# Effect of River Stratification on Black-Fly Population Following Application of *Bacillus Thuringiensis Israelensis* (Bti) on Domasi River in Zomba

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## Abstract

*Zomba District in southern Malawi was reported to have been infested with black-fly in 2006. By the year 2007, the Ministry of Health (MoH) had initiated a larviciding program using Bacillus thuringiensis Israelensis (Bti) to mitigate the effects of black-fly bites. The study aimed at assessing the impact of stratification on black-fly through monitoring of adult and larvae fly populations following Bit application. The Domasi River was divided into three strata and data on the population of black-fly was collected before and after Bit application. Larva monitoring was done using four different substrate types: nylon strips, rocks, debri and boards on upper and lower sections of the river. Adult monitoring was carried out by human landing catches. All samples collected were preserved in 75% alcohol as voucher specimen. It was concluded that stratification of the river did not have statistically significant effect on the impact of Bit on black-fly populations.*

**Key words:** Stratification, *Bacillus Thuringiensis Israelensis (BTI)*, Larviciding, Substrate, Black-fly, Larva monitoring, river.

## *1. Introduction*

The modern history of black-flies in Malawi dates to the 1980's when studies conducted in the Shire Highlands in Thyolo district showed availability of black-flies and its health implications of which the symptoms of the disease they cause include skin rash, eye lesions, squamous cell cancer and subcutaneous bumps under the skin. The most serious manifestation consists of lesions in the eye that can progress to blindness (River blindness) (Burnham, 1991; Morris, 2004). The skin snip studies of 23,373 people living in the area showed infection with *O.volvulus* distributed evenly in the highlands. However the infection was light as it did not exceed geometric mean of 8 microfilarias/mg of skin (Burnham, 1991). Until 2006, Thyolo District was the only known focus with autochthonous transmission of *O. volvulus* in Malawi (Courtright *et al,* 1994). This area is now receiving ivermectin to treat patients infected with *O.volvulus* to kill the young microfilaria.

To control black-fly infestation in Zomba, the Ministry of Health uses *Bacillus Thuringiensis Israelensis* (*Bti)* as the insecticide of choice to suppress the fly population and consequently protect the human population from fly bites and potential Onchocerciasis outbreak. According to Pemba (2006) Malosa area around Zomba mountain has an Onchocerciasis prevalence averaging 14% but this does not warrant mass community ivermectin administration since it is less than the 20% recommended by the African Programmer for Onchocerciasis Control (APOC) standards. Other alternatives, like the use of repellants, seem to be expensive due to the high importation cost hence making it prohibitive. In South Africa, the Onchocerciasis control programme done by South Africa National Department of Agriculture using *Bti* in larviciding has proved to be cheaper effective alternative (Rivers-Moore, 2008a).

In addition to being inexpensive, larviciding using *Bti* was considered especially useful because it poses minimal threat to non-target organisms (Palmer, 1997). The main objective of this study was to assess the effects of stratification by altitude on the impact of *Bacillus thuringiensis israelensis (Bit)* on black-fly population on Domasi River on Zomba Mountain in southern Malawi by monitoring the impact of *Bti* on the three strata of the river. The Domasi River was selected because it has the highest risk of Onchocerciasis vector sources warranting black-fly control as shown by the Rapid Epidemiology Assessment (REA) that was carried out in 2006. Stratification by altitude was selected because Black fly breeding occurs at higher altitudes with fast flowing waters (Palmer, 1997).

## 2. *Methods*

### 2.1 Study area

The study was done in Domasi part of Zomba District located in southern part of Malawi at $15^0$ $23^{'}$ $0^{''}$ South and $35^0$ $20^{'}$ $0^{''}$ East. Zomba has a land area of 2,580 $km^2$, comprising 3% of the total land area of Malawi. According to the 2008 Population and Housing Census (NSO, 2008), the District has a total population of 591,903 representing about 5% of the national population. The population density is estimated at more than 209 persons per square kilometer but the density is higher in the urban area (NSO, 2008). Areas affected by black-flies are those around Zomba mountain-slopes. Specific locations include: Likangala, Katsonga, Matengula, Malemia, Chingamuzi, Nawimbi forest tower area, Zomba Mental Hospital, St Mary's, Three miles, Zomba RTC, Mtiya, Mangasanja, Zilindo, Zomba Plateau and Old Naisi (Pemba and Alezuyo, 2006). Unpublished reports show that the affected areas have substantially expanded more than those previously considered. More new cases of bites have been reported in areas like Chancellor College and other areas not previously mentioned (Mkumpha, 2007). From this, it seems that the entire population of Zomba municipality and surrounding areas is at risk of black-fly bites. The general topography varies from mountainous hilly regions to broad flat plains in the Upper Shire Valley and Lake Chilwa to the east. The diverse topographical characteristics result in local climatic variations which in turn result into different ecological diversity. The river flows over areas of natural vegetation of trees and grasses on the upper part through to areas under cultivation on the lower section with open fields and less natural vegetation. Rivers and streams that arise from springs in the upper portion of Zomba plateau are relatively short and swift, and flow over stones and boulders, within short distances before joining other tributaries and later Lake Chilwa. These features are characteristic breeding places for black-flies (Pemba and Alezuyo, 2006). The study area is shown on Figure 1.

