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A rapid method of estimating the abundance of immature blackflies (Diptera: Simuliidae)

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ABSTRACT

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A ten-point visual method of estimating the abundance of immature blackflies in the field is proposed and tested. The method is based on the comparison of larvae and pupae found on natural substrates, with ten diagrammatically prepared abundance classes. When estimates were based on the abundance of blackflies within a 4 x 4 cm area of highest density, there were no significant differences between estimates based on the ten-point visual method and those based on actual counts ($P > 0,05$). The time taken to assess the abundance of larval blackflies on 30 substrates was about 15 min, depending on substrate accessibility. Personal bias was assessed independently by four people, and was negligible when estimates were based on the highest densities within a 4 x 4 cm quadrat. The method tends to overlook very small larvae, and is not recommended for estimating overall population densities. However, the method provides a reliable, practical and rapid index of blackfly abundance suitable for use in blackfly control programmes.

INTRODUCTION

Blackfly larvae, like many stream invertebrates, are notoriously non-uniform in distribution (Hynes 1970; Crosskey 1990), and large numbers of samples are usually required for reliable estimates of abundance (Needham & Usinger 1957; Chutter & Noble 1966; Chutter 1972; Resh 1979; Morin 1985). The traditional three Surber (Surber 1936) or Box samples (Doeg & Lake 1981; Merritt, Cummins & Resh 1984) used in stream surveys provide reasonable estimates of species occurrence, but provide only semi-quantitative abundance estimates because of large sampling variability (Chutter & Noble 1966; Resh 1979). Sampling variability is reduced by the use of artificial substrates of a standard size and texture (Morin 1987).

Substrates which have been used for sampling immature blackflies include ceramic tiles, bricks, gauze strips, plastic strips, plastic balls, plastic cones, PVC tubing, wooden blocks, rope, string and lengths of fabric (Tarshis 1968; Disney 1972; Lewis & Bennett 1974; Boobar & Granett 1978; Fredeen & Spurr 1978; Colbo 1987; Morin 1987). However, artificial substrates pose several problems. Firstly, substrates may be tampered with or stolen. In West Africa theft was minimized by the use of semi-mummified monkey heads (Disney 1972), but the method has not been accepted widely. Substrates may also be washed away by high water, or left exposed by low water. Furthermore, substrates require two visits per study site, which therefore increases the time and cost of sampling. Their use also poses problems regarding species substrate selectivity, and assumptions about colonization times and equilibrium populations (Disney 1972; Resh 1979; Pegel 1981; Morin 1987). In addition, their position in the water, particularly in relation to flow, is critical for uniform colonization (Colbo 1987; McCreadie & Colbo 1991). Artificial substrates are therefore not the panacea for

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sampling blackfly larvae, and the use of natural substrates has been recommended (Morin 1987).

Blackfly larvae attached to stones (cobbles) have been sampled by simply removing the stones from the water and counting the specimens (Chutter 1968; Carlsson, Nilsson, Svensson, Ulfstand & Wotton 1977; Colbo 1985; Gíslason 1985; Gíslason & Gardarsson 1988). Measurements of stone-surface area enable population densities to be determined. Approximate stone surface area may be determined from measurements of maximum length x maximum width (Calow 1972; Carlsson *et al.* 1977; McCreadie & Colbo 1991). More accurate methods involve wrapping tin foil (Morin 1987) or a thin plastic film (Doeg & Lake 1981) over the stone, and comparing its mass to that of foil or film of known area. Despite the accuracy of these measurements, errors are likely to arise because total stone surface area does not reflect the surface area available for blackfly colonization (McCreadie & Colbo 1991). A method of estimating the potentially suitable area for blackfly colonization involves tracing onto graph paper the area of each stone as it was positioned in the water (Carlsson *et al.* 1977; Gíslason 1985; Gíslason & Gardarsson 1988).

In many rivers, however, blackflies are found predominantly on aquatic vegetation. Semi-quantitative samples may be obtained by filling a standard-sized cup with plants, and then counting the blackflies in each cup (Niesiolowski 1980; Simmons 1991). To overcome the problem of non-uniform distribution of immature blackflies, Wotton (1977) has proposed the use of "removal sampling", in which substrates on which blackflies are found are removed from the stream in repeated searches of equal duration, and then counted. The method is suited to streams where blackflies are scarce, and cannot be used when population densities are high (Wotton 1977).

