# Efficiency of Bti-based floodwater mosquito control in Sweden – four examples

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**Abstract:** Mass-occurrence of floodwater mosquitoes, mainly *Aedes sticticus*, in the River Dalälven floodplains in central Sweden has caused public health issues and economic losses for many decades. In the summer of 2000, the problem escalated and the Biological Mosquito Control project was initiated with the aim of reducing mosquito nuisance. Larviciding, based on *Bacillus thuringiensis israelensis* (Bti), was chosen as the optimal method. However, high abundance of blood-seeking floodwater mosquitoes after Bti-treatments on some occasions raised questions about the effectiveness of the treatments. This study evaluated the effect of Bti-larviciding on abundance of larval and adult floodwater mosquitoes in four selected study areas, each represented by a CDC-trap site and a 5 km radius. The four areas differed with respect to their mosquito control history and the coverage of larval habitats with Bti-larviciding. The Bti-treatments provided a significant reduction of mosquito larval abundance, and normally 100% reduction was achieved. Thus, high abundance of blood-seeking mosquitoes could not be explained by insufficient larval control by Bti. However, a significant negative correlation was found between high numbers of blood-seeking floodwater mosquitoes and the coverage of larval habitats with Bti-larviciding within 5 km around the trap site. Consistently low numbers of mosquitoes (less than 1000 per trap/night) were only found in the two areas with high treatment coverage of larval habitats (97-100%). Evaluating the mosquito control efficiency showed that larval habitat coverage of at least about 95% is required in order to accomplish consistent low floodwater mosquito numbers. The conclusion from this analysis is that the coverage of larval habitats with Bti-larviciding in parts of the River Dalälven floodplains has to increase in order to guarantee an improvement of the public health problems caused by *Aedes sticticus* and other floodwater mosquitoes to both humans and animals in the region.
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## Introduction

Mosquitoes have plagued mankind for centuries, both as nuisances and as vectors of mosquito-borne diseases, causing indescribable human suffering and economic losses (Becker *et al.*, 2010). Species of floodwater mosquitoes (Schäfer *et al.*, 2004) are a common cause of nuisance along rivers, lakes and coastlines and the cause of public health problems in a large number of countries, e.g. Germany, France, Croatia, Serbia, Canada and in many states in the U.S.A (Becker & Ludwig, 1981; Minar *et al.*, 2001; Merdic & Lovakovic, 2001). Also in Sweden, public health issues and economic losses due to mass-occurrence of floodwater mosquitoes could no longer be denied when the problem in the River Dalälven floodplains escalated in the summer of 2000.

In the floodplains of the River Dalälven in Central Sweden, large inundation areas with irregular occurrence of floods both in spring after snowmelt and during summer after heavy rainfall provide extensive larval habitats for floodwater mosquitoes. The predominating species in this floodplain area is *Aedes (Ochlerotatus) sticticus* Meigen (Schäfer *et al.*, 2008), known for its mass-production and long-range dispersal behaviour (Brust, 1980). In the summer of 2000, more than 60,000 mosquitoes were sampled in one trap in one night (Schäfer *et al.*, 2008). Documentation of the extreme mosquito abundance, identification of the main nuisance species and the willpower of the local population were the driving forces that initiated the "Biological Mosquito Control" project in 2000. Mr Kjell Larsson, Minister for the Environment, visited the Lake Färnebofjärden area of the floodplains during the worst period of mosquito nuisance in August 2000 and decided to support the peoples' desperate need for help. The massive mosquito nuisance problems needed to be solved without harming the protected environments in the area. Therefore, mosquito larval control using *Bacillus thuringiensis israelensis* (Bti) was suggested as the optimal method to drastically reduce mosquito numbers while not harming any other non-target organisms (Boisvert & Boisvert, 2000; Schnetter *et al.*, 1981; Becker & Margalit, 1993; Miura *et al.*, 1980; Mulla *et al*,. 1982). Worldwide, Bti is probably the most commonly used mosquito control agent due to its environmental safety, ease of handling, cost-effectiveness and lack of potential for resistance development (Becker *et al.*, 2010).

The Swedish routine Bti-applications started in 2002 with permit for 1,170 ha of temporary flooded areas near the villages of Österfärnebo and Tärnsjö, requiring very detailed and comprehensive applications to national and regional authorities. The Swedish authorities were in general very restrictive with permits, and were strictly against use of Bti in any protected area including Nature Reserve, National Park, and Natura 2000. In 2008, the question about permit for floodwater mosquito control using Bti in the Nature Reserve Ista first became a case for the Environmental Court, and then also for the Superior Environmental Court, resulting in the first permit to use Bti as a floodwater mosquito larvicide also in protected areas. Since July 2009, the permit for Bti-based mosquito control includes protected areas and now the total mosquito control frame area comprises 9,246 ha of inundation areas in the River Dalälven floodplains, and in some adjacent inundation areas flooded by local streams. However, some highly productive areas for *Ae. sticticus* at the edge of Lake Färnebofjärden are still not included in the mosquito control. Also, economic problems and lack of long-term financing have occasionally led to incomplete treatments (no more money available for helicopter costs or purchase of Bti).

