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Research Article

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The Effectiveness of Asian Bush Mosquito (*Aedes japonicus japonicus*) Control Actions in Colonised Peri-urban Areas in the Netherlands

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Abstract

The Asian bush mosquito (*Aedes japonicus japonicus (Theobald)*) is an invasive mosquito species in Europe. In 2012, it was for the first time detected in the Netherlands, in the municipality of Lelystad. After further research, thousands of specimens were found in the surrounding peri-urban areas of the city. A targeted mosquito control campaign began in 2015 with the objective of reducing populations in locations with the highest concentrations of *Ae. japonicus* breeding sites: allotment garden complexes. Mosquito control consisted of source reduction combined with application of the larvicide Vectomax in breeding sites. At eight complexes, mosquito control effectiveness has been systematically measured by sampling larvae from breeding sites. Six measurements were performed between 2015 and 2016. Results show that the effectiveness of mosquito control actions was similar in all treated allotment gardens and resulted in a significant reduction in *Ae. japonicus* larval abundance. Rain barrels at the allotments represent the most frequent breeding site in Lelystad, but every water filled artificial container is a potential breeding site for the species. *Ae. japonicus* was not found in the samples taken in other allotment gardens in the province of Flevoland; however, the collection methodology used proven to be effective in detecting this species when it has newly colonized surrounding areas. Targeted mosquito control actions at the breeding sites are crucial for successful reduction of populations of an invasive mosquito species, and systematic measurements of the effectiveness, is in this case, the base to understand the dynamics of *Ae. japonicus* populations after mosquito control.

Key words: Mosquito, *Aedes japonicus*, vector, control, VectoMax

The Asian bush mosquito *Aedes japonicus japonicus* (Theobald, 1901) is an invasive mosquito species (IMS) that originates from Japan, Korea, and Southern China (Tanaka et al. 1979) and has also been found in south-eastern Russia (Gutsevich and Dubitskyi 1987). The first interception of the species in Europe occurred in France in the year 2000 (Schaffner et al. 2003). Afterward, established populations of this species have been found in Belgium (Versteirt et al. 2009), Switzerland (Schaffner et al. 2009), Germany (Becker et al. 2011) (Kampen et al. 2012) (Werner and Kampen 2013), Austria, and Slovenia (Seidel et al. 2012). Since the first interceptions in the 1990s, *Ae. japonicus* has also successfully colonized numerous states of the United States, and by 2000, it was already reported from south-eastern Canada (Kampen and Werner 2014).

Under laboratory conditions, this species has been shown to be a competent vector of Japanese encephalitis virus (Takashima and Rosen 1989), West Nile virus (Sardelis and Turell 2001), La Crosse virus (Sardelis et al. 2002a), Eastern equine encephalitis virus (Sardelis et al. 2002b), St. Louis encephalitis virus (Sardelis et al. 2003), chikungunya virus, and dengue virus (Schaffner et al. 2003). It has never been proven that *Ae. japonicus* is an efficient vector of pathogens in the field, but recent investigations in the United States have found La Crosse virus in field-infected *Ae. japonicus* (Harris et al. 2015, Westby et al. 2015).

Extensive mosquito surveillance in 2013 confirmed the existence of a widely established population of *Ae. japonicus* in the municipality of Lelystad (Province of Flevoland, the Netherlands) (Ibañez-Justicia et al. 2014). This population seemed to be thriving in allotment gardens and in the surrounding patches of forest. Within the allotment gardens, *Ae. japonicus* larvae and pupae were found in multiple types of artificial containers. In 2014, a specific mosquito monitoring program in the province of Flevoland was implemented with the purpose of detecting populations of this IMS in municipalities adjacent to Lelystad. Results showed that populations of *Ae. japonicus* in the province, seem to be confined to the municipality of Lelystad.

With the aim of preventing further spread of the population in the Netherlands, in 2015, the Ministry of Public Health decided to implement mosquito control actions in Lelystad (source reduction and control of larvae using larvicides) to reduce the population hotspots in allotment gardens. In combination with the mosquito surveillance at the province of Flevoland to detect spread of the species, this control strategy was considered to be adequate for maintaining the populations of *Ae. japonicus* at low levels to prevent further spread. The ultimate goal of this control action is to achieve a medium/long-term reduction of the populations to reduce the possible risk of vector-borne diseases outbreaks.

