Institute of Freshwater Ecology

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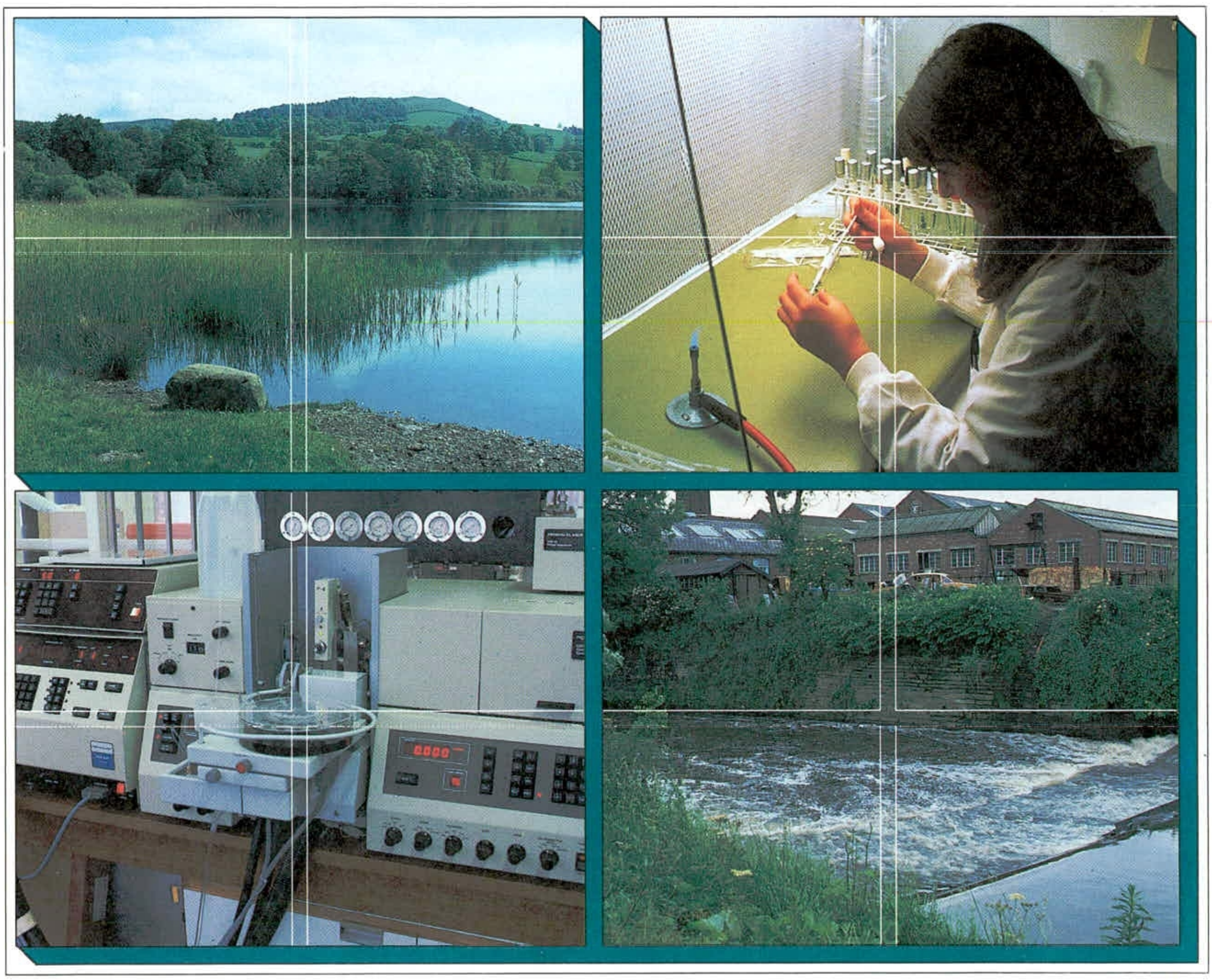
Report To: North Dorset District Council
IFE Report Ref. No: T04073C7



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CONFIDENTIALITY STATEMENT

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The Institute of Freshwater Ecology is part of the Centre For Ecology and Hydrology Directorate of the Natural Environment Research Council.