Thus, treatment coverage of floodwater mosquito producing areas varied between areas and between years. On some occasions, the abundance of blood-seeking floodwater mosquitoes was higher than expected from the strong larval abundance reduction, raising questions about the effectiveness of Bti-based larviciding.

In the present study, we address the question of the effect of Bti-applications on the target-species *Ae. sticticus* and the associated floodwater mosquitoes *Ae. vexans*, *Ae. cinereus*, and *Ae. rossicus* (Schäfer *et al*., 2008). We use data from floods with and without Bti applications between 2000 and 2012, and evaluate the effect of the larviciding on the larval numbers in treated temporary wetlands and on the consequent abundance of blood-seeking *Ae. sticticus* and other floodwater mosquitoes. The relative treatment coverage of larval production areas in each mosquito control treatment was included in the evaluation, and we selected four study areas as examples with varying proportions of the actual floodwater mosquito larval production areas included in the control measurements.

Our aim is to evaluate how the abundance of blood-seeking *Ae. sticticus* and other floodwater mosquitoes relate to both the efficiency of the Bti-larviciding and the relative control coverage within the flight range of the target species.

## Materials and Methods

*Study areas*

The floodplains of River Dalälven in Central Sweden are located along a 120 km stretch of the river from the city of Avesta in the West to the town of Älvkarleby in the East, with the river forming a series of lakes on its way. Most subject to flooding are the inundation areas around Lake Färnebofjärden, where up to four flood events can occur between April and August. These floods are caused by melting snow and/or rainfall, both locally and in the large catchment area of the River Dalälven. In addition, some floodwater mosquito breeding sites are located along the Stream Vretaån, and these flood-prone areas become flooded after local rain and/or melting snow.

For evaluating the effect of Bti-application on larval and adult floodwater mosquito numbers we selected four study areas with major nuisance caused by the floodwater mosquito *Ae. sticticus*, with a different background of mosquito control, and where blood-seeking female mosquitoes have been collected both before and after the start of mosquito control operations. Each study area is represented by a mosquito trap site and a circle with a 5 km radius around it. Within this 5 km radius around each trap site, the extent of temporary flooded areas with permit for mosquito control for different years and any additional potential larval production areas were extracted. This radius was chosen because it is within the flight range of *Ae. sticticus* (Brust, 1980; Bogojevic *et al*., 2011), and because the Swedish Environmental Protection Agency uses this radius around a trap as an area of delimitation. The number of mosquitoes in a trap has to exceed 5000 per trap/night in order to get permit for mosquito control in breeding sites within the area covered by a 5 km radius around the trap.

Within areas with a permit for mosquito control, the mosquito control frame areas, Bti treatments are allowed without necessarily including the complete area at an actual application. Extraction of mosquito control frame areas within each 5 km circle around trap sites was based on our own data. Additional floodwater mosquito production areas were extracted based on flood modelling using a high resolution digital elevation model (based on laserscanning) in MapInfo Professional 11.5 (Pitney Bowes Software Inc. 2012) and Encom Discover version 2013 (Pitney Bowes Software Inc. 2013). Based on Lake Färnebofjärden water level measurements at the Ista automatic station, a maximum water level of 57.20 m a.s.l. (RH70) was chosen for flood modelling of Lake Färnebofjärden. Locations of mosquito control frame areas and potential mosquito production sites not included in mosquito control permits are shown in Figure 1 for the early years (2002-2004) while Figure 2 shows the actual situation from July 2009 to 2012. The proportion of untreated breeding sites in relation to treated areas was assumed to be similar for all flood events independent of the extent of flooding.

The four study areas differ mainly with respect to relative coverage of floodwater mosquito production areas in the Bti-based larviciding, in relation to the total floodwater mosquito production area within a 5 km radius.

**Hallsjön** (60° 4' 4.0" N; 17° 1' 0.0" E), with the CDC-trap site located in the wetland flooded by the creek Vretaån, and near the village of Huddunge. This area is located 13 km south of the River Dalälven floodplains, without connection to the river. The previous lake Hallsjön was drained in the 19th century to increase agricultural land. However, as a consequence, the deeper parts of the former lake turned into a marsh surrounded by temporary flooded wet meadows. Flooding is caused by melting snow and/or heavy rainfall within the local catchment area of the creek Vretaån. Karbosjön, a similar drained former lake area with wet meadows, is located downstream Hallsjön and within 5 km distance from the CDC-trap. No additional potential floodwater mosquito breeding sites have been found within 5 km distance. Floodwater mosquito larviciding with Bti started in 2005 and the mosquito control frame area includes 197 ha, covering 100% of the floodwater mosquito breeding sites within 5 km.