All the methods mentioned above are time-consuming because they involve actual counting. A rapid index of immature blackfly abundance, used by the World Health Organization's Onchocerciasis Control Programme in West Africa, involves ranking blackfly abundance on a four-point scale (absent, rare, numerous and very numerous; Kurtak, personal communication 1993). The method is limited because it does not provide data on population densities, and personal bias is likely to arise, especially with inexperienced users. The aim of this paper is to propose and test a rapid method of classing the abundance of immature blackflies for use in blackfly control. The method is based on a visual comparison of blackflies on hand-held, natural substrates, with ten diagrammatically prepared abundance classes. The abundance classes were based on a semi-logarithmic scale, and provide rough estimates of population densities. The method was developed for use in the control of pest blackfly, *S. chutteri* Lewis, along the Orange River, South Africa.

MATERIALS AND METHODS

Two sets of diagrammatic representations for populations of small (2 mm) blackfly larvae and pupae in each of ten abundance classes were prepared (Fig. 1–4). One set was prepared for use on large, flat surfaces, such as stones and broad leaves (Fig. 1 and 2), and the other was prepared for use on thin, cylindrical surfaces, such as twigs, roots, grass and trailing vegetation (Fig. 3 and 4). The classes ranged, on a semi-log scale, from no individuals (Class 1) to an excess of 500 000/m^2 for larvae, and 250 000/m^2 for pupae (Class 10; Table 1). The classes were based on visual distinguishability between population densities within a 4 x 4 cm quadrat. The upper limit (Class 10) was based on the maximum population densities which are likely to be encountered in the field.

The diagrams were of such a size that when the highest nine classes were placed on a single A4 sheet, the sheet could be held in one hand, and conveniently used in the field. The diagrams were photocopied onto plastic sheets which, together with a coloured backing, were taped onto perspex plates (counting plates are available at cost from the author). For the sampling procedure, natural substrates in fast-flowing water were lifted by hand, and population densities were visually compared with the diagrammatic abundance classes (Fig. 1–4). Sampling variability was reduced by choosing substrates from areas with similar hydraulic conditions (i.e. random stratified method; Chutter & Noble 1966; Cuff & Coleman 1979; Resh 1979; Merritt *et al.* 1984). A wax crayon was used to mark scores (expressed as frequencies) onto the perspex plate.

For flat surfaces, estimates of overall blackfly abundance were obtained by "averaging" numbers over the habitable surface of each substrate. This involved visually "smoothing" numbers over the area which was potentially available for blackfly colonization. Because of the difficulty in deciding the area potentially available for blackfly colonization, and the difficulty in visually "smoothing" the numbers, estimates were also based on the abundance of blackflies in the area of highest density. A 4 x 4 cm quadrat, cut out from a piece of hard

TABLE 1 The number of larvae and pupae per 16 cm^2 in each of 10 abundance classes used for estimating the abundance of immature blackflies. Ranges for the classes are given in brackets

Class	Larvae	Pupae
1	0	0
2	1 (1–2)	1 (1–2)
3	3 (3–4)	3 (3–4)
4	6 (5–9)	6 (5–8)
5	16 (10–22)	11 (9–15)
6	36 (23–58)	25 (16–35)
7	88 (59–120)	55 (36–80)
8	202 (121–310)	120 (81–180)
9	500 (311–800)	280 (181–400)
10	1 050 (> 800)	600 (> 400)