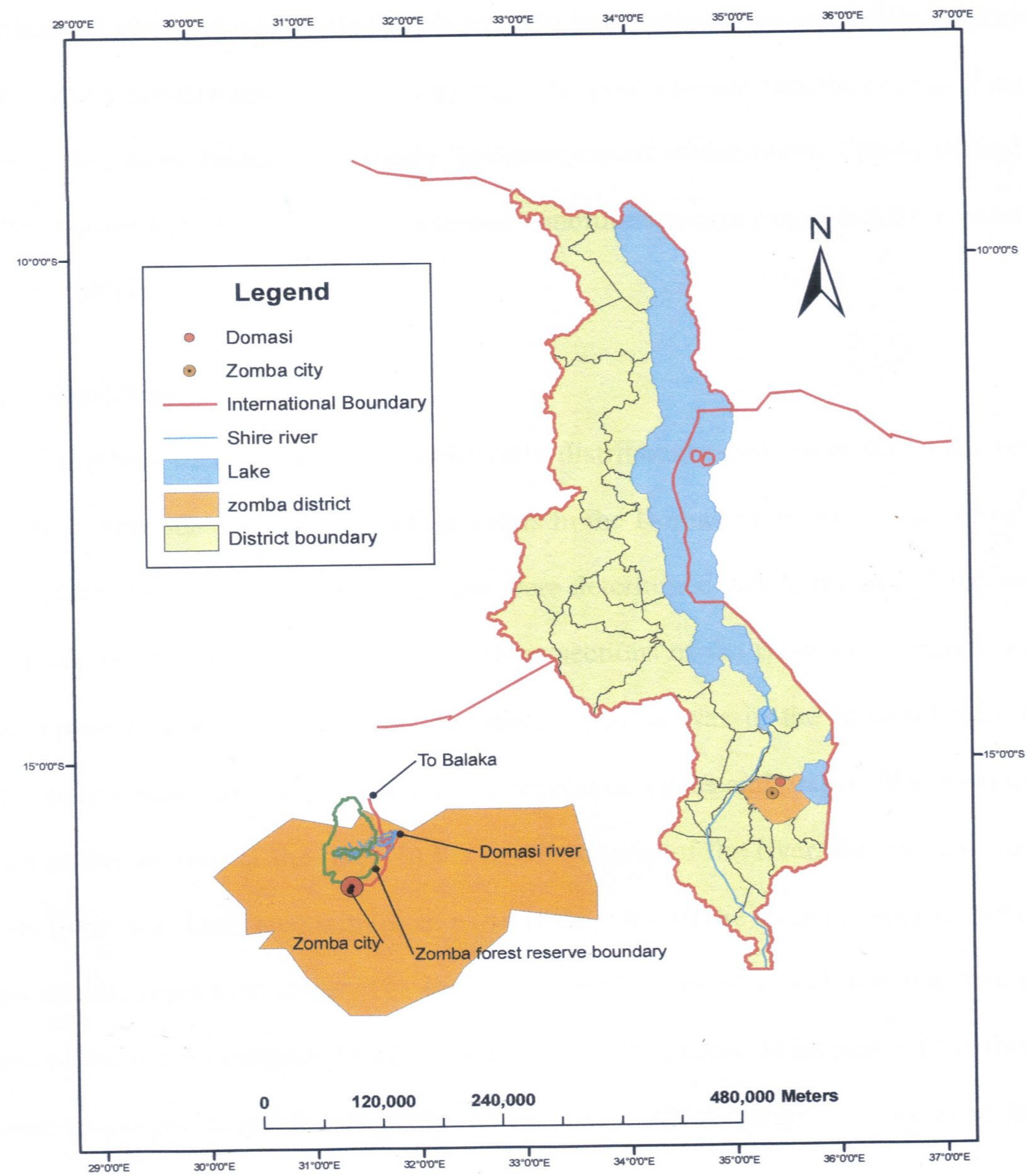


**Figure 1: Map of Malawi showing the location of Zomba District in Southern Malawi**

## 2.2 Sampling

The current study was done on the middle stretch of Domasi River which has reportedly been known to contain larva breeding sites (Pemba, 2006). The selected stretch of the river was stratified by altitude of the breeding sites and then arbitrarily divided into three strata: upper (900 - 1000 m above sea level), middle (800 – 900 m above sea level) and lower (700 – 800 m above sea level) because of the differences in the flow of the river. The upper stratum had steep slopes and high water velocity. The middle stratum had a gentle slope and relatively lowers water velocity. The lower stratum had the least in terms of steepness and water velocity. On this stretch seven breeding sites were identified to determine the impact of *Bti* on larvae population density. On each of the sampled breeding sites three samples were taken using purposive sampling.

This was done to ensure that the monitoring covered the entire sampled stretch of the river having breeding sites taking into account the inherent environmental conditions that affect species density and composition. Water and air temperature, and flow rate of the river were recorded at sampling sites because they both affect larva occurrence and *Bti* performance (Figueiró *et al.,* 2006). Adults were captured on the same strata where larva monitoring was done. This was done because adult black-fly tends to concentrate close to their breeding sites (Bukaciski and Bukacinska, 2000).

### 2.3 Larvae monitoring

Data on black-fly larvae were collected before *Bti* application in July 2008 to provide baseline data. Thereafter data were collected weekly for the entire study period of four months (August to November 2008). This is a period when black fly populations grow and becomes a nuisance to the community in this area. A total of 21 samples were collected on each monitoring visit. 20 monitoring visits were made on each site and a total of 420 samples were collected for analysis. Four substrates were used to monitor larva in this study: rocks, debris, strings and plastic boards and mean larva concentration was calculated to increase objectivity of the results. Choice of these substrates was partly determined by the nature of the study site, duration of the study and their cost. The substrates were used to obtain quantitative information on the population of black-fly larva. At every sampling point all 4 substrate types were deployed to sample larva. The colonized substrates were scored on a semi-logarithmically defined abundance scale (index classes) of 2-10 representing larva density according to the method of Palmer (1994) on larva of 2-3mm long. Data collected before and after *Bti* application was also used to determine changes and establish trends in the larva density.