**Österfärnebo village** (60° 18' 35.0" N; 16° 47' 56.0" E), with the CDC-trap positioned in the centre of the village located north of Lake Färnebofjärden near the most flood-prone part of the River Dalälven floodplains. Within a 5 km radius, large floodwater mosquito production areas including wet meadows and alder swamps are present in all directions except to the North. When the water level in Lake Färnebofjärden rises as a consequence of increased flow in the river, the water is pushed into Lake Fängsjön and the creek Norrån causing extensive flooding near Österfärnebo village. Mosquito control in the surroundings of Österfärnebo village commenced in July 2002, covering 595 ha and approximately 43% of all mosquito breeding sites within a 5 km circle. From July 2009 onwards, mosquito control has been allowed in protected areas, increasing the mosquito control frame area within 5 km radius from the CDC-trap to 1,352 ha and increasing the relative coverage of Bti-larviciding to 97% of all presently identified floodwater mosquito production areas.

**Fågle** (60° 14' 42.0" N; 16° 43' 36.0" E), with the CDC-trap positioned in an alder swamp. The area within 5 km radius from the trap includes large areas of both wet meadows and swamps adjacent to and influenced by the River Dalälven. During the first years of mosquito control, an area of 237 ha (16% coverage of breeding sites) at Lake Fangsjön located more than 3 km east of the trap site was included in Bti treatments. From July 2009 to 2012, the mosquito control frame area within 5 km radius increased to 1,159 ha, increasing the relative coverage of Bti-larviciding to 78% of all presently identified floodwater mosquito production areas.

**Ormpussen** (60° 7' 30.0" N; 16° 46' 50.0" E), with the CDC-trap located in a swamp with mixed coniferous and deciduous trees, is located at the southern beach of Lake Färnebofjärden, and at the brim of Färnebofjärden Nationalpark which is also a Natura 2000 site. The trap site is surrounded by large areas of wet meadows and swamps and the area is directly influenced by the water level in Lake Färnebofjärden. Until July 2009, no larval habitats within the 5 km radius of this trap were included in the floodwater mosquito larviciding with Bti, and thus the relative control coverage of floodwater mosquito production areas was 0%. From July 2009 to 2012, the mosquito control frame area within 5 km radius increased to 632 ha, resulting in a relative coverage of Bti-larviciding to 58% of all presently identified floodwater mosquito production areas.

*Floodwater mosquito control by larviciding*

Floodwater mosquito control in the River Dalälven floodplains and adjacent areas is solely based on application of VectoBac G® (manufactured by Valent BioSciences Corp., U.S.A.), a granule formulation with ground corn-cobs as the carrier for the Bti protein crystals and a potency of 200 UTI/mg for *Aedes aegypti*. VectoBac G® is applied by helicopter using a sling system and a bucket with rotating disc. The helicopter is equipped with a navigation system and a customised software, allowing for precise application and automatic logging of treatments as well as all flight movements. An average dosage of 13-15 kg VectoBac G® per hectare is applied.

In the years 2002 to 2012, 25 floods with floodwater mosquito production occurred and Bti-larviciding was executed at 22 flood occasions, mainly in May and July (Table 1). However, several required treatments were not performed, or only partially performed, due to restrictions of permits, lack of money, insufficient amount of VectoBac G® in storage, or denial by the executive board.

*Measuring larval abundance*

Abundance of floodwater mosquito larvae were measured, both before and 24 hours after larviciding with Bti, using a standard 250 ml white enamel mosquito dipper and with 10 samples per sampling site. The actual number of sampling sites varied with extent of flooding but included at least 20 sampling sites. The mean number of larvae per litre was extracted for the sampling performed within the 5 km circle of each trap site. Based on number of larvae before and after application, the percentage reduction of larval abundance was calculated. Larval sampling was only performed in connection with the Bti-larviciding, thus no data exists for floods without treatments.

*Measuring blood-seeking female abundance*

Since 2001, surveillance of blood-seeking female mosquitoes are performed regularly in approximately 22-35 sites located in the River Dalälven floodplains, and some adjacent flood-prone areas producing floodwater mosquitoes. Mosquitoes are collected every second week between May and September using CDC miniature light traps baited with carbon dioxide (see also Lundström *et al.*, 2013). The total number of mosquitoes per trap/night was estimated by weight. For some areas, trap data was available from the year 2000 as well (Schäfer *et al.*, 2008). For evaluation of the effect of mosquito control measures on blood-seeking female abundance, only floodwater mosquito species (*Ae. sticticus, Ae. vexans, Ae. cinereus* and *Ae. rossicus*) were considered in this study since these are the target species. The number of collected floodwater mosquitoes per trap/night usually reaches a peak 2-4 weeks after a flood event, and this maximum number of mosquitoes is used for analyses. For all areas, maximum numbers of floodwater mosquitoes were available for flood events with and without Bti-larviciding.