To achieve the goal of reducing the population of *Ae. japonicus* in Lelystad, Integrated Mosquito Management (IMM) is used. An IMM uses a combination of methods such as: mosquito surveillance, source reduction, control of larvae and pupae, and control of adults, to prevent and control mosquito vectors of diseases. Application of a targeted IMM at the locality of Natoye in Belgium (source reduction and larviciding) eliminated the existing population of *Ae. japonicus* between 2013 and 2015 (Direction Générale Opérationnelle Agriculture Ressources naturelles et Environnement 2017).

The aim of the study is 1) to evaluate the effectiveness of the mosquito control actions conducted in 2015 and 2016 in reducing the populations of *Ae. japonicus* at the mosquito hotspots in Lelystad, 2) to identify the most preferred breeding site in the allotment gardens, 3) to evaluate the reduction of the mosquito populations the Lelystad allotments compared to the populations in allotments from adjacent municipalities in the province, and 4) to study the population dynamics of *Ae. japonicus* through longitudinal mosquito surveillance at the infested locations.

Material and Methods

Study Area

The study area for evaluating the effectiveness of the control actions against *Ae. japonicus* consisted of allotment garden complexes ($n = 8$) totalling 22.68 hectares (Fig. 1) in Lelystad (Table 1). Allotments in other municipalities within the province of Flevoland ($n = 31$) were also included in the study with the main objective of promptly detecting new populations of this IMS.

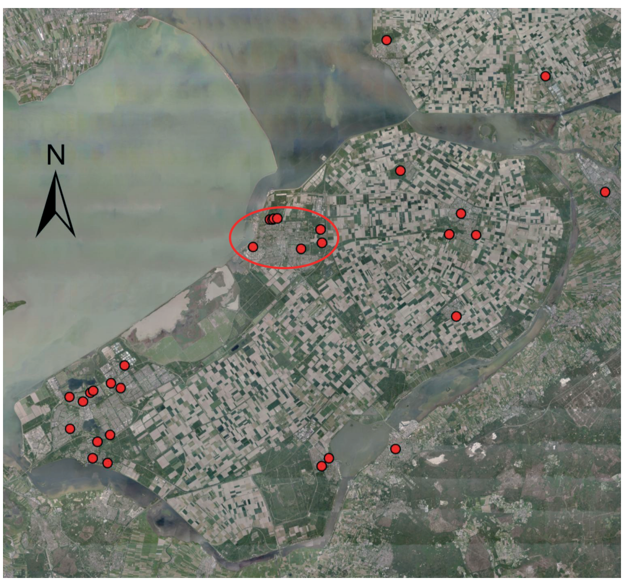

Fig. 1. Location of the 31 monitored allotments in the Province of Flevoland and surroundings 2015–2016 (red points: allotments, red circle: Lelystad municipality).

Sampling Methodology in Lelystad

At eight allotments, mosquito control effectiveness was systematically measured. Using ArcMap 10.2.2 (ESRI, Redlands), a grid of squares of 20 × 20 m (400 square meters) was created for each allotment. Each square of the grid received a unique number. For each allotment, 30% of the total of squares were randomly chosen for study. At each random square, all potential breeding sites (containers) were sampled for presence and abundance of mosquito larvae. Six measurements were performed between 2015 and 2016, and at every measurement, sample squares were randomly selected from the grid. Measurements were performed between the months of March and November. The first larval measurement before the control actions started, the so called 'zero measurement,' was done in September 2015.

At the chosen squares, the field inspector sampled all potential breeding sites (containers). At every container, and using aquarium fish nets (with fine mesh), the inspector collected all living invertebrates present. These were transferred to a white plastic container, and using a plastic pipette, all mosquito immatures (larvae and pupae) were collected and transferred to a plastic vial containing 70% alcohol. Each vial was labeled with a barcoded sample number, and samples were transferred to the laboratory. Each sample was accompanied by a paper form containing basic information about the sample including: date, location, square number, container type (rain barrel, bucket, litter, etc.), XY coordinates, and inspector name. Information about the total number and types of container in the measured square was also recorded.