CONCLUSION

1. In 1996, as in previous years, **TEKNAR HP-D (*Bti*) was found to be an effective simuliicide** when used against the larvae of *Simulium posticatum* under the conditions prevailing in the River Stour.

2. The populations of "**non-posticatum**" simuliids had, as usual, mostly emerged as adults by the end of March.

3. *Bti* application was again scheduled for the end of March when the overwintering populations had emerged and last instar larvae of *S.posticatum* had been found (showing that further delay in treatment would be undesirable).

4. The full river survey identified a range of sites with populations of *S. posticatum* larvae, these were treated on 1 April 1996.

5. Statistical analysis of samples of larvae, taken before and after application of *Bti*, showed that **the mean mortality was 54.3 % at Blandford and 53.2% at Longham.** However, at the Longham treatment site a massive, *Bti* induced, mortality of *S. posticatum* had occurred at the control site (due to exceptional carry of TEKNAR HP-D from upstream) and because of the method of calculation this greatly reduced the apparent mortality at the treatment site. A simple 'before and after' calculation indicated that **the true mortality at the Longham treatment site was probably closer to 92%.**

6. Samples of pupae were no longer required, by HSE, this year and none were taken, but details of the data for pupae obtained in 1995 were supplied to HSE in September 1996.

1. 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 was given. In 1993, 1994, 1995 and 1996 successful treatments were carried out and samples were taken from sites down to 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, have been treated.

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

2. RECONNAISSANCE SURVEYS

2.1 Introduction

These surveys are designed to monitor the status of over-wintering populations of simuliids and the increase in density of *S. posticatum* larvae in order to determine the best 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, particularly, 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 1996 a slightly different approach to the early season monitoring of fly populations was taken. Following three extremely successful annual treatments of the Stour system with *Bti*, as a control measure for the Blandford Fly, very few people were bitten by the insects in April to June 1995 (Ladle and Welton, 1995). However, North Dorset District Council reported a small focus of biting activity (seven individuals bitten) in the vicinity of Sturminster Newton, several kilometres upstream of the uppermost treatment site at Blandford. At the request of the NDDC, a survey of sites along the River Stour and in some of the main tributaries was carried out in March 1996. The purpose of the survey was simply to establish whether the population of *Simulium posticatum* extended beyond the normal region being treated annually for their control (Blandford to Throop). (See report to NDDC by Ladle and Welton 1996).

2.2 Methods

As mentioned above a survey of sites along the River Stour and in some of the main tributaries was carried out in March 1996. The results of this survey were used to provide an indication of the status of the fly populations prior to treatment. (See report to NDDC by Ladle and Welton 1996).

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.

On the the 1 and 3 April quasi-quantitative samples of weed were taken, as usual, from the standard treatment and control sites at Blandford and Longham. In the laboratory, the simuliid larvae were identified and the numbers of *S. posticatum* larvae were recorded separately from the numbers of other simuliid larvae. First and last instars were noted. The wet weight of each sample of weed was recorded and the density of larvae was determined as numbers per gram of weed.

2.3 Results

2.3.1 Survey of River Stour for additional foci of *S. posticatum* larvae.

For maps and map references see previous reports.

Very few *S. posticatum* were present in the untreated reaches of the River Stour upstream of Blandford. However, at Throop there were more *S. posticatum* larvae present than at any of the previously treated sites apart from Wimborne, at Julians Bridge. It appears, however, that the lower reaches of the River Allen now hold a significant population of these insects. The small size of the river and the relatively sparse nature of the submerged vegetation suggest that the total number of flies emerging from the River Allen is likely to be quite small, but in view of the proximity of the Wimborne conurbation and the reducing numbers of *S. posticatum* originating from the main river, it will be worth treating the section up to High Hall (NGR SU 006 025) if permission can be obtained. It is probable that the high density of larvae at Wimborne, relative to some of the sites treated in earlier years, could be due to the untreated sub-population in the River Allen. This could provide a reservoir of insects to sustain the numbers in the River Stour locally.

