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Treatment of *Simulium posticatum* Larvae with TEKNAR HP-D (*Bti*) at selected sites on the River Stour, Dorset, 2006

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1. CONCLUSION

1. In 2006 **TEKNAR HP-D (*Bti*) was NOT found to be an effective simuliicide** when used against the larvae of *Simulium posticatum* under the conditions prevailing in the River Stour. This was probably due to the product being stored for over 12 months prior to use. It is essential that fresh TEKNAR HP-D is used in future treatments.

2. The reconnaissance survey was completed in March. As a result, the treatment was delayed until mid-April due to cold conditions resulting in slow larval growth and late hatching of *S. posticatum*.

3. Larval monitoring and treatment with TEKNAR HP-D was carried out in the period 11-13 April under almost ideal conditions.

4. Statistical analysis of samples of larvae, taken before and after application of TEKNAR HP-D, showed that **the mean mortality was 49% at Blandford and 54% at Longham.**

5. Retreatment at selected sites with fresh TEKNAR HP-D showed increased mortality of larvae and, perhaps more importantly, suggested that the application of 'fresh' TEKNAR HP-D was more effective than that of older material.

6. The value of the preliminary reconnaissance survey has been confirmed. Without it, treatment could have been too early, overwintering generations of non-target species would have been killed and late-hatching individuals of *S. posticatum* would have escaped treatment.

7. The pre and post treatment monitoring has also proved its worth. Without this, the apparent weakened impact of the use of one year old TEKNAR HP-D would not have been detected, mortality in future years may have been markedly reduced and the possibility of the appearance of flies resistant to treatment would have been increased.

2. INTRODUCTION

In 1993, the Health and Safety Executive (HSE) gave permission to treat the River Stour with *Bti*, where necessary, along the whole length of the river. Previously, restrictions to the areas treated were imposed by the HSE but following the successful experimental treatments in 1989, 1991 and 1992, clearance to treat for an experimental period of 4 years (subsequently extended) was given. In each of the years 1993-2004, successful treatments were carried out and samples were taken from sites at Blandford and Longham. Previous trials had shown no adverse effects on any fauna in the river apart from the target species *Simulium posticatum* (The Blandford Fly).

Treatment sites are no longer limited to a maximum of eight and the restriction of no spraying within 7 Km of the intake of Bournemouth Water Co at Longham has been lifted. Despite the lifting of this latter constraint no sites between Canford School and Longham were treated due to the unsuitable nature of the river as a larval habitat. No sites were treated downstream of Longham due to Bournemouth Council's decision not to fund control of *S. posticatum*. However, this year, CEH undertook to survey suitable sites in this area to ascertain whether this lower river was acting as a source of *S. posticatum.*

The conduct of the present treatment took into account the "Guidelines for Biological Monitoring" put forward by the Pesticides Registration Section, 28 February 1990.

3. RECONNAISSANCE SURVEY

3.1 Introduction

This survey is designed to monitor the status of over-wintering populations of simuliids and to provide an assessment of the abundance of *S. posticatum* larvae in order to determine the optimal time for treatment of the pest species. This is normally after the pupation and emergence of the overwintering larvae of other species and after the hatching of all of the eggs of *S. posticatum* but before pupation of that species.

In order to identify the above phase in the life cycle of the Blandford Fly, it is necessary to recognise first and last instar larvae. The presence of first instar larvae would indicate that individuals were still hatching and that recruitment was continuing. Treatment at this stage would not affect the entire population. The presence of last instar larvae, in contrast, would indicate that pupation and emergence were imminent. Treatment must then be applied as soon as possible.

In practice, the reconnaissance survey is timed so as to determine whether all larvae have hatched (as indicated by a lack of first instar larvae). The size and developmental stage of the larvae present then indicates the appropriate time for treatment.

In 2006, a reconnaissance survey of sites was carried out in order to establish that the larvae of *Simulium posticatum* were distributed in the usual manner and were at an appropriate state of development for treatment. A check was also made to establish whether the overwintering species had mostly pupated and emerged as adult flies. Samples were taken from 9 sites known to be suitable for simuliid larvae between Blandford and Longham. In addition, samples were taken from Muscliffe and Throop, sites downstream of Longham to assess the infestation level in areas controlled by Bournemouth Council.