Sampling Methodology in Other Allotment Complexes in the Flevoland Province

To be able to detect *Ae. japonicus* in other allotments in the province of Flevoland, another monitoring programme was set up in 31 allotment complexes. (Fig. 1). In these allotments, visual inspection of breeding sites was carried out. Among all mosquito breeding sites present at each allotment, rain barrels situated along the border of the allotment in proximity to a forested area were preferred for sampling. At each allotment, five open blue rain barrels containing water were selected and sampled for mosquito larvae. Sampling for mosquito immature stages was carried out following the same methodology mentioned before.

Longitudinal Adult Sampling

In 2016, longitudinal adult sampling of *Ae. japonicus* was carried out between April and November at two allotments. Two CO_2-baited Mosquito Magnet Liberty Plus traps (Woodstream, Lititz; hereafter MM trap) were checked weekly at each allotment. Traps were situated in similar conditions at each allotment but separated at least 100 m to avoid trap interference. Trapping nets from the MM trap with collected mosquitoes were sent to the laboratory for morphological identification and were stored at −20°C until analysis. Field sampling information including: inspector name, site number, trap start date, trap end date, and XY coordinates recorded with a Global Positioning System (GPS) were documented and sent together with the sample.

Table 1. Study areas (allotments) measured to analyse the effectiveness of control measures in the municipality of Lelystad

Allotment name	Area (hectare)	Total number 20 × 20 m grid squares	Area 20 × 20 m squares (hectare)	Number squares measured	% of total squares	Area measured (hectare)
Bosweg	0.9	21	0.8	6	29	0.2
Gelderse hout	4.4	113	4.5	34	30	14
Karveel	1.9	46	1.8	14	30	0.6
Ons Genoegen	3.8	93	3.7	28	30	1,1
Runderweg	3.6	90	3.6	27	30	1.1
Milieuvriendelijk tuinieren	3.0	77	3.1	23	30	0.9
Visarend	2.6	64	2.6	19	30	0.8
Zuigerplas	2.4	60	2.4	18	30	0.7
Total	22.7	564	22.6	169		6.8

Table 2. Breeding site measurements and container indices at the allotments of Lelystad

All allotments	Sept. 2015	Oct. 2015	April 2016	June 2016	Aug. 2016	Nov. 2016	Total
No. of potential breeding sites inspected	808	592	818	627	585	639	4,069
No. of breeding sites larvae absent	410	576	780	542	455	633	3,396
No. of breeding sites with larvae	398	16	38	85	130	6	673
No. of breeding sites with *Ae. japonicus*	242	10	36	7	57	1	353
General container index, % (GCI)	49.25	2.70	4.64	13.55	22.22	0.93	na
Ae. japonicus container index, % (JCI)	29.95	1.68	4.40	1.11	9.74	0.15	na

na (not applicable).

Mosquito Diagnostics

Field collected samples were sent to the National Reference Centre (NRC) laboratory of the Netherlands Food and Consumer Product Safety Authority (NVWA) for diagnostics. In the laboratory, mosquitoes were counted and morphologically identified by specialists using the electronic identification key of Schaffner (Schaffner et al. 2001), the key of Becker (Becker et al. 2010), and the key of Haren&Verdonschot (Haren and Verdonschot 1995). Except for the target species (*Ae. japonicus*), larvae and pupae were identified to genus level (e.g., *Culex* sp., *Culiseta* sp., etc). Adults captured with the MM traps were identified to species level.

Mosquito Control

In 2015, two actions were planned (in September and in October). In 2016, the first control action was conducted in April, and repetitions of the control actions were implemented every 4–5 wk. Mosquito control consisted of elimination and treating of breeding sites with a larvicide (VectoMax granules FG, Libertyville). If a breeding site could not be removed, granules were added to the water using a handheld dispenser. A standard dose of approximately 2 g was applied to each container in the allotment. The dose was increased 2–3 fold for large containers (e.g., water tanks).