3. DISCHARGE AND VELOCITIES

Discharge values were required for calculation of *Bti* dilution factors.

3.1 Methods

The Wessex region of the National Rivers Authority (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 a considerable distance upstream of Blandford and a second at Throop - potentially the furthest downstream site for treatment. The NRA 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 and the fact that more sites need to be treated, interpolation of the NRA gauging stations results is now used, as routine, to calculate quantities of *Bti* to be added at each site.

3.2 Results

The discharge of the River Stour at Hammoon and Throop, in March 1996, is given in Figure (1).

On treatment day (2 April 1996) the flow at Hammoon was **>4.5 m^3s^{-1}** and at Throop **>14.2 m^3s^{-1}** .

4. *BTI* APPLICATION

4.1 Methods and quantities

The above flows would require TEKNAR HP-D loadings of **2.16 l** and **6.82 l** respectively to achieve the desired concentration of **0.8ppm**. Blandford is well downstream of Hammoon and under the prevailing stable flow conditions of the time it was estimated that the discharge would exceed that at Hammoon by at least 50%. As in previous years treatment levels were conservative and ranged from a minimum of **3 l** of *Bti* at Blandford to a maximum of **6 l** of *Bti* at Throop.

The TEKNAR HP-D was carried to the sites as measured doses in closed containers and mixed in 20 l knapsack sprayers with sieved river water. The material was sprayed, by a qualified operative, who, when possible, traversed the river approximately ten times during the application period. The jet of the spraying equipment was totally submerged beneath the water surface to avoid spray drift or loss. At some points, where conditions rendered access to the river dangerous, mixing was achieved by introducing the required quantity of TEKNAR HP-D to a turbulent sluice or weir from a bridge or other vantage point. The sites were treated sequentially starting at Blandford, the furthest upstream at 10.00 hr and ultimately treating , Throop, the lowest site on the river, at 19.00 **hr** on 2 April.

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

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

- Blandford main river	3 l
- Langton Long	3 l
- Charlton Marshall	3 l
- Clapcott's Farm	
Middle channel	2 l
West channel	2 l
- Spetisbury	5 l
- Millmore Farm	5 l
- Corfe Mullen	5 l
- Little Pamphill	6 l
- Wimborne	6 l
- Canford School	
Main river	6 l
Carrier	0 l

- Longham	6 l
- Longham control	0 l
- Muscliffe	6 l
- Throop	6 l

A total of 64 litres of TEKNAR HP-D was added to the river on treatment day, 2 Apr 1996. This is about the same as in the previous year (62 l in 1995).

5. MONITORING THE EFFECTS OF *Bti* ON *SIMULIUM POSTICATUM*

5.1 Methods

30 weed samples were taken from each of the control and treatment sites at both Blandford and Longham on pre-treatment day (1 April 1996). At Blandford, the weed samples at the treatment site were taken 50 m below the proposed application point and at Longham this distance was also 50 m. The sites chosen were dependent on site conditions such as presence of weed, suitability of conditions for larvae and safety of access.

Sampling was repeated on 3 Apr 1996, the day after treatment.

Samples were transported to the laboratory and the number of living larvae on each piece of weed was counted after identification into *S. posticatum* and other simuliid species. Weed samples were weighed after blotting dry. The method used was identical to that in previous years.

5.2 Results

5.2.1 Dead larvae

At Blandford, following treatment, 17 dead *S. posticatum* larvae were recorded from the 30 weed samples at the treatment site on 3 Apr 1996. The number from the equivalent samples at Longham was 119 dead larvae per 30 samples. No dead larvae were found in the Blandford control site samples but 126 dead larvae were present in the Longham control site samples. As noted in 1993, 1994 & 1995 little interpretation of these results is possible as it is not known what (variable) proportion of dead larvae remain attached to the weed. It does show, however, that larvae were again killed by *Bti*.