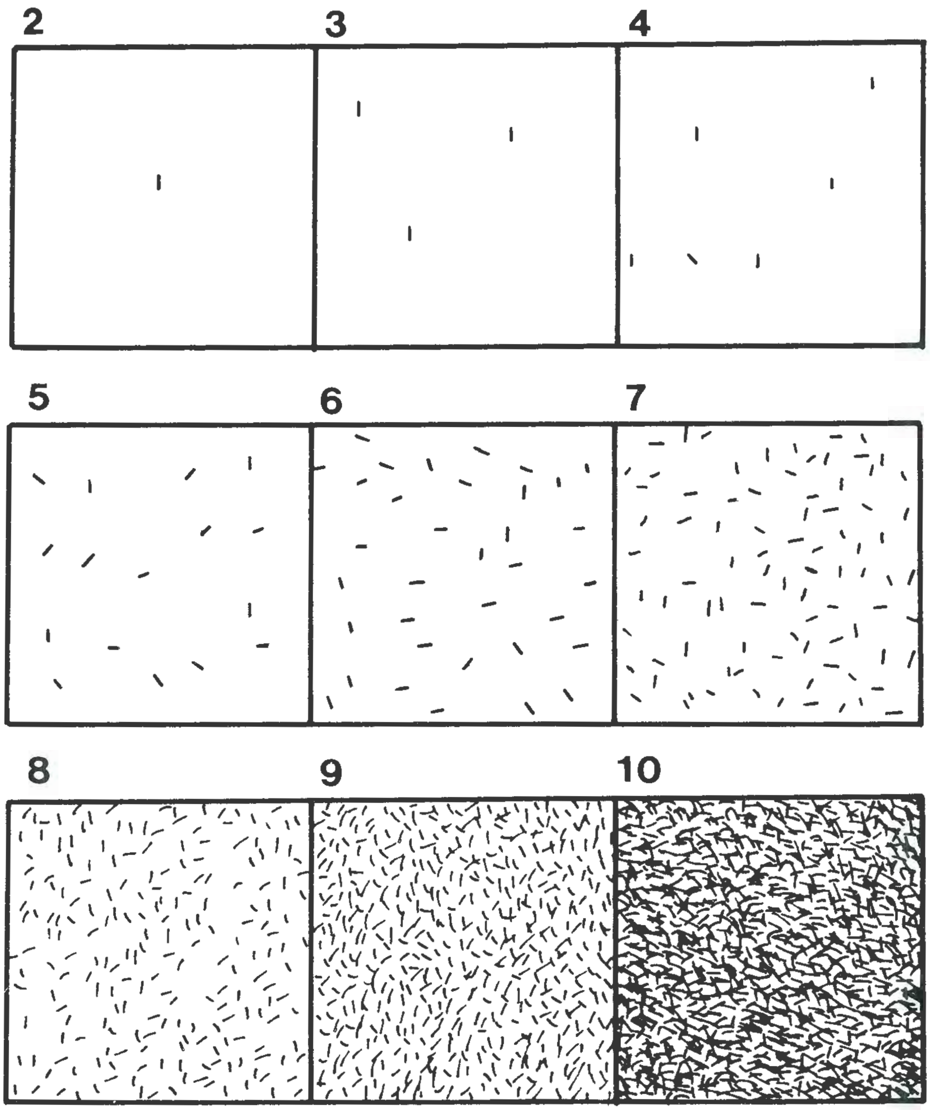

FIG. 1 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of larval blackflies about 2 mm in length found on flat substrates, such as stones or leaves. Classes correspond to the numbers shown in Table 1

plastic, was used to demarcate the area. Separate areas were used for larvae and pupae.

For cylindrical surfaces, no attempt was made to estimate overall abundance because of the difficulties involved in determining total surface areas. Instead, abundance was estimated by choosing, in each handgrab sample, a length of vegetation in which the abundance of blackflies was highest, and their distribution uniform. Ideally, a length of about 12 cm of vegetation was used (Fig. 3 and 4). Often, however, larvae and pupae were tightly clumped, and a smaller length (down to 1 cm) of vegetation was used. Separate lengths of vegetation were used for larvae and pupae.

Accuracy

To compare estimates of blackfly population based on the ten-point visual method with estimates based on actual counting, forty stones (cobbles) were removed from the fourth rapids downstream of the Gifkloof Weir on 1 November 1993. Stones ranged between 10 and 20 cm in diameter. Larval and pupal densities for each stone were assigned a class in the manner described above. For "overall" counts, the "true" (expected) values were obtained by counting all larvae and pupae on each stone, and estimating the area available for blackfly colonization.