Data Analysis

The general container index (GCI) and *Ae. japonicus* container index (JCI) were calculated at the eight Lelystad allotments for each of the six data collection time points as:

$$\mathrm{GCI} = \left(\begin{array}{l}\text{number of breeding sites containing larvae /}\\ \text{total number of breeding sites}\end{array}\right) \times 100$$

$$\mathrm{JCI} = \left(\begin{array}{l}\text{number of breeding sites containing } \textit{Ae. japonicus}\text{larvae /}\\ \text{total number of breeding sites}\end{array}\right) \times 100$$

To test the mosquito control effects on the mosquito larval populations at the allotments, an analysis of the variance (ANOVA), was carried out on the GCI and JCI indices. Analysis was performed using software Genstat (version 17.1, 64 bit). To test for differences in mean number of larvae between Lelystad and the province of Flevoland, a Mann–Whitney *U* test was carried out with SPSS (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc).

Results

Effectiveness Mosquito Control in Lelystad Allotment Gardens

The total area of the eight allotments included in the mosquito control effort represented 22.68 hectares (Table 1). At every measurement, 6.8 hectares (30% of the squares) were sampled for mosquito-breeding sites. During the study in 2015–2016, mosquito larval presence was measured at six time points, two in 2015 (September and October) and four in 2016 (April, June, August, and November). Two rounds of mosquito control (source reduction and larviciding) were conducted in 2015 and seven in 2016. The period between mosquito control actions was planned to be a maximum of 5 wk, corresponding to the maximum period of VectoMax larvicidal activity. Except for the period between the fifth and sixth control action in 2016 that lasted for 6 wk, mosquito control actions were performed as planned.

Table 2 shows that the number of breeding sites found during the measurements in September 2015 (n = 808) and April 2016 (n = 818) are more numerous than the measurements in October 2015 (n = 592), June (n = 627), August (n = 585), and November (*n* = 630) 2016. A total of 4,069 potential breeding sites were inspected, of which, 3,396 sites were negative for the presence of mosquito immatures (83.46%) (Table 2). Of the 673 breeding sites where mosquito larvae were detected (16.53%), *Ae. japonicus* was recovered from 353 sites (52.45%).

Results of the six measurements for the presence of mosquito larvae and specimens of *Ae. japonicus* at each allotment are presented in Table 3. At the zero measurement in September 2015, GCI varied from 1.53% to 100% in the allotments. It can be noticed that *Ae. japonicus* was absent at the 'Visarend' allotment at each measurement in the study. The maximum values of JCI at the zero measurement were recorded in the 'Zuigerplas' (61.73%) and 'Bosweg' (60.87%) allotments. Maximum values of GCI after the start of the mosquito control actions were recorded in August 2016 (32.43% in 'Zuigerplas' allotment and 32.35% in 'Bosweg' allotment). At the last measurement in November 2016, all allotment complexes were negative for the presence of *Ae. japonicus* mosquito immatures except in the 'Runderweg' allotment (JCI 1.33%).

In the 673 breeding sites containing larvae, a total of 44,461 mosquito larvae were identified. Larval identifications for each allotment are presented in Table 4. *Culex* sp. larvae were the most abundant in the containers in the allotments of Lelystad (*n* = 37,319, 83.93%) followed by *Ae. japonicus* larvae (*n* = 6,509, 14.63%), and *Culiseta* sp. (*n* = 586, 1.31%). *Anopheles* sp. larvae and *Aedes/Ochlerotatus* larvae (other than *Ae. japonicus*) were also found in a lesser extent. Table 5 shows the total number of larvae mosquito specimens identified at each of the six measurements. During the zero measurement in September 2015, the highest number of larvae were identified (*n* = 18,738), including the highest number of *Culex* sp. (*n* = 14,354) and *Ae. japonicus* (*n* = 4,335). The last measurement in November 2016 provided the lowest results in collection of mosquito larvae (*n* = 225). At the measurement in April 2016, larvae of *Culex* sp. were absent in the samples and *Ae. japonicus* was present (*n* = 862).

During the study period, *Ae. japonicus* larvae were found in a total of 353 breeding sites. Rain barrels at the allotments were the most frequent breeding site found for *Ae. japonicus* in Lelystad. As shown in Fig. 2, 47% (*n* = 166) of the containers where *Ae. japonicus* has been found were rain barrels. Other frequent breeding sites were buckets (*n* = 49) and small bins (*n* = 48), but it was also found breeding in a variety of different containers.