Although the numbers of dead larvae on pre-treatment and control site samples are generally very small (much less than 1 per sample) again, as in 1995, there was an anomalous result with dead larvae recorded from thirty control weed samples at Longham on post-treatment day (3 April 1996). Dead larvae examined had adopted the characteristic "stretched" appearance which we have come to associate with death following ingestion of *Bti*. It is thought that under the prevailing conditions of low flow/low turbidity/minimal weed growth/small larval population (reduced filtering capacity), the carry of the *Bti* was, as in 1995, much greater than previously encountered. In other words the larvae must have been killed by material carrying downstream from the Canford treatment point, a total distance of 7 km. In early trials substantial mortalities were recorded over carry distances of a little over 1km but in 1993 and 1994 there had been no evidence of mortality in the Longham control samples.

5.2.2 Density of living larvae

The densities at the four sites on pre-treatment day was, as in previous years, very patchy in distribution of larvae (Table 1). Control and treatment sites were within 50 m of each other at both locations (Blandford and Longham) and there was relatively little within location variation. There were however large differences between Blandford and Longham (Table 1).

The changes in density following treatment were tested statistically to see if they were significant. Initially the odds ratio method was applied and a t test used to compare means (Table 2). A non-parametric method (Mann-Whitney) comparing medians was used as confirmation (Table 3).

5.2.3 Odds ratio method

This works on the premise that the ratio of the larval density before and after the treatment date should be the same at the control and treatment sites if there is no effect of the *Bti*, thus q, the 'odds ratio' coefficient, is determined as follows;

$q = R_T/R_C = 1$ where $R_T = \bar{x}_{ta}/\bar{x}_{tb}$ and $R_C = \bar{x}_{ca}/\bar{x}_{cb}$

$\bar{x}_{ca}$ = mean density in the control site after treatment

$\bar{x}_{cb}$ = mean density in the control site before treatment

$\bar{x}_{ta}$ = mean density in the treatment site after treatment

$\bar{x}_{tb}$ = 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

$D_C = \bar{y}_{ca} - \bar{y}_{cb}$ for the control sites

and $D_T = \bar{y}_{ta} - \bar{y}_{tb}$ 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 $\bar{y}_{cb}$ does not equal $\log_{10} \bar{x}_{cb}$, 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.

$$Q = (\bar{y}_{ta} - \bar{y}_{tb}) - (\bar{y}_{ca} - \bar{y}_{cb})$$

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) 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

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

The $\log_{10}$ x+1 values for mean density are given in Table 2.

The results were calculated for pre- and post- the retreatment as follows

Q = (0.39 - 0.63)-(0.52-0.42)
= -0.35

The proportion of pre-treatment density remaining is
antilog Q = 0.457 or 45.7%.

Thus the percentage kill at Blandford was 54.3%.

Limits can be calculated from the formula, antilog (Q ± t SE(Q))
The SE(Q)= 0.210, therefore the limits are 1.14 and 0.174 (or 115%) and (17.4%).

The limits of the percentage kill are therefore 0.0%% and 82.6%.

t = 1.66 with 116 df. p=0.1

This shows that the reduction in density at the Blandford treatment site is not significant p=0.1.

As the densities have changed at Longham control, the best estimate of the proportion of pre-treatment density left after application of *Bti* at the treatment site is

$$Q = (0.29-1.23) - (0.72 - 1.33)$$
$$= -0.33$$

$$SE(Q)= 0.209$$

$$\text{antilog } (Q \pm t\ SE(Q)) = \text{antilog } (-.33\pm2.24*0.209)$$
$$= 0.468 \text{ with limits of } 1.37 \text{ and } 0.159$$

As a percentage this equates to 46.8% with limits of 137% and 15.9%. **Thus the best estimate of mortality of larvae at Longham is 53.2%**

with limits of 0.0% and 84.1%.

and **t = 2.24 with 116 df. p = 0.02***

This shows that the reduction in density at the Longham treatment site is significant.

6.2.4 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. The calculations were performed twice, firstly assuming unequal within-time variability in log density and secondly assuming equal variances. At Blandford, there was no significant difference between pre- and post-treatment control site samples nor was there any significant decrease in density at the treatment site for the equivalent period. At Longham, there were highly significant decreases in density at both the control and the treatment sites (Table 2).

