju smanjenja populacije azijskih tigrastih komaraca *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), 2020 godine je organizovana kampanja kontrole komaraca od strane pet slovenačkih opština. Fokus kampanje je bio na primeni Aquatain AMFTM u javnim odvodima za vodu i kišnicu. Osnovni cilj našeg istraživanja bio je da se direktnim pregledom vode u odvodima ispita njegova efikasnost na larve komaraca kao i praćenje gustine populacije komaraca. Šahte za vodu su kontrolisane pre i posle aplikacije produkta, od maja do septembra. Uzorci vode su sakupljani pomoću posebnih posuda za uzorkovanje (dipper). Abundanca je određena prebrojavanjem broja jaja i odraslih komaraca od juna do oktobra.

Do dve nedelje nakon primene proizvoda primetili smo smrtonosni efekat Aquatain-a na larve, lutke i odrasle komarce. Nakon padavina, proizvod je ispran, a živi komarci su uzorkovani u kontrolnim odvodima za vodu. Prosečan broj prikupljenih jaja po lokaciji bio je dva puta veći u oblasti bez kontrole komaraca, u poređenju sa kontrolnim lokacijama. Ipak, kampanju suzbijanja komaraca smo ocenili kao delimično uspešnu, zbog toga što je broj komaraca na lokacijama na kojima je obavljeno suzbijanje komaraca i dalje ostao visok. Uprkos tome, smatramo da su naši rezultati dobra polazna osnova za buduća istraživanja kao i da bi kontrolu komaraca trebalo nastaviti, uz kampanju podizanja svesti kod građana.