The ANOVA analysis to check the differences on GCI and JCI at the eight allotments indicated that there were no significant differences in the calculated indices between the allotments (ANOVA GCI: df = 6, $F = 1.14$, $P = 0.364$; ANOVA JCI: df = 6, $F = 2.25$, $P = 0.06$). However, there is a significant difference ($P < 0.001$) between the sampling dates. Mean percentage of GCI and JCI data and least significant difference grouping is presented in Table 6. Statistically significant differences on GCI and on JCI were noted between the zero measurement and the rest of the sampling dates ($P < 0.001$).

Comparison Lelystad With Measured Allotments in Flevoland

In 2015 and 2016 in a maximum of 28 allotments in the province of Flevoland (including Lelystad), samples were taken that could be used for assessing the effect of the mosquito control in the municipality of Lelystad. A total number of 42,706 mosquito larvae were collected and identified in the samplings conducted in May and in September of the years 2015 and 2016. Measuring only five rain barrels in this survey in the allotments in Lelystad, it was possible to detect *Ae. japonicus* in almost all samples. All samples taken in the allotments outside Lelystad were negative for the presence of *Ae. japonicus*. Results show

Table 3. Results of general container index (GCI %) and *Aedes japonicus* container index (JCI %) at the eight allotments included in mosquito control

	Bos GCI	Bos JCI	Gel GCI	Gel JCI	Kar GCI	Kar JCI	Ons GCI	Ons JCI	Mil GCI	Mil JCI	Run GCI	Run JCI	Vis GCI	Vis JCI	Zui GCI	Zui JCI
Sept. 2015	69.5	60.8	20.3	2.7	45.0	27.5	44.0	31.1	55.8	41.4	100.0	40.9	1.5	0.0	70.4	61.7
Oct. 2015	4.5	3.0	0.0	0.0	2.2	0.0	0.0	0.0	9.5	6.7	2.7	1.4	0.0	0.0	0.0	0.0
April-2016	5.9	5.9	0.0	0.0	3.6	1.8	6.0	6.0	10.8	10.8	4.0	4.0	0.0	0.0	4.2	4.2
June-2016	12.9	3.2	17.3	0.0	21.4	0.0	17.2	2.1	11.5	0.0	12.4	2.5	0.0	0.0	10.5	0.0
Aug. 2016	32.3	26.4	20.5	0.9	19.7	9.1	18.2	6.3	21.4	11.4	20.4	12.6	1.8	0.0	32.4	16.2
Nov. 2016	2.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	1.3	1.3	0.0	0.0	6.1	0.0

Bos = Bosweg; Ge = Gelderse Hout; Kar = Karveel; Ons = Ons genoegen; Mil = Milieuvriendelijk tuineren; Run = Runderweg; Vis = Visarend; Zui = Zuigerplas.

Table 4. Number of samples and number of larvae of *Aedes japonicus* and other mosquitoes at the eight allotments in Lelystad included in mosquito control in 2015 and 2016

Allotment complex name	Number of samples	*Culex* sp.	*Aedes japonicus*	*Culiseta* sp.	Other *Aedes* sp.	*Anopheles* sp.	Total larvae
Bosweg	40	2,559	623	190	0	1	3,373
Gelderse Hout	71	7,958	9	84	0	0	8,051
Karveel	49	3,557	250	105	12	2	3,926
Ons Genoegen	156	8,993	1,485	39	0	8	10,525
Milieuvriendelijk tuinieren	106	3,395	1,918	157	0	14	5,484
Runderweg	159	4,808	1362	8	4	4	6,186
Visarend	2	28	0	0	0	0	28
Zuigerplas	90	6,021	862	3	0	2	6,888
Total	673	37,319	6,509	586	16	31	44,461

Table 5. Number of *Aedes japonicus* larvae and larvae of other genera in the eight Lelystad allotments included in mosquito control in 2015 and 2016

Measurement moment	*Culex*. sp	*Aedes japonicus*	*Culiseta* sp.	Other *Aedes* sp.	*Anopheles* sp.	Total
Sept. 2015	14,354	4,335	40	0	9	18,738
Oct. 2015	220	233	1	0	2	456
April-2016	0	862	4	12	0	878
June-2016	11,436	92	50	0	2	11,580
Aug. 2016	11,287	980	295	4	18	12,584
Nov. 2016	22	7	196	0	0	225
Total	37,319	6,509	586	16	31	44,461