### 2.4 Adult monitoring

Human landing catches was conducted to determine adult black-fly population. The flies were captured using a pooter. A trend in the population changes after larviciding was also determined. Data was collected on each of the three strata within a radius of 1km from the river. A radius of 1km was used because black-flies tend to concentrate close to the breeding sites (Hutchinson, 2008; Bukaciski and Bukacinska, 2000). A total of three sites were used in the adult monitoring and the number of adult flies caught was recorded and the flies were kept as voucher specimen. The sites were purposively chosen in relation to the breeding sites and were unchanged throughout the study period**.** A total of 20 monitoring visits were made and 60 samples were collected.

### 2.5 Data management and analysis

Data analysis included descriptive summaries of adult numbers and larva mean density, t-tests, regression and Analysis of Variance (ANOVA) of larva density against adult numbers. All the statistical analysis were done at 5% significance level. Data collected in the study area after larviciding was compared to the baseline data to determine the impact of *Bti*. Data collected after larviciding was analyzed to determine if there were any changes in population trend.

## *3. Results And Discussion*

### 3.1 Descriptive summaries

The average air temperature before and after application was 20ºC and 26ºC respectively. There was also an increase in water temperature from an average of 16ºC before application to 20ºC after the application. The increase in temperature was attributed to progression of the season. Before *Bti* application the mean Larva density on the semi-logarithmically defined abundance scale (number per $16cm^2$) was 6.89 and it dropped to 4.08 after application showing a significant decrease (P= <0.001). The adult flies captured had a mean of 19.6 (±6.8) per hour before *Bti* application and dropped to 1.8 (±3.8) after application. This indicates a significant decrease in the mean adult numbers captured after *Bti* application (p<0.001). The summaries throughout the study show a decrease in the larva density. Larvae were abundant before *Bti* application and significantly dropped after application with the lowest densities.