6.2.5 Non-parametric method

The Mann-Whitney test is a two sample rank test for the difference between population medians. It assumes that the data are independent random samples from two populations that have the same variance.

This year there were no significant changes, between the densities before and after treatment day, in either control or treatment sites at Blandford. Significant changes occured in the control and treatment sites at Longham (Table 3). .

This year the results of the treatments were rather different to previous years. At Blandford initial densities of larvae at both control and treatment sites were very low even before treatment. Many individual samples were devoid of larvae and, because of this high variability, the simple statistics represented by t tests and Mann-Whitney tests were unable to detect significant changes following treatment. Even the more sophisticated odds ratio method, which takes into account changes in population at the control site showed a marked (53%) but not significant decrease in numbers of larvae ($p > 0.1$) at the treatment site. However, the presence of substantial numbers of dead larvae in the post-treatment samples showed that the treatment had been effective.

At Longham the situation was much clearer. Initial population densities were greater and, as a result, all the statistical tests showed large and significant reductions in numbers of larvae at the treatment site. As reported in 1995 there was also a significant mortality of larvae at the 'control' site (76%) due to *Bti* which must have originated from the treatments further upstream (Canford). The latter result affects the odds ratio calculation, reducing the apparent mortality (53%) at the Longham Treatment site. However a simple calculation, based on the reduction in mean density indicated a much higher mortality (in the order of 93%).

6.2.6 Other periphyton

Although there was no longer any requirement to examine periphyton this was done in the course of monitoring the larvae of *S. posticatum*. Again only one family of periphyton invertebrates (Chironomidae) was found on the weed samples. None were present at Blandford before treatment and only one was found after treatment. Chironomids were more numerous at Longham (but numbers were too small for meaningful statistical comparisons) with numbers remaining more or less constant after treatment. Densities were not calculated due to the very low numbers involved.

7. EMERGING ADULTS

The requirement to monitor emerging adults (pupae) has been lifted.

8. DISCUSSION

Conditions for treatment of the river Stour in 1996 were again almost ideal. Steady flows made survey and treatment of the river easy. As usual a conservative approach to TEKNAR HP-D application was adopted. On 2 April 1996 several sites, including the monitoring sites at Blandford and Longham, were treated by the standard wading technique (see previous reports).

The low population densities of larvae at the two Blandford sites, even before treatment, were associated with high variability and a great many zero values in the thirty samples. As a result, even though dead larvae were detected at the treatment site following the application of the pesticide, it was not possible to demonstrate a significant decrease in numbers between samples.

At Longham the densities of larvae at control and treatment sites were relatively high prior to treatment and levels of variability were similar to previous years. There was a large reduction in densities following treatment but because of an unexpected high mortality at the control site the application of the odds ratio method, which takes into account any changes in control samples, gave the impression that treatment had been ineffective. The condition of the observed dead larvae (characteristic form of rigor mortis) confirmed that the control mortalities were undoubtedly due to the effects of TEKNAR HP-D and the high figure presented below is based on this premise.

The mean percentage kill achieved was less than usual at **Blandford (53%)**. At **Longham** the apparent kill was only **53 %** but the figure of **93%** which is obtained by ignoring control mortalities may be more realistic.

The impression gained from local reports and from direct enquiries is that the cumulative effect of treatment, over the past four years has been very successful and once again far less people had been affected by the bites of the fly than in previous years. Figure (3) shows the reduction in biting activity based on medical reports to North Dorset District Council (figures kindly supplied by Mr Dave Morgan). It may be significant that, although 1996 appeared to be a year of poor weather conditions for fly biting activity, a survey by Cherwell District Council in Oxfordshire reported 69 bites from the vicinity of the River Cherwell.

Acknowledgements

Our thanks go to R.T.Clarke for statistical advice. Discharge data was kindly supplied by the South Western NRA (EA) (Wessex Region).

Table 1. Densities of *S. posticatum* larvae and percentage changes following treatment at control and treatment sites 1996.