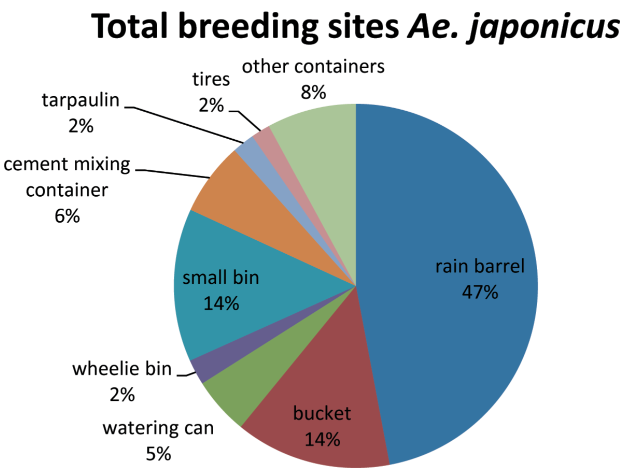

Fig. 2. Chart showing proportion of breeding site types with *Ae. japonicus* larvae, at the monitored allotments in 2015 and 2016 in Lelystad.

a clear decrease in the average number of mosquito larvae in Lelystad allotment rain barrels from May 2015 onward, ranging from a maximum of 205.19 larvae per rain barrel in May 2015 to a minimum of 6.99 in September 2016. In the allotments of Flevoland (outside Lelystad), the average number of larvae per rain barrel decreased from 165.42 in May 2015 to 20.79 in May 2016. However, in September 2016, unlike in Lelystad, the average number of larvae per rain barrel increased to 42.44 (Table 7). Only the data of Lelystad and the adjacent municipality of Almere were used in the analysis because the allotments in these municipalities were most extensively sampled. The data from other municipalities in the province of Flevoland appeared to be insufficient for the analysis. The Mann-Whitney U test revealed that the average number of larvae found in Lelystad (Mean Rank = 4.75, n = 8) was significantly lower than in Almere (Mean Rank = 13.3, n = 10) (Mann-Whitney U = 2.00, P = 0.001 and $P < 0.05$) only at the measurement in September 2016. All the other measurements (May and September 2015, and May 2016) showed no significant differences in the average number of larvae collected.

Longitudinal Data

The first adults captured in the MM trap were recorded on 13 May 2016 (week 19). Since that collection, adults of *Ae. japonicus* were captured every week in the traps placed in Lelystad through 28 October 2016 (week 43). A total of 416 adults were captured in the four traps. The maximum number of *Ae. japonicus* (37) captured in a single trap was recorded between 14 July and 22 July, followed by a capture of 27 specimens between 15th and 22th of September (Fig. 3).

Discussion

In this article, we report the measured effectiveness of the largest active mosquito control action taken in the Netherlands since the vector control measures against malaria vectors (van Seventer 1969). To date, actions taken against *Ae. japonicus* have only been effective in Belgium, eliminating this mosquito vector species around the town of Natoye (Direction Générale Opérationnelle Agriculture Ressources naturelles et Environnement 2017). In an area of more than 22 hectares in Lelystad, source reduction combined with the application of larvicide was performed, and in more than 6 hectares, the effectiveness of these treatments was measured. For this task, a GIS-based methodology was applied. We consider that the calculated larval indices at the allotment complexes during the different measurements have been proven to be effective for statistical comparison. In other parts of Europe, the number of mosquito pupae per hectare (PHI) has been applied as an index and could provide useful data (Petrić et al. 2014). Pupal indices exploit the strong correlation between the number of pupae and the number of adults in a defined area, based on the low natural mortality of the pupae (Baldacchino et al. 2015). Due to the large amount of specimens collected in our study, it was decided to focus the efforts only on the larval indices.

An especially relevant measurement was the measurement zero in September 2015, which showed the extent of the population. In some allotments, *Ae. japonicus* larvae were present in more than 60% of the containers sampled. It can be noticed that at the 'Visarend' allotment, that larval indices were always very low, and *Ae. japonicus* was absent at each measurement in the study. Larvae of *Ae. japonicus* were found in the allotment during the intensive surveillance of the municipality in 2013 (Ibañez-Justicia et al. 2014). The 'Visarend' allotment is situated in the vicinity of a large used tire company that, since 2013, has been found positive for introductions of *Aedes albopictus (Skuse)* every year. Control measures applied in this location after finding an invasive mosquito include, among others, larviciding an area of 500 m surrounding the site. As a result, this allotment has been treated since 2013 with larvicide, resulting in a reduction in mosquito populations breeding in the containers and in the absence of *Ae. japonicus* during this study.