For stones with low populations of blackflies, counting was done in the field. Stones with high populations were scrubbed in a bucket of water, and all larvae and pupae were collected by sieving through a 60-μm mesh, and preserved in 80 % ethanol. Counting was done in the laboratory with the aid of a dissecting microscope. The area on each stone suitable for blackfly colonization was determined by wrapping a thin plastic film

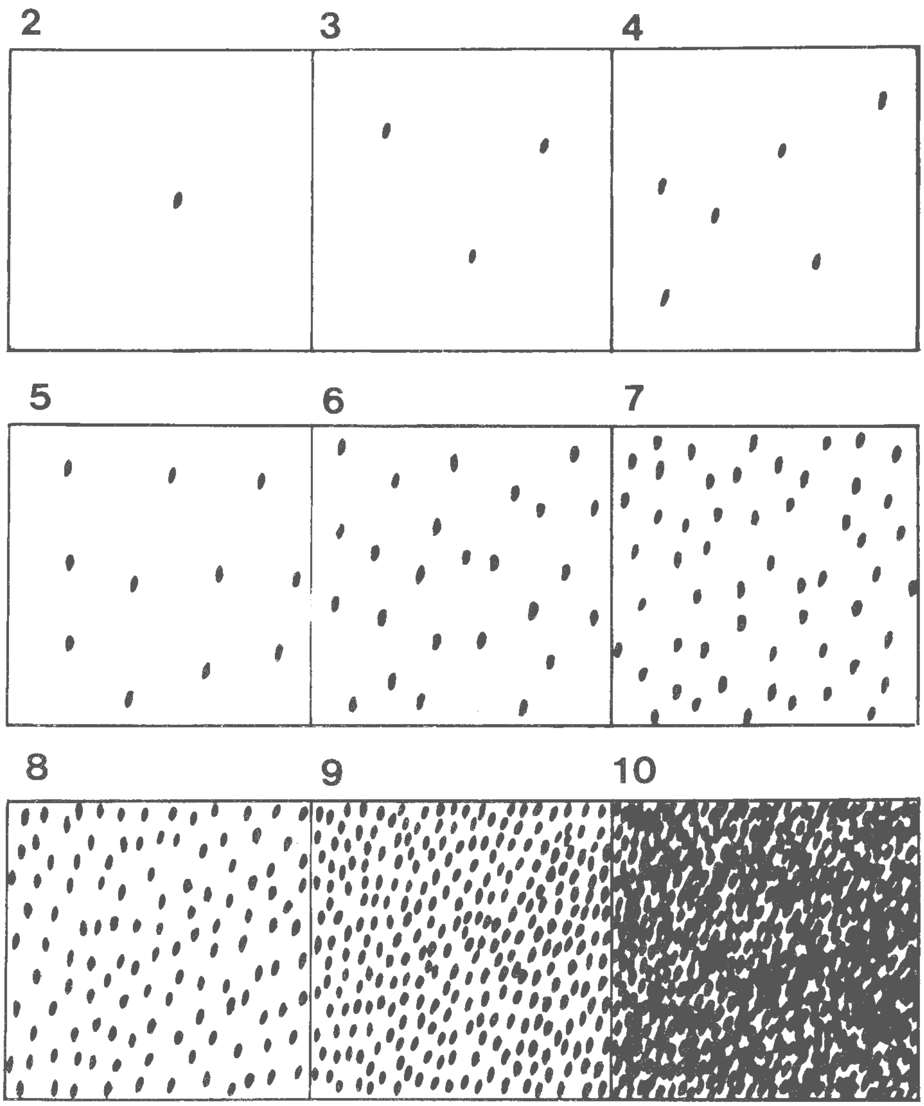

FIG. 2 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of pupal blackflies 2–3 mm in length found on flat substrates, such as stones or leaves. Classes correspond to the numbers shown in Table 1

(Gladwrap®) over the area of rock which was exposed to flowing water, and comparing its mass with that of a known area of plastic film. Weighing was done with a Masskof® balance accurate to 0,1 mg. For "high-density" counts, larvae and pupae within each 4 x 4 cm quadrat where removed by fine forceps, and counted to give a "true" (expected) value. This value was then assigned a class (by use of the values given in Table 1), and compared to the class obtained from a visual assessment (observed value).

To test the agreement between classes assigned by use of the ten-point visual method and classes assigned by actual counting, stones and trailing vegetation were removed from various sites in the Orange River during 1992 and 1993. The area of highest blackfly density on each stone or handful of vegetation was assigned a class in the manner described above. Vegetation samples were based on the roots and stems of the reed *Phragmites australis*. The length and breadth of each vegetation sample was measured so that population densities could be determined. Samples containing too many larvae to be counted in the field were preserved in 80 % ethanol, and larvae were counted in the laboratory with the aid of a dissecting microscope.