3.2 Methods

A quasi-quantitative sample of weed was taken by hand from fast flowing water at each site. In the laboratory, the weed was thoroughly washed once in tap water and the water then poured through a 125 µm sieve before examination under stereo microscope. Simuliid larvae were identified and the numbers of *S. posticatum* larvae were recorded separately from the numbers of other larvae. First and last instars were also noted. Assessment of population density of larvae was made as High, Medium, Low or None. Approximate size/ developmental state of larvae was judged by eye. Weed cover was assessed in terms of proportion of river bed covered at each site.

3.3 Results

3.3.1 2006 Reconnaissance Survey of River Stour for *S. posticatum* larvae

Eleven sites were visited and samples of weed were collected on 8 March.

At the time of sampling the river was clear with very low flows for the time of year.

Blandford - Main River

50% weed cover, same as last year.

Simulium posticatum – low density (<10/sample) - all small, second-third instar.

Small number of medium and large larvae of other species – *S. ornatum*, 2; *S. lineatum /equinum* >10/sample.

One pupa of *S. lineatum* was found.

Blandford Carrier

30% weed cover, much higher than last year.

Simulium posticatum - high density (>100/sample) - mainly small, second-third instar.

High density of other species - *S. erythrocephalum*, >100/sample , *S. ornatum*, >10/sample.

Pupae; *S. ornatum*, 1, *S.lineatum*, 6.

Charlton Marshall

80% weed cover, similar to last year

Simulium posticatum – high density (>100/sample), second-third instar.

Medium densities of large sized larvae of other species – *S. erythrocephalum*, >50/sample; *S. lineatum/equinum*, >10/sample, *S.ornatum* >10/sample.

No pupae were found.

Clapcott's Farm (upstream Spetisbury)

5% weed cover, same as last year.

Simulium posticatum – medium density (>50/sample), second-third instar.

S. erythrocephalum, >100/sample; *S. lineatum/equinum*, >100/sample, large larvae.

Pupae; *S.lineatum* 20/sample.

Spetisbury Bridge

60% weed cover, same as last year.

Simulium posticatum – medium density (50/sample) - all small, second instar.

High numbers of large and medium sized larvae of *S. erythrocephalum*,>100/sample.

Pupae; *S. lineatum/equinum*, 8, *S. erythrocephalum* 3/sample.

White Mill Bridge

5-10% weed cover, much lower than last year.

Simulium posticatum – very low density (5/sample) - all small, second instar.

High numbers of large and medium larvae of other species – *S. erythrocephalum*, >100/sample; *S. lineatum/equinum*, >10/sample.

Pupae; *S. lineatumequinum*, 5/sample.

Julian's Bridge Wimborne

5-10% weed cover, lower than last year.

Simulium posticatum – none found.

Medium numbers of large larvae of other species– *S. lineatum/equinum*, >10/sample; *S. erythrocephalum*, >50/sample, *S, ornatum* >10/sample.

Pupae; *S. lineatum/equinum*, 5/sample; *S. ornatum*, 3/sample.

Canford School

80% weed cover, higher than last year.

Simulium posticatum – medium density (>50/sample), all very small, first-second instar.

Very high numbers of large larvae of other species– *S. lineatum/equinum*, >10/sample; *S. erythrocephalum*, >100/sample.

Pupae; *S. lineatum/equinum*, 7.

Longham control site

0% weed cover.

Longham Treatment Site

5% weed cover, similar to last year.

Simulium posticatum – low density (<10/sample) all second-third instar.

Medium numbers of large and medium larvae of other species – *S. lineatum/equinum*; <10/sample; *S. erythrocephalum*, >50/sample.

Pupae; *S. lineatum/equinum*, 2/sample, *S. erythrocephalum*, 5/sample.

Muscliffe

40% weed cover

Simulium posticatum – low density (<10/sample) all second-third instar.

Medium numbers of large and medium larvae of other species – *S. lineatum/equinum*; >10/sample; *S. erythrocephalum*, >50/sample.