*Efficiency of Bti larviciding on larvae and adults*

The efficiency of the Bti larviciding was calculated taking both the relative coverage of actual floodwater mosquito production areas with larviciding in relation to all production areas within 5 km radius, and the actual relative reduction of larval abundance in the treated areas into consideration. The relative coverage of actual areas with larviciding in relation to all floodwater mosquito production areas within 5 km radius around CDC-trap sites varied from 0 (Bti-larviciding not done in any of the larval production areas) to 1 (all larval production areas included in the larviciding). Relative reduction of floodwater mosquito larval abundance varied from 1 (no alive larvae found after Bti-larviciding) to 0.8 (20% of larvae/pupae survived the larviciding).

The product of the relative larviciding coverage and the relative larval reduction is used to calculate the efficiency of Bti treatments, with efficiency = larviciding coverage x larval reduction. The efficiency of Bti-treatments ranged from 0 (no control performed) to 1 (combination of complete coverage of breeding sites and complete larval reduction). The efficiency of Bti-treatments is then set in relation to the maximum number of adult floodwater mosquitoes collected after the flood event.

*Statistics*

Basic statistics including t-tests and correlation tests were computed using Statistica 11 (StatSoft Inc. 2012).

## Results

*Abundance of floodwater mosquito larvae before/after Bti-larviciding*

Number of floodwater mosquito larvae per litre before treatments varied both between areas and within areas, ranging from 8 to 504 larvae per litre. In years with several flood events, the number of larvae per litre before treatments was always highest at the first annual flood event. A total of 48 Bti-treatments were evaluated, and for 41 treatments no living floodwater mosquito larvae were observed 24 hrs after application of Bti. For seven treatments a few live larvae up to a maximum of 14 larvae/pupae per litre were observed. Larval abundance reduction thus was usually 100% but lower at a few occasions with 80% as the lowest reduction. A t-test showed highly significant differences between the mean number of larvae per litre before and after treatments ($t=6.39$, $df=47$, $p=0.0000$). More detailed data on larval abundance reduction is provided below for each of the four study sites.

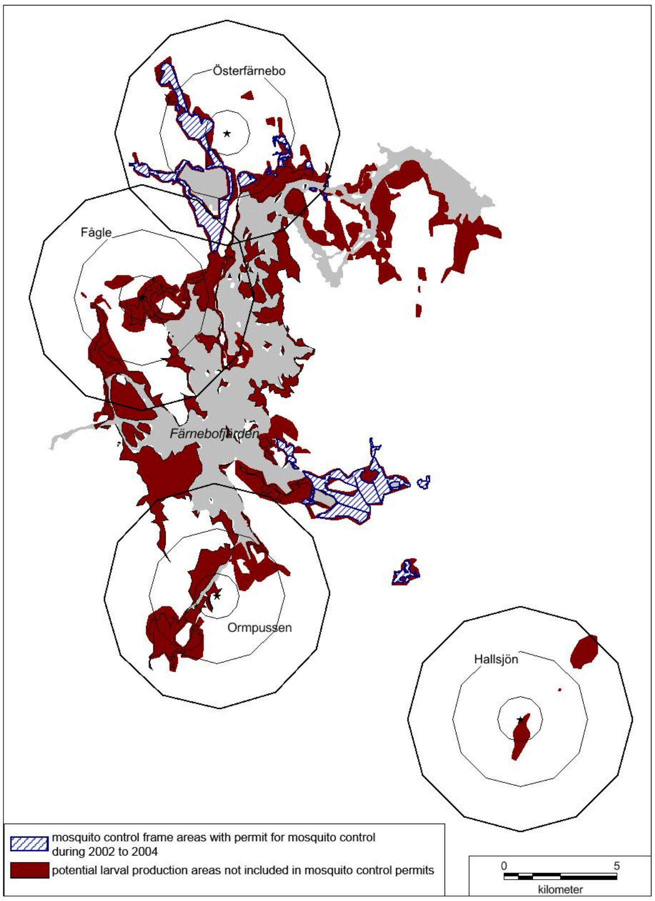


**Figure 1.** Map showing the mosquito control frame areas in the year 2002 to 2004 and the potential mosquito larval production areas not included in mosquito control permits within 5 km radius around selected trap sites at Hallsjön, Österfärnebo, Fågle and Ormpussen in the lower River Dalälven region, Central Sweden. For a better overview, circles with 1 and 3 km radius around trap sites are shown as well.