	Blandford		Longham	
Date	Control	Treatment	Control	Treatment
1-Apr-96	5	6.59	37.13	38.92
3-Apr-96	12.8	3.61	8.73	2.81
% change	156%	-45%	-76%	-93%

Table 2. Densities (Log10+1), Student t values and significance levels by site. *=significance level NS= not significant.

	Pre-treatment	Post-treatment	t	p	Significance
Blandford Control	0.42	0.63	-1.47	0.15	NS
Blandford Treatment	0.52	0.39	1.19	0.24	NS
Longham Control	1.33	0.72	4.55	0	***
Longham Treatment	1.23	0.29	6.49	0	****

Table 3. Mann-Whitney test for significance of differences in densities *=significance level, NS= not significant.

	Pre-treatment	Post-treatment	w	p	Significance
Blandford Control	0	5.26	945	0.265	NS
Blandford Treatment	1.74	0	972	0.356	NS
Longham Control	19.6	7.6	1175	0.0001	***
Longham Treatment	21.6	0	1229	0	****

Figure 1. Measured discharges in River Stour, March 1996 (data supplied by Environment Agency).

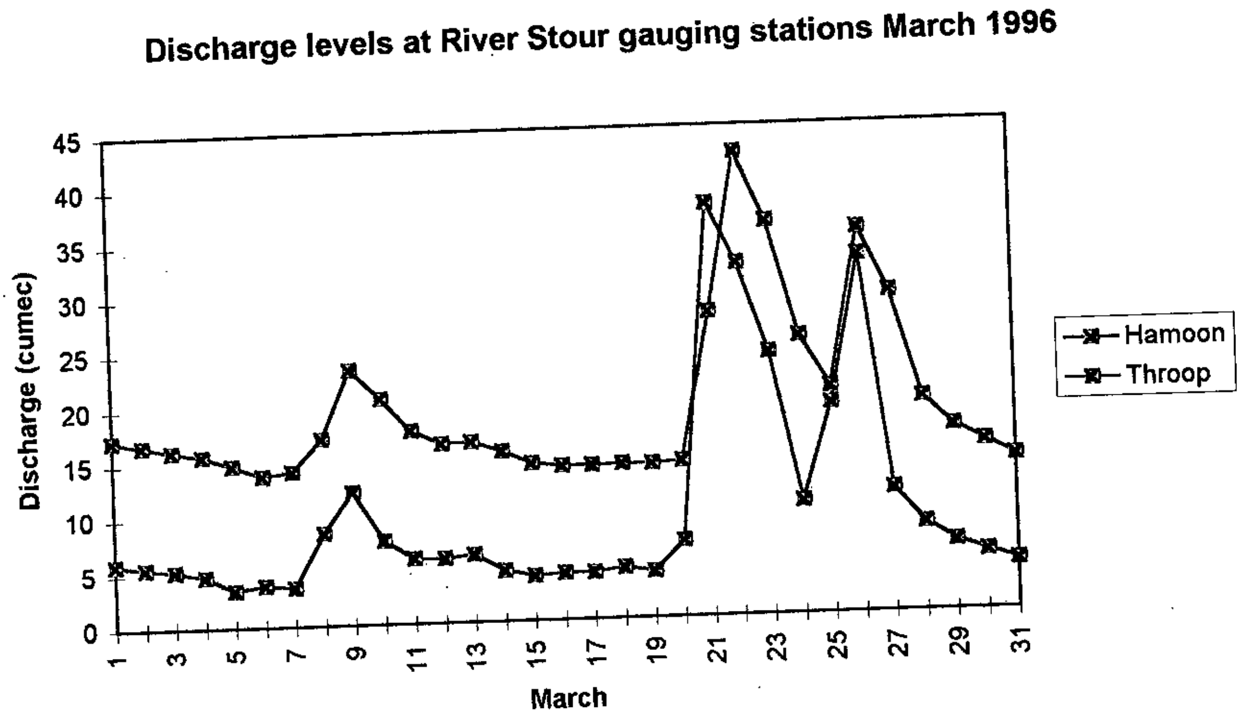

Figure 2. Histograms with 95% confidence limits showing densities of larvae before and after treatments