Pupae; *S. lineatum/equinum*, 6/sample.

Throop

40% weed cover

Simulium posticatum – low density (<10/sample) all second-third instar.

High numbers of large larvae of *S. erythrocephalum*, >100/sample.

Pupae; *S. erythrocephalum*, 12/sample.

In general, weed cover was similar to last year at many sites, again high probably due to the very low winter flows and consequently little washout of the weed beds. Higher weed cover was seen at Blandford in the carrier and at Canford School. In contrast, lower percentage cover was apparent at White Mill Bridge and at Julian's Bridge, Wimborne. In contrast to last year, there were more Blandford Fly larvae at the upper sites with very high densities at Blandford in the carrier and at Charlton Marshall with generally medium to low densities elsewhere in the main river with good weed cover. Very low densities were recorded at White Mill Bridge (between Spetisbury and Corfe Mullen) and no larvae were found at Julian's Bridge, Wimborne.

Two sites were surveyed from the lower river downstream of Longham in Bournemouth Council's area. Although weed coverage was greater here than some of the upstream sites, densities of *S. posticatum* were low. This suggests that in 2006 there will not be an excessive reservoir of untreated larvae in the river, although this is likely to vary year on year.

Individuals were small to medium in size and first instar larvae were found which indicated that hatching was incomplete. This is probably due to the relative lack of winter flooding preventing larvae from entering the river from the oviposition sites high in the river banks. There were no last instars of *S. posticatum* but there were high numbers of other species. Treatment was thus not considered to be urgent, and with the low temperatures, larval growth was expected to be slow and treatment was delayed until the majority of the overwintering larvae were expected to have emerged. It was further hoped that there would be a flood before treatment allowing any remaining larvae into the river. It was anticipated that the last of the overwintering larvae would have hatched by mid April and that *S. posticatum* larvae would be a suitable size for treatment at this time. Monitoring and treatment was planned for the 11-13 April.

4. DISCHARGE AND VELOCITIES

Discharge values are required for calculation of TEKNAR HP-D (*Bti*) dilution factors.

4.1 Methods

The South West region of the Environment Agency are unable to provide discharge values at the prescribed sampling/application points as there are only two continuous gauging stations on the Stour, one at Hammoon (NGR ST 820 147) a considerable distance upstream of Blandford and a second at Throop (SZ 112 958) - potentially the furthest downstream site for treatment. The Agency were, however, extremely helpful having, in previous years, supplied maps and graphs which established that**,** with care, approximate interpolation between gauging stations is reasonable. With the experience gained, interpolation of the results of the Environment Agency gauging stations is now used, as routine, to calculate quantities of TEKNAR HP-D to be added at each site.

4.2 Results

The discharge of the River Stour at Hammoon and Throop is given in Figure 1.

The planned date of treatment coincided with low flow conditions in the river and flows were similar to the previous year. Treatment followed a period of high flows when the last of the eggs in the river bank should have hatched and larvae would have had access to the river.