*Abundance of blood-seeking floodwater mosquitoes with and without Bti-larviciding*

The number of blood-seeking floodwater mosquitoes reported after individual flood events in the four study sites ranged from 0 to 76,669 individuals per CDC-trap/night, and showed variations for the different sites in relation to their mosquito control background. Significantly lower numbers of blood-seeking floodwater mosquitoes per trap/night for flood events with in comparison to flood events without Bti-larviciding were found for Hallsjön ($t=2.49$, $df=11$, $p=0.03$) and Österfärnebo ($t=3.47$, $df=17$, $p= 0.003$). For the study areas Fågle and Ormpussen, neither a significant reduction nor an increase of blood-seeking mosquito numbers by Bti-treatments was found.

**Hallsjön** – 0% and 100% relative treatment coverage of larval areas.

From the study area Hallsjön, close to the village of Huddunge, adult mosquitoes have been sampled twice before any mosquito larviciding with Bti was allowed. In August 2002, 8,600 floodwater mosquitoes per trap/night were collected, and in August 2004, 16,200 floodwater mosquitoes per trap/night were caught.

Mosquito control using Bti-larviciding and covering all potential floodwater mosquito larval production areas within a 5 km radius around the trap site, commenced in June 2005 and showed a larval abundance reduction rate of 100% at all occasions except once in June 2007. In June 2005, the mean number of floodwater mosquito larvae before Bti-larviciding was 504 per litre, and no live larvae were observed 24 hrs after treatment, indicating high treatment efficiency. A maximum of 2,765 blood-seeking floodwater mosquitoes per trap/night was measured at Hallsjön after the first treatment. During the years 2005 to 2012, all treatments except for June 2007 (see below), resulted in 100% larval abundance reduction and complete treatment efficiency. The number of blood seeking floodwater mosquitoes per trap/night after successful Bti-larviciding varied between 0 and 338

In the Hallsjön study site, it was clear that a small but productive area can produce very large numbers of floodwater mosquitoes. In June 2007, little but continuous rain created a collection of small pools and puddles but no continuous water surface in the Hallsjön study area. This waterlogged area was discovered too late and emergence of adult mosquitoes had already started. Some 40 hectares with an average of 68 larvae per litre were instantly treated with Bti but only an 80% abundance reduction of larvae/pupae was achieved, resulting in a treatment efficiency of 0.8. The production of mosquitoes from this small area resulted in measured abundance of 18,139 blood-seeking floodwater mosquitoes per trap/night.

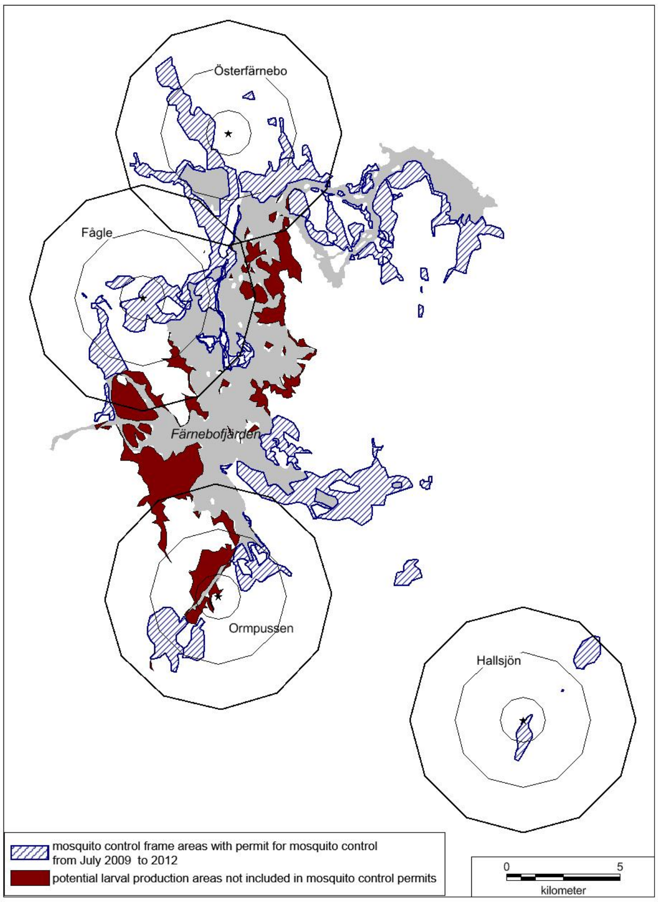


**Figure 2.** Map showing the mosquito control frame areas from July 2009 to 2012 and the potential mosquito larval production areas not included in mosquito control permits within 5 km radius around selected trap sites at Hallsjön, Österfärnebo, Fågle and Ormpussen in the lower River Dalälven region, Central Sweden. For a better overview, circles with 1 and 3 km radius around trap sites are shown as well.