### 3.2 Impact of stratification on black-fly population

#### 3.2.1 Larva density on different strata

ANOVA on density across the stratum showed no statistically significant difference (p=0.987) and there was no significant interaction between time of *Bti* application and strata (p=0.834). However, there was statistically significant variations in density before and after *Bti* application (p<0.001) (Figure 2).

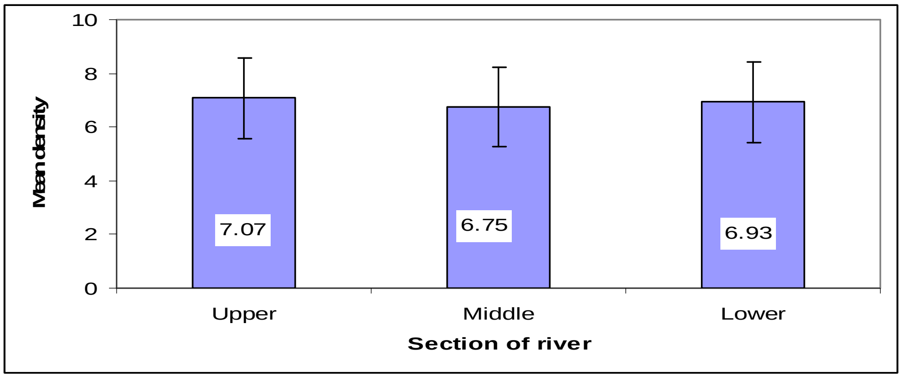


**Figure 2:** Mean larva density on strata before application

There were almost no differences in the mean Larva density across the strata before *Bti* application (Figure 3).

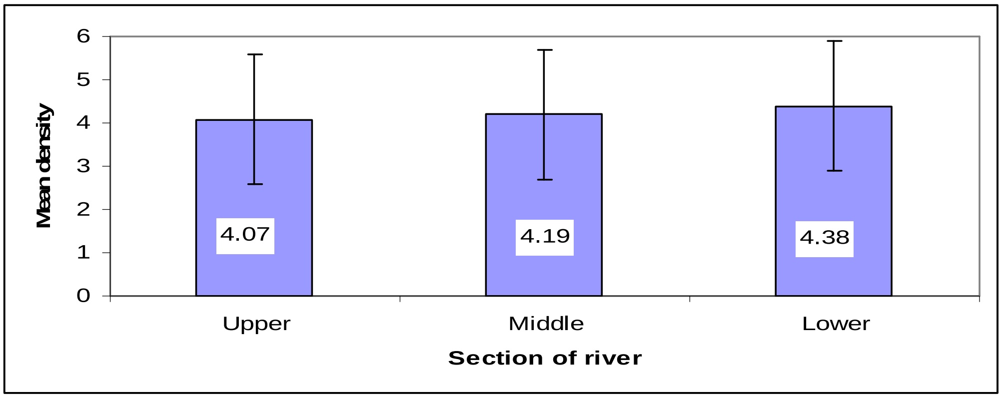


**Figure 3:** Mean larva density after *Bti* application

On the other hand there were little differences in the mean densities across the strata after *Bti* application. The densities were slightly higher in the lower stratum than middle and upper strata.

### 3.2.2 Adult numbers captured on different strata

ANOVA on adult numbers captured across the strata showed no statistically significant difference both before and after *Bti* application (p=0.670 and 0.414 respectively). However, after the application there were statistically significant differences in the numbers captured within each of the strata ($p<0.001$) (Figure 4).

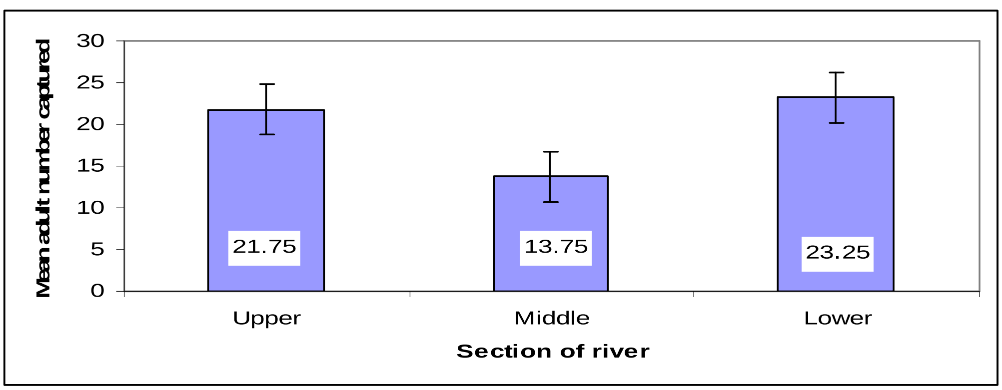


**Figure 4:** Mean adult numbers captured before application

Before application more adults were captured on the lower stratum followed by upper stratum. The middle stratum had the least number of adults captured (Figure 5).