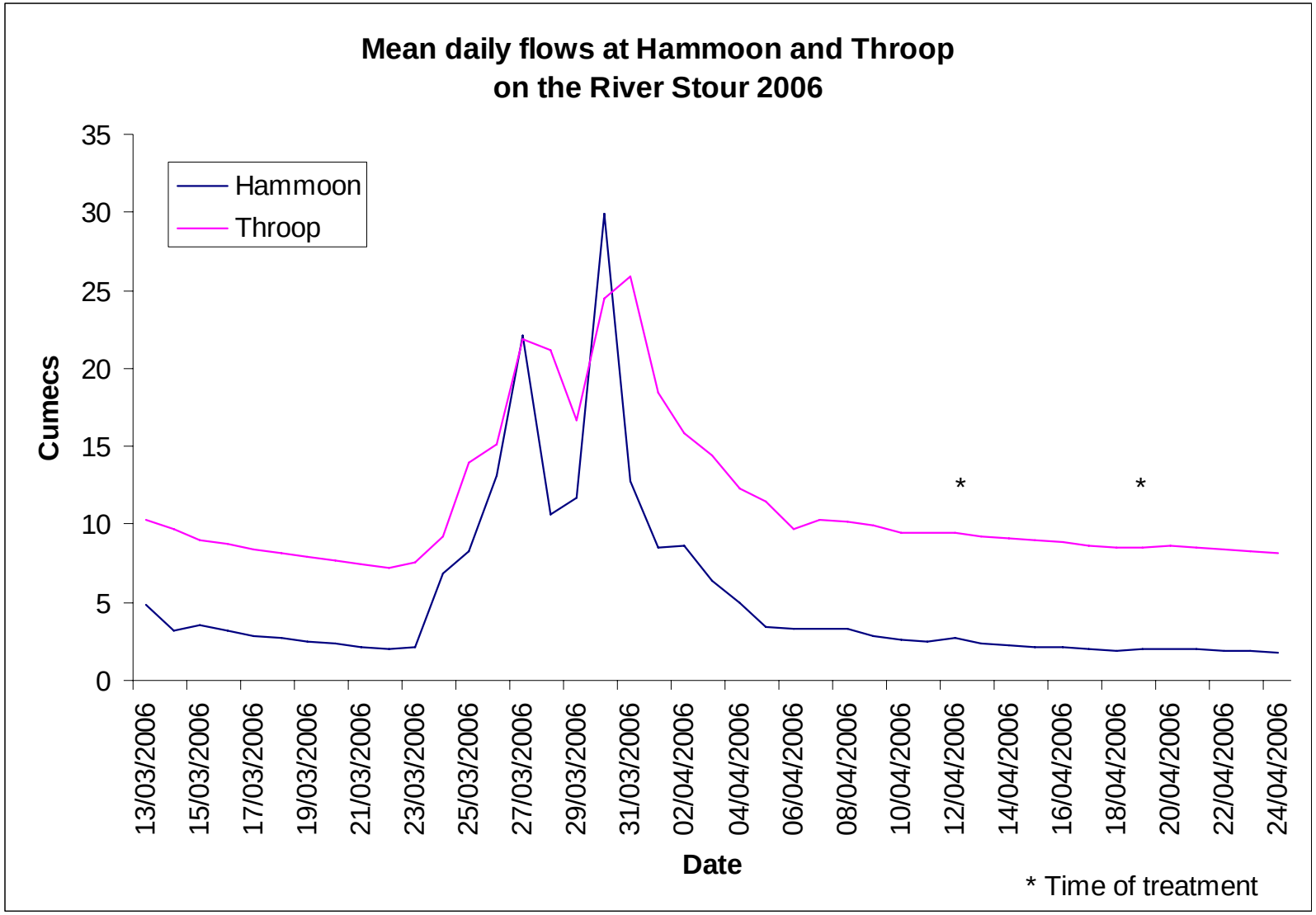

Figure 1 Mean daily discharge (cumecs) at Hammoon and Throop on the River Stour

5. *Bti* APPLICATION

Permission to treat was sought from the Environment Agency (by NDDC) and Bournemouth Water Company was also notified by NDDC of the impending treatment.

5.1 Methods and Quantities

The flows at Hammoon and Throop on 12 April would have required TEKNAR HP-D loadings of 1.3 l and 4.7 l respectively to achieve the desired concentration of 0.8 ppm. As a consequence, treatment levels ranged from a minimum of 3 l of TEKNAR HP-D at Blandford to a maximum of 5 l of TEKNAR HP-D at Longham.

Mixing was achieved by introducing the required quantity of TEKNAR HP-D to the river by knap sack sprayer at Blandford, Spetisbury and Longham, a turbulent sluice or weir (Clapcott's Farm, middle channel upstream of Spetisbury, Corfe Mullen, Wimborne and Canford School) or upstream of a natural riffle (Charlton Marshall, Clapcott's Farm, west channel, Shapwick and White Mill Bridge) or was dispensed by throwing small quantities of diluted material from the bank (Langton Long) over a 10 minute period.

The Control site and carrier at Blandford were subsequently treated on 13 April, after post-treatment samples had been taken. The control site at Longham had to be moved to immediately downstream of the bridge due to the lack of weed at the usual site, as was the case in 2005.