**Table 1:** Overview of the 25 flood events and 22 Bti-treatments between 2002 and 2012 for the whole River Dalälven region, Central Sweden.

| Year | Treatment date | Total area treated (ha) | comment |
|---|---|---|---|
| 2002 | May | 0 | Permit not ready |
| 2002 | 13-14 July | 443 | |
| 2002 | 29 July – 1 August | 955 | |
| 2003 | 13-15 May | 583 | |
| 2003 | July | 0 | Lack of money |
| 2005 | 16-18 June | 579 | |
| 2005 | 3 August | 150 | |
| 2005 | August | 0 | Denial by executive board |
| 2006 | 11-14 May | 840 | |
| 2007 | 22 June | 40 | Puddles detected too late |
| 2008 | 1 May | 49 | |
| 2008 | 10-13 May | 1,037 | |
| 2008 | 21-22 August | 385 | |
| 2009 | 22-25 June | 1,585 | |
| 2009 | 15 July | 479 | |
| 2009 | 22-26 July | 2,150 | |
| 2009 | 1-2 August | 858 | |
| 2010 | 24 May -2 June | 3,656 | Insufficient amount of VectoBac G |
| 2010 | 21-26 June | 2,578 | |
| 2010 | 26-28 July | 683 | |
| 2011 | 24 July | 29 | Artificial flood due to reparations at Söderfors (outside Lake Färnebofjärden) |
| 2012 | 18-25 May | 3,530 | |
| 2012 | 14 June | 61 | |
| 2012 | 5 July | 214 | |
| 2012 | 19-21 July | 1,448 | Incomplete treatment due to lack of money |

**Österfärnebo village** – 0%, 43% and 97% relative treatment coverage of larval areas.

From the village of Österfärnebo, a maximum number of 20,914 floodwater mosquitoes per trap and night were recorded in August 2000 before the start of the Bti-based larviciding for floodwater mosquito control in 2002.

Mosquito control covering 43% of all potential floodwater mosquito larval production areas within a 5 km radius around the trap site, commenced in July 2002 with larval abundance reduction of 100% at all occasions. The treatment efficiency was 0.43 for the time period June 2002 to June 2009. The measured abundance of blood-seeking floodwater mosquitoes during this time period varied between 1 and 1,248 individuals per trap and night.

From July 2009 to 2012, the relative treatment coverage of larval areas increased to 97%, with respective treatment efficiency of 0.97, except for May 2010. Measured abundance after treatments with 0.97 efficiency varied between 1 and 274 floodwater mosquitoes per trap/night. In May 2010, larval abundance reduction was 90%, resulting in a treatment efficiency of 0.87, and a maximum of 1,361 blood-seeking floodwater mosquitoes per trap/night were collected.

**Fågle** – 0%, 16% and 78% relative treatment coverage of larval areas.

At Fågle, high numbers of floodwater mosquitoes per trap and night were observed before the start of Bti-based floodwater mosquito control with 10,791 individuals per trap/night in June 2000 and 25,392 individuals per trap and night in August 2000.

Between July 2002 and June 2009, with 16% relative treatment coverage of larval areas, the number of blood-seeking mosquitoes per trap/night varied between 140 (in August 2008) and 76,669 individuals (in May 2008). In May 2008, the mean number of larvae before treatments reached 302 per litre. Larval reduction was 100% at all larviciding occasions, resulting in a treatment efficiency of 0.16.

From July 2009 to 2012, the relative Bti treatment coverage within 5 km radius increased to 78% and accordingly treatment efficiency increased to 0.71 (larval reduction 91%) to 0.78 (larval reduction 100%). The maximum number of blood-seeking floodwater mosquitoes varied between 1,238 and 15,687 individuals per trap/night. At occasions with more than 10,000 floodwater mosquitoes per trap/night, larval sampling before treatments showed more than 100 larvae per litre.

**Ormpussen** – 0% and 58% relative treatment coverage of larval areas.

During 2002 to June 2009, the area within 5 km around the trap site at Ormpussen was not included in Bti-based floodwater mosquito control operations. The number of collected floodwater mosquitoes varied from 680 to 38,451 individuals per trap/night.

From July 2009 to 2012, approximately 58% of all breeding sites within 5 km around the trap site were included in mosquito control operations. Mean numbers of larvae before treatments varied between 21 and 236 per litre. Larval abundance reduction was 100% with treatment efficiency of 0.58 between July 2009 and July 2012. Adult floodwater mosquito samples contained more than 10,000 individuals per trap/night at two occasions where high numbers (more than 200) larvae per litre before treatments were reported. In July 2012, the mean number of mosquito larvae per litre before treatments was relatively low (22 larvae per litre), but almost 15,000 adult floodwater mosquitoes per trap/night were caught. However, the Bti-larviciding in this part of Lake

Färnebofjärden in July 2012 was incomplete due to lack of money. Thus, the relative coverage for that Bti-larvicide treatment was less than the assumed 58% coverage.