Sample number

The number of samples required for reliable estimates of blackfly abundance depends on the abundance of blackflies (Morin 1985). The abundance of blackfly larvae on 30 stones at Gifkloof was estimated weekly throughout 1992. The minimum sample size was defined as the point at which the 95 % confidence limits of the median were within one class on either side of

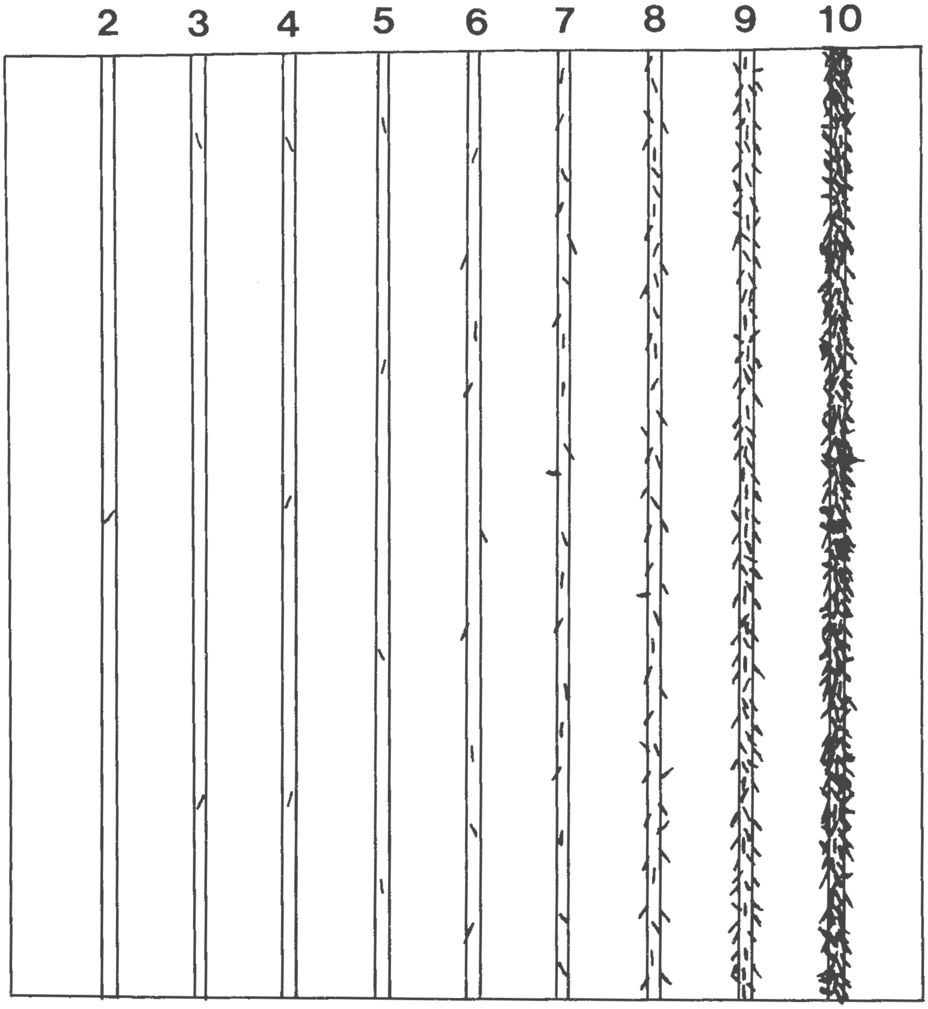

FIG. 3 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of larval blackflies about 2 mm in length found on cylindrical substrates. Classes correspond to the numbers shown in Table 1

the median. Confidence limits were determined from binomial test tables (Zar 1984). For a sample size of 30, the 95 % confidence limits of the median are located at ranks 10 and 20.

Personal bias

To test personal bias in the use of the visual method, four individuals independently estimated the abundances of larval blackflies for the same 30 stones at Prieska Bridge in the Orange River (29°39'26"S; 22°44'45"E). Two individuals were inexperienced in river ecology, and so they were shown how to distinguish blackfly larvae from other invertebrates, such as chironomid larvae, but they were not given the opportunity to practise using the method.