In order to assess the effectiveness of the treatment, samples should not be taken within 24 hours of treatment. However, as larvae are very mobile and move position relative to flows, post-treatment samples should not be unduly delayed otherwise differences would be more likely to occur in the controls. Treatment occurred between 1030 hours and 1400 hours on 12 April (with monitoring sites treated first) and post-treatment samples were taken at 1130 hours at Longham and 1230 hours at Blandford.

The quantities of TEKNAR HP-D required, in litres, to achieve concentrations of 0.8 ppm over 10 minutes was calculated from the manufacturer's formula:

Volume (litres) = 0.48*Flow (cumec) and was as follows:

- Blandford main river	3.0 l
- Langton Long	3.0 l
- Charlton Marshall	3.0 l
- Clapcott's Farm	
Middle channel	3.0 l
West channel	1.0 l
- Spetisbury	4.0 l
- Shapwick	4.0 l
- White Mill bridge	4.0 l
- Corfe Mullen	4.0 l
- Wimborne (weir above Julians Bridge)	5.0 l
- Canford School main river	5.0 l
- Longham	5.0 l
- Blandford carrier and control	3.0 l

A total of 47 litres of TEKNAR HP-D was added to the river on 12 and 13 April 2006.

6. MONITORING THE EFFECTS OF TEKNAR HP-D (*Bti*) ON *Simulium posticatum*

6.1 Methods

The standard sites at Blandford (NGR ST 886 062) and Longham (NGR SZ 065 973) were again chosen for the pre- and post-treatment samples, as they are known to have held large numbers of larvae in previous years and are near two of the main residential areas affected by the fly. In addition, the site information is now building into a long term data base of treatment effects. The reconnaissance survey in March indicated low densities of larvae on the weed at both Blandford and Longham which potentially causes problems in assessing confidence of the results.

Thirty weed samples were taken from each of the control and treatment sites at both Blandford and Longham on pre-treatment day (11 April 2006). Sampling was repeated on 13 April 2006, the day after treatment allowing a minimum of 24 hours following the introduction of TEKNAR HP-D.

Samples were transported to the laboratory and the number of living larvae on each piece of weed was counted. Numbers of dead larvae were also counted. Weed samples were weighed after blotting dry with tissue. The method used was identical to that in previous years.

Statistical procedures are detailed in the appropriate results section.

6.2 Results

6.2.1 Dead larvae

No dead larvae were found which had adopted the characteristic ‘stretched’ appearance which we have come to associate with death following ingestion of *Bti* although dead larvae were recorded at both Blandford and Longham, following treatment. This was a major change to the results normally found and indicated that the treatment had not worked as effectively as in all previous years. Initial thoughts were that the TEKNAR HP-D had not killed larvae quickly and effectively. More attention was paid to this assumption following the analysis of the results.

6.2.2 Density of living larvae

In contrast to the previous year, the density of larvae in the upper river was generally medium to high and in the lower river it was low. Although density depends on weed cover, this year’s weed cover was very similar to that in 2006 and observed densities were considered to be a true reflection of numbers in the river. The absence of high densities may be due to the very low winter flow conditions and the lack of floods which allow access of larvae to the river. This is borne out by the presence of first instar larvae during the survey at a time when hatching should have been completed.

The percentage change in larval density was calculated for each of the four sites (Table 1). This shows that the *Bti* treatment was not particularly effective **with decreases in density of 47% at Blandford and 71% at Longham.** However, these results do not take into account

any changes that occurred at the control sites. When these additional data are incorporated into the calculations, the true kill rates observed were 49% at Blandford and 54% at Longham.

Table 1 Mean density of *S. posticatum* larvae (numbers per gram of weed) at control and treatment sites and percentage change following application of TEKNAR HP-D

	Blandford		Longham	
	Control	Treatment	Control	Treatment
11 April 2006	18.9	38.6	24.4	15.8
13 April 2006	17.1	20.4	10.3	4.6
% change	-10	-47	-58	-71

Again, there was a large decrease in density of larvae at the Longham control site calculated from pre- and post-treatment samples. Ideally, control samples should remain stable, the discrepancy was again probably due to carry of TEKNAR HP-D from sites upstream.