*Relative treatment coverage of larval areas*

Taking only data from treatments with 100% larval abundance reduction into consideration, the number of floodwater mosquitoes per trap/night was negatively related to the relative treatment coverage of larval areas with mosquito control (Pearson correlation -0.37, p<0.05). The correlation trend-line (Figure 3) showed that coverage of at least about 60% is needed to achieve less than 5,000 blood-seeking floodwater mosquitoes per trap/night, while less than 1,000 blood-seeking floodwater mosquitoes per trap/night require a relative larvicide treatment coverage of at least about 95%.

*Treatment efficiency*

Treatment efficiency and number of floodwater mosquitoes per trap/night were significantly and negatively correlated (Pearson correlation -0.33, p<0.05). The correlation trendline (Figure 4) showed that a treatment efficiency of at least about 0.75 is needed in order to reduce the abundance of blood-seeking floodwater mosquitoes per trap/night to less than 5,000 individuals. Even lower abundance of blood-seeking floodwater mosquitoes per trap/night requires mosquito control efficiency to exceed 0.95. Complete relative treatment coverage but with low larval abundance reduction can result in high abundance of blood-seeking mosquitoes (e.g. Hallsjön June 2007:18,139 floodwater mosquitoes per trap/night with a treatment efficiency of 0.8). Similarly, a 100% larval abundance reduction in treated larval areas, but low relative treatment coverage can also result in high abundance of blood-seeking mosquitoes (e.g. Fågle May 2008: 76,669 floodwater mosquitoes per trap/night with treatment efficiency of 0.16).

## Discussion

The analysis clearly showed that larviciding with Bti strongly and significantly reduced the abundance of both the larval and consequently the blood-seeking adult mosquitoes, which is in accordance with the literature (Becker & Bozhao, 1989; Becker & Margalit, 1993; Margalit *et al.*, 1995; Becker, 1997). In addition, we could also show the importance of high relative coverage of larval production areas with larviciding in order to achieve a sufficient success rate.

Larval reduction was 100% in most cases and occasionally between 90% and 99%, with a significant reduction of the mean number of larvae per litre after Bti-larviciding. Thus, very high numbers of blood-seeking floodwater mosquitoes could not be related to insufficient larval reduction by Bti. In one case, Hallsjön in June 2007, pupation and emergence of adults had already started before the treatment. Thus, reduction of remaining larvae was only 80% which indeed led to very high numbers of adult floodwater mosquitoes. This emphasizes the importance of high larval reduction (at least 90%) to get an appropriate reduction of blood-seeking mosquito numbers.

The abundance of blood-seeking floodwater mosquitoes collected per trap/night could be related to the relative treatment coverage of larval production areas. In areas with high relative treatment coverage, such as Hallsjön and Österfärnebo, the number of blood-seeking floodwater mosquitoes per trap/night significantly and strongly decreased with the implementation of Bti-treatments in comparison to untreated flood events. In these two study areas with 100% (Hallsjön) and 97% (Österfärnebo July 2009 to 2012) treatment coverage of larval areas, the number of blood-seeking floodwater mosquitoes was very low. In Österfärnebo between July 2002 and June 2009, the relative coverage of larval habitats with Bti-larviciding was 43% and the abundance of blood-seeking mosquitoes was at most 1,248 individuals per trap/night.

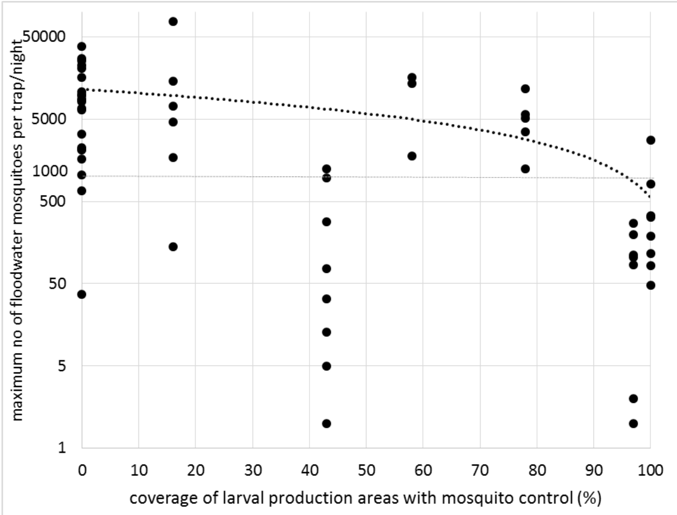


**Figure 3**. Relation of maximum number of blood-seeking floodwater mosquitoes per trap/night after flood events and the relative Bti-larviciding coverage of larval production areas with Bti-larviciding in four study areas in the lower River Dalälven region, Central Sweden, in the years 2000 to 2012.