Finding lower numbers of breeding sites present during the measurements after the first mosquito control actions in 2015 (September) and in 2016 (April), confirm the expected results of the source reduction at the allotments. However, following the measurement in June 2016, the number of potential breeding sites did not decrease any further. The use of rain barrels is the most common practice in the allotments to collect and store water. At least one or two rain barrels are encountered at each parcel and even in a mosquito control campaign to reduce populations of an IMS, these potential breeding sites could not be easily removed. Since the number of rain barrels could not be further reduced, those remaining were treated with larvicide.

Table 6. Fisher's protected least significant difference test grouping results for General Container Index (GCI) and *Aedes japonicus* Container Index (JCI) for the eight Lelystad allotments included in mosquito control in 2015 and 2016

GCI			JCI		
Moment	Mean	Group	Moment	Mean	Group
Nov. 2016	1.6	a	Nov. 2016	0.2	a
Oct. 2015	2.7	a	Oct. 2015	1.6	ab
April 2016	4.9	ab	June 2016	2.6	ab
June 2016	15.0	bc	April 2016	4.7	ab
Aug. 2016	23.4	c	Aug. 2016	10.4	b
Sept. 2015	57.9	d	Sept. 2015	38.0	c

Table 7. Average number (and standard deviation) of mosquito larvae found in rain barrels in Lelystad and Flevoland surveillance for *Aedes japonicus* in 2015–2016

Year	Location	Number allotments	Moment	Average number of mosquito larvae in rain barrel	SD standard deviation
2015	Flevoland	21	May	165.4	165.6
2015	Flevoland	19	Sept.	91.1	74.3
2016	Flevoland	20	May	20.8	27.9
2016	Flevoland	20	Sept.	42.4	41.8
2015	Lelystad	7	May	205.2	150.0
2015	Lelystad	7	Sept.	48.4	30.3
2016	Lelystad	8	May	24.4	29.1
2016	Lelystad	8	Sept.	7.0	10.9

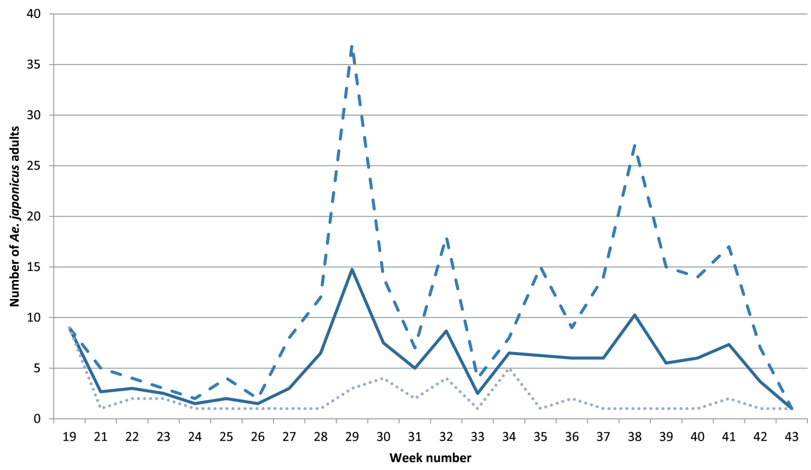

Fig. 3. Minimum, maximum, and average number of *Ae. japonicus* adults captured in longitudinal survey in Lelystad in 2016. Dotted line indicates minimum number; dashed line indicates maximum number; and solid line indicates average number.

In comparison with the other potential breeding sites, *Ae. japonicus* larvae were most frequently found in rain barrels. Other breeding sites where *Ae. japonicus* frequently occurred were buckets and small bins. In addition to these breeding sites, *Ae. japonicus* was also found in a variety of different containers such as watering cans, wheelie bins, cement mixing containers, tarpaulins, and used tires. In the measurements at the allotments, all water-filled artificial containers were considered to be potential breeding sites for *Ae. japonicus*.