The changes in density following treatment were tested statistically to see if they were significant. Initially, a two-sample t test was used to compare means.

6.2.3 Two-sample t test

The t value tests for significance of the difference between two means. Samples are assumed to be independent and to come from normal distributions. As this is not the case the data requires log transformation. Results show statistically significant decreases in density at both Longham and Blandford treatment sites (Table 2). The lack of significance in the decrease in density at the Longham control site (as has been apparent previous years) again adds weight to the concern over reduced efficacy of the TEKNAR HP-D.

Table 2 Results and significance of two-sample t tests between pre- and post-treatment log (x+1) densities of *S. posticatum* larvae
NS = not significant, *** = highly significant

	Pre-treatment	Post-treatment	t	p	significance
Blandford control	1.15	0.99	1.24	0.22	NS
Blandford treatment	1.52	1.07	3.95	<0.001	***
Longham control	0.99	0.79	1.30	0.10	NS
Longham treatment	0.96	0.42	3.80	<0.001	***

6.2.4 Odds ratio method

The advantage of this method is that it gives error estimates to the percentage kill. It works on the premise that the ratio of the larval density before and after treatment should be the same at the control and treatment sites if there is no effect of the TEKNAR HP-D, thus q, the 'odds ratio' coefficient, is determined as follows;

$$q = R_T/R_C = 1 \quad \text{where } R_T = x_{ta}/x_{tb} \text{ and } R_C = x_{ca}/x_{cb}$$

x_{ca} = mean density in the control site after treatment

x_{cb} = mean density in the control site before treatment

x_{ta} = mean density in the treatment site after treatment

x_{tb} = mean density in the treatment site before treatment

The data is log transformed as it is not normally distributed and the logarithm of x+1 is taken (where x is the density) owing to the presence of zero counts in some samples, giving $y = \log_{10}(x+1)$. The ratio now becomes the difference between before and after, D, (because we are dealing with logs), simply

$$D_C = y_{ca} - y_{cb} \text{ for the control sites}$$

and $D_T = y_{ta} - y_{tb}$ for the treatment sites.

If no treatment effect exists then, on average, $D_C = D_T$ or

$$Q = D_T - D_C = 0$$

Mathematically, $D_C = \log_{10} R_C$, $D_T = \log_{10} R_T$ and $Q = \log_{10} q$
so testing Q = 0 is equivalent to testing q = 1.

In practice the two tests are not the same since y_{cb} does not equal $\log_{10} x_{cb}$, etc., because they are geometric means. However, the test of Q = 0 is preferable because it is effectively a test of differences rather than ratios, the latter being difficult to analyse.

$$Q = (y_{ta} - y_{tb}) - (y_{ca} - y_{cb})$$

and the standard error of Q is given by

$$SE(Q) = \sqrt{(SE_{TA}^2 + SE_{TB}^2 + SE_{CA}^2 + SE_{CB}^2)}$$

The test of Q = 0 is

$$t = Q/SE(Q) \text{ with 116 degrees of freedom (n-1 for each of the four sites)}$$

If densities have changed at the control site from before to after, then the best estimate of the proportion of pre-treatment density left after application of *Bti* at the treatment site is

$$q = R_T/R_C$$

which is estimated by q_1 to q_2, where

$$(q_1,q_2) = 10^{(Q \pm tSE(Q))} = \text{antilog } (Q \pm t\ SE(Q))$$

Values calculated from these equations are shown in Table 3

Table 3 Results of odds ratio method for assessing the effectiveness of the treatment with TEKNAR HP-D

	Q	Test of Q=0 t	Significance	Proportion of pretreatment density remaining (antilog Q)	% Kill	95% CL	
						Lower	Upper
Blandford	-0.29	-1.48	NS	0.51	48.9	0	79.3
Longham	-0.33	-1.58	NS	0.46	53.7	0	82.5

Thus the mean kill of *S. posticatum* larvae at Blandford was 49% and at Longham was 54% but these kills are not considered to be statistically significant.