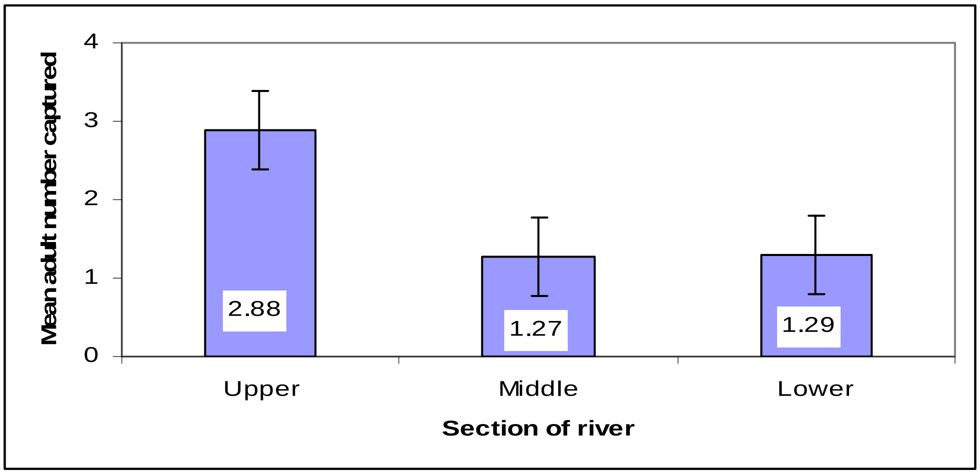


**Figure 5:** Adult numbers captured after application

After application of *Bti* despite the drop in adult numbers, there were more adults captured on the upper stratum than the middle and the lower. There were little differences in the numbers of adults captured on the middle and lower stratum.

### 3.3 Impact on stratification on black-fly population

The results show a decrease of adult numbers captured from being high before application and low after application. It was also observed that *Bti* application affected the adult numbers captured on the lower and middle strata more than the upper stratum. This suggests that when populations are lower in the environment adult flies are more concentrated on the upper part of the river. However, the study did not determine the cause for this. As such it can only be speculated that the upper stratum was more environmentally friendly attributed to temperature, shade, water quality favoring fly survival and migration. The results also show a decrease of Larva density from being high before application and dropping down after application. However, the lower stratum had slightly higher densities than the rest after application. The lower densities on upper and middle strata of the river were due to the fact that *Bti* application was done on these two strata and was killing the larvae decreasing their abundance. This observation may also mean that *Bti* has no carry over or downwash effect.

The *Bti* applied in the upper and middle strata of the river had little effect on the lower strata where no application was done. This could be due to high dilution factor on *Bti* and also settling and adherence of *Bti* particles as they move down the stream. This observation agrees with Palmer (1995) working on a larvicide trial in Thyolo, Malawi who found that live larvae were scarce one kilometer downstream from the point of application. However, 4 km downstream no larva mortality was observed. For effective treatment the whole stretch of the river has to be treated and all the tributaries included to avoid the situation where larvae would drift (Hutchinson 2008) from other areas into the control area resulting in higher densities. However, the small differences in densities on the strata suggest minimal effect of drifting. Overall ANOVA on density across the stratum showed no statistically significant difference ($p=0.987$). The limitation of the study included the fact that it did not take a long time to include seasonal variation in the fly population. Also the study did not use a wide range of altitude in the stratification due to the nature of the river.

## *4. Conclusion*

Stratification of the river did not have statistically significant effect on the impact of *Bti* on black-fly larvae populations. Larva densities were slightly high on the lower stratum of the river as compared to middle and upper strata after *Bti* application. *Bti* was found to have no carry over effect downstream. The application on the upper sections of the river did not significantly affect larva densities on the lower section. On the other hand, more adult numbers were captured on upper stratum after *Bti* application than on the lower and middle strata but the difference was not statistically significant. This mean that larviciding programs using *Bti* should be done across all sections of the river that contain breeding sites as stratification has no significant effect on the impact of *Bti* on blackfly population especially the larva.

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