Culex sp. larvae were the most abundant in the containers at the allotments of Lelystad followed by *Ae. japonicus* larvae. It needs to be noticed that at the measurement in April 2016, larvae of *Culex* sp. were absent in all samples taken, and almost all larvae found were *Ae. japonicus*. These *Ae. japonicus* larvae were probably larvae from late 2015 that passed the winter in the containers. This information is important because mosquito control (source reduction or larviciding) applied at this moment will only affect larvae of the target IMS.

The effect of the mosquito control actions (source reduction and larviciding) on population reduction at the eight allotments in Lelystad has been proven for all larvae species (GCI) and for *Ae. japonicus* (JCI). Both larval indices were high at the zero measurement and low in the remaining sampling periods. The effects of the treatments were proven to be similar at the eight locations. Lowest values of larval indices were found, and lowest amount of larvae were collected after the last treatment (November 2016). As published by (Schaffner et al. 2013), limiting the available sites for oviposition through source reduction also affects the distribution of native mosquitoes such as *Culex* sp. in a locality. According to the statistical analysis (Fisher's protected LSD test), the low value of JCI found at the last measurement in November 2016 (0.190%) was not significantly different from the value of JCI in October 2015, June 2016, or April 2016. At these moments, values of JCI can be also considered low. The value of JCI found in August 2016 (10.441%) is significantly higher than the value of JCI in November 2016 (0.190%), but it is not significantly different from the values of June and April 2016, and October 2015.

The methodology applied for sampling larvae at the allotments in Flevoland province proved to be appropriate to detect the presence of *Ae. japonicus*. Using this sampling methodology (larval sampling in five rain barrels containing larvae preferably nearby forested areas), *Ae. japonicus* was detected in Lelystad even after the control actions when populations were lower. We consider that if *Ae. japonicus* was present at the other allotments in the province, it would have been detected during the study. Results from collections in Lelystad using this sampling methodology also show a clear decrease in the average number of mosquito larvae in the rain barrels in the allotments from May 2015 onward. This decreasing larval density trend was not seen in the sampled allotments in province of Flevoland, where the average of larvae per rain barrel decreased from May 2015 to May 2016 but increased in September 2016. This indicates that the control actions taken in Lelystad do have an effect on the mosquito population at the 'hot spots'.

Longitudinal data of adult *Ae. japonicus* captured with MM traps revealed important biological facts for this species in the Netherlands. Traps were deployed at the end of April, but first captures were recorded after the second weekly control. We can consider that the first adults collected in these allotments originated from overwintering larvae that developed to adults when an appropriate temperature was reached to complete their life cycles. From that collection forward, adult specimens were captured in the traps every week with low numbers from May until early July and reaching a peak in the middle of July. Adults emerging in July could have originated from eggs laid by the adults that emerged in May or remaining eggs from 2015 that hatched in 2016 when ecological conditions were appropriate for survival of the larvae (contact with water, appropriate temperature, and appropriate photoperiod). Two months later, in September, another peak in the population was recorded. In this case, most of the adults probably originated from eggs laid by the adult females emerging in 2016.

For the first time, results of effectiveness of the mosquito control actions performed to reduce populations of *Ae. japonicus* are reported in Europe. Populations of the target species were considerably reduced after the applied mosquito control actions. As recommended by (Baldacchino et al. 2015), source reduction methods, should involve the public in a community-based approach. Effective source reduction requires scrupulous and repeated cleaning or treatment of containers for everyday use and so relies on extensive homeowner collaboration (Unlu et al. 2013). A community-based participation program, could significantly reduce the cost of control measures and contribute greatly to the decrease of the IMS populations in Lelystad in the future. However, it is not expected that source reduction in combination with larvicide intervention will completely eliminate the populations at the allotments, due the presence of untreated cryptic containers in the surrounding areas. Effective and targeted mosquito control actions at the breeding sites are crucial for successful reduction of populations of an IMS, and well-planned and systematic measurement of the effectiveness, is in this case, the base to understand the dynamics on the populations of *Ae. japonicus* after mosquito control. For this reason, monitoring in allotments of the Flevoland province is recommended for promptly detecting the possible spread of the species from Lelystad and, if necessary, applying control measures.

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