7. DISCUSSION

The lack of dead larvae with the characteristic ‘stretched’ appearance along with the poor percentage kill and lack of statistical significance in the results at both Blandford and Longham led to the conclusion that the TEKNAR HP-D was not as effective as in previous years. Conditions for treatment were as near perfect as could be hoped for. For the first time this year, the TEKNAR HP-D had been stored for over 12 months before use. This was as a consequence of the late delivery in 2004 and the consequent delay in treatment that year. This led to NDDC ordering the product well in advance presumably after consultation with the manufacturer. However, it is clear to us that the product has deteriorated and this presents two problems. Firstly, that the kill is not as high as it could be with the consequent result of more biting flies emerging. Secondly, and more importantly, there is a real risk that many larvae were subjected to a sub-lethal dose of TEKNAR HP-D which could result in a resistant strain appearing if this practice is continued.

CEH advised NDDC of the results and suggested using product already in store for the 2007 treatment for retreatment of areas of the upper river where medium/high population densities of larvae coincided with conurbations.

8. RETREATMENT

Sites chosen for re-treatment were Blandford, Charlton Marshall, Spetisbury and Wimborne. On 19 April, these sites were treated with similar quantities of TEKNAR HP-D as before. Although a full scale monitoring of this treatment was not planned, samples were taken at Charlton Marshall and Spetisbury the following day. Results showed dead larvae with the characteristic 'stretched' appearance at both sites (Table 4).

Table 4 Results of retreatment at Charlton Marshall and Spetisbury

Charlton Marshall	21 dead	6 alive	77.8% kill
Spetisbury	33 dead	4 alive	89.2% kill

Although only one sample was taken at each site, the indications are that the percentage kill was high at both sites. It is clear that the new batch of TEKNAR HP-D worked well confirming the conclusion that product kept for more than 12 months may deteriorate and should not be used on a regular basis.

9. SUMMARY

Conditions for treatment of the river Stour in 2006 were ideal having recovered from a major flood two weeks previously. Flows were low and were stable over the treatment period. As usual, a conservative approach to TEKNAR HP-D application was adopted. On 12 and 13 April 2006, 13 sites between Blandford and Longham, including the control site and carrier at Blandford, were treated.

The reconnaissance survey in early March showed that *S. posticatum* larvae were generally very small in size and the presence of first instars showed that hatching was not complete. Due to cold conditions and consequent slow growth of larvae, a decision was made to treat the river in mid-April when the high numbers of large overwintering larvae were expected to have pupated or emerged.

Reductions in density were observed at both Blandford and Longham treatment sites and both were statistically significant. The mean percentage kill achieved at **Blandford** was **49%**. At **Longham** the kill was **54%**. This poor kill together with the lack of dead larvae in the characteristic 'stretched' posture suggested that the efficacy of the TEKNAR HP-D which had been stored for more than 12 months had decreased. This has potentially serious consequences in that larvae had almost certainly been subjected to sub-lethal doses which if continued annually would possibly produce a resistant strain of flies.

Re-treatment with TEKNAR HP-D already in stock for the 2007 application showed a high percentage kill and dead larvae of characteristic appearance. It is concluded that future treatments must be made with new product.

It is anticipated, from the above results, that the number of adult Blandford Fly females (potential biters) in 2006 at Blandford may be higher than last year due to the poor kill in the main treatment. The size of the larvae suggests that the majority of biting insects will not be on the wing until mid-late May. Both the intensity of biting and the seasonal range of activity are of course subject to weather conditions.

Discussion with interested parties in recent years has looked at ways of reducing the cost of the annual treatment. Suggestions have included dispensing with the survey and/or the monitoring of the percentage kill. This year, the survey highlighted the fact that hatching was late and larval growth was slow. Treatment at the usual time would have killed large numbers of larvae of non-target over-wintering species. Clearly, if monitoring had been abandoned, the deterioration of TEKNAR HP-D stored for more than 12 months would not have been realised and a resistant strain of fly could have developed in subsequent years if the practice of ordering TEKNAR HP-D a year in advance had continued.

10. ACKNOWLEDGEMENTS

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