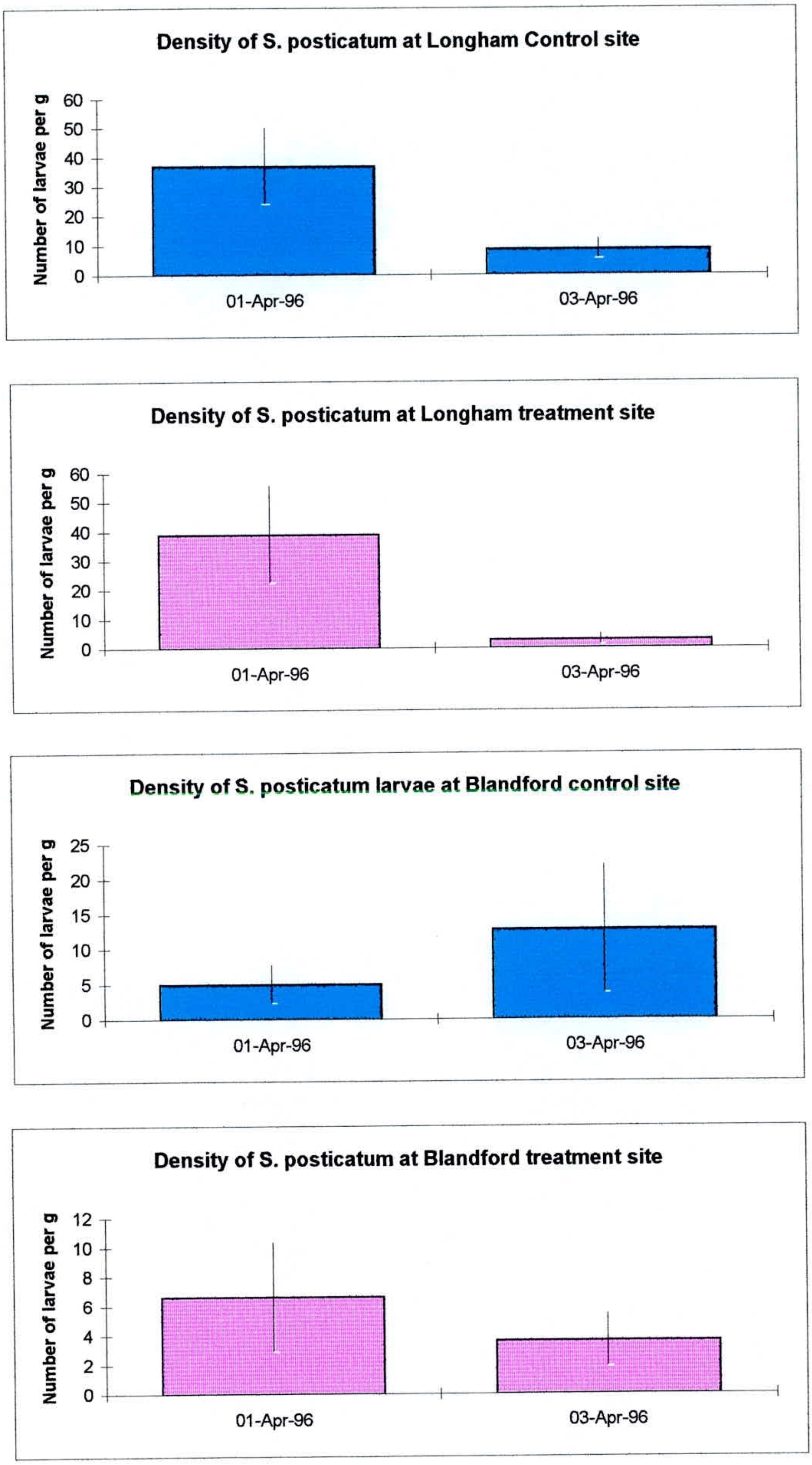

Figure 3. Numbers of individuals treated for bites at selected health centres in the North Dorset area.