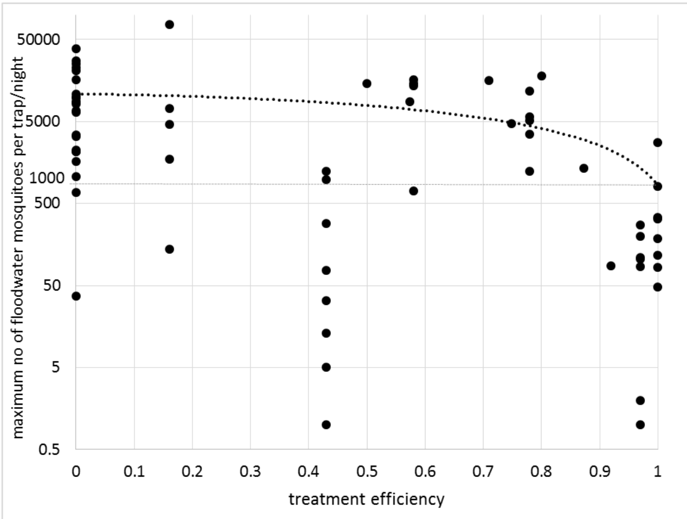


**Figure 4.** Relation of maximum number of blood-seeking floodwater mosquitoes per trap/night after flood events and mosquito control treatment efficiency (relative larval abundance reduction x relative coverage of larval production areas with larviciding) for four study areas in the lower River Dalälven region, Central Sweden, in the years 2000 to 2012.

In contrast, 78% relative treatment coverage of larval production areas in the study area Fågle between July 2009 and 2012 could still result in very high abundance of blood-seeking floodwater mosquitoes per trap/night, with up to almost 16,000 individuals per trap/ night. At these occasions, high numbers of larvae per litre were observed, indicating that this study area is extremely productive for floodwater mosquito larvae. This could also be observed in May 2008 when 302 larvae per litre were observed, and only 16% of the larval production areas within 5 km radius were included in Bti-larviciding. After the treatment, a maximum of 76,669 blood-seeking floodwater mosquitoes per trap/night were collected in this study area.

The predominant species *Ae. sticticus* is known for its long-range dispersal behaviour covering distances of at least 10 km (Brust, 1980; Bogojevic *et al.*, 2011). Thus, blood-seeking *Ae. sticticus* females could also originate from larval habitats outside the 5 km radius study area around each trap site. The extent of the dispersal is dependent on population size (Becker, 1984), thus high larval numbers and low relative treatment coverage of larval habitats could lead to the occasionally observed very high abundances of blood-seeking floodwater mosquitoes. Adult floodwater mosquitoes usually use forested areas as resting habitats but can disperse over open areas, using the forest edge for orientation (Bildlingmayer & Hem, 1981). Schäfer *et al.* (2006) also showed that floodwater mosquito species are influenced by the amount of forest cover. The River Dalälven region is characterised by forested areas with open wet meadows close to the river, and thus dispersal of floodwater mosquitoes to nearby human settlements does not seem to be restricted by any barrier.

In Germany, the Bti-larviciding is performed in similar floodplain environments as the River Dalälven floodplains and against the same species of floodwater mosquitoes, resulting in a reduction of the mosquito population (comparison of mosquitoes per trap/night in treated versus untreated areas) by >90% per year (Becker *et al.*, 2010). At the River Dalälven, similar mosquito population reduction could only be achieved for the areas with high relative treatment coverage.

Low floodwater mosquito abundance is desirable to guarantee an improvement of the situation for the human and animal residents. Thus, a treatment efficiency of at least 0.95 should be the goal to achieve consistent low abundance of blood-seeking mosquitoes (less than 1,000 floodwater mosquitoes per trap/night). Assuming measured larval abundance reduction to normally vary between 95-100%, successful floodwater mosquito control measurements require at least 95% coverage of larval production areas within 5 km. This treatment coverage is definitely not achieved for all areas in the lower Dalälven region. With respect to the long-distance dispersal ability of the predominating species *Ae. sticticus*, it is hardly a surprise that these mosquitoes disperse from surrounding untreated areas into treated areas and spoil the local mosquito reduction achieved by Bti-larviciding.

With respect to occasional very high numbers of blood-seeking floodwater mosquitoes per trap/night after Bti-larviciding, some scepticism about the successfulness of Bti-applications is understandable. Here we have shown that Bti-larviciding has had the desired effect on mosquito larval numbers with normally about 100% larval reduction, and that insufficient coverage of larval production areas with mosquito control explained the observed high numbers of blood-seeking floodwater mosquitoes. The conclusion from this analysis is that the treatment coverage of larval production areas in the lower River Dalälven region has to increase in some areas in order to guarantee consistent floodwater mosquito control efficiency and an improvement of the public health issues caused by floodwater mosquitoes to both humans and animals in the region.

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