Worked example

The visual method of estimating blackfly larval abundance was used to test the efficacy of larvicides used in the control of pest blackflies (mainly *S. chutteri*) along the Orange River. Results of this control programme are reported elsewhere (Edwardes, Palmer & Nevill 1993; Palmer 1993), but the results of one trial are reported here to illustrate the method used. Thirty-two litres of Abate® 200-EC (temephos), supplied by S.A. CYANAMID, were applied (undiluted) over a period of 3 min across the width of the river from the Upington Bridge (28°27'52"S; 21°14'35"E). Discharge at the time of application was 107 m^3/s, giving a theoretical concentration of 0,1 mg/ℓ (ppm) over 10 min. The abundance of blackfly larvae on 30 substrates (stones and trailing vegetation) found in five rapids downstream of the bridge, and one rapids upstream of the bridge (the control site), were assessed immediately before and 3 d after application. The change in larval abundance before and after application was tested using the One-tailed Mann-Whitney U-Test (Zar 1984). No attempt was made to sample the same substrata before and after application. The difference

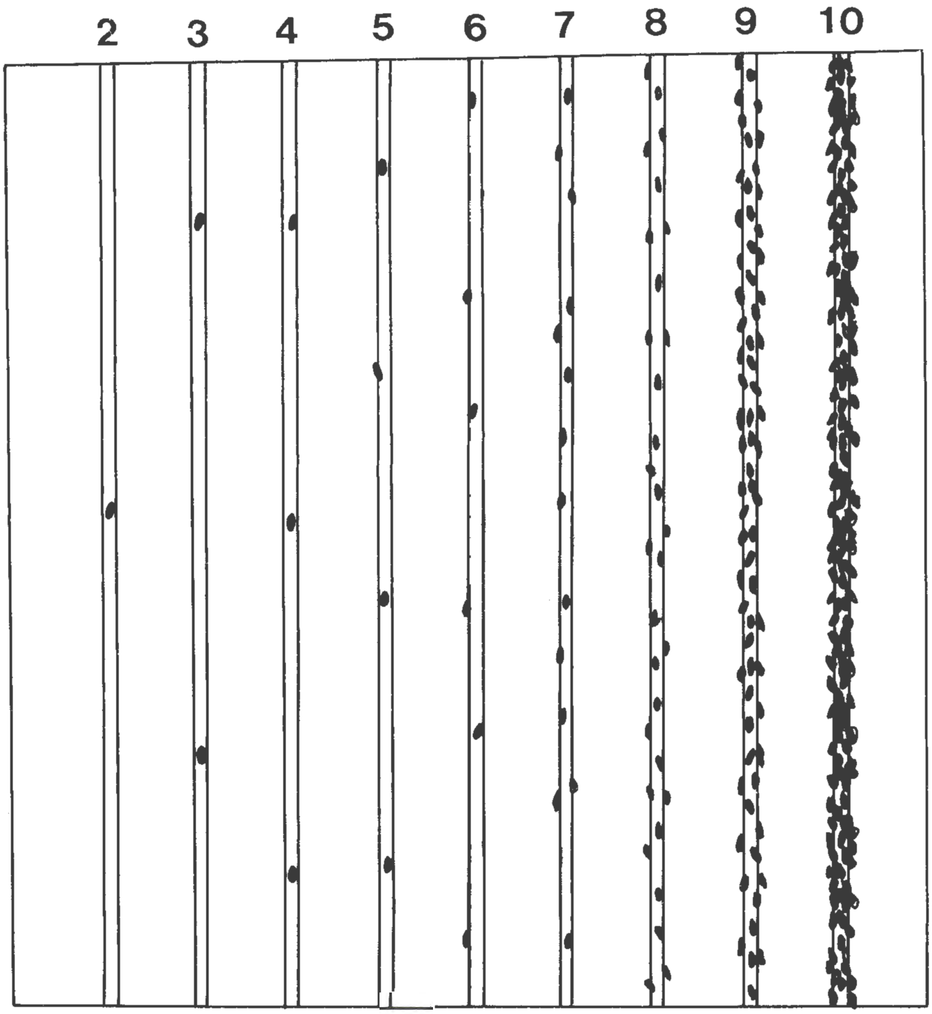

FIG. 4 Diagrammatic presentation of semi-logarithmically defined abundance scale for classing population densities of pupal blackflies 2–3 mm in length found on cylindrical substrates. Classes correspond to the numbers shown in Table 1

between the median abundance class before and after application was used to determine the percentage mortality as follows:

$$\text{Difference} = \frac{Y-X}{Y} \cdot 100$$

where Y = the number of individuals of the median class for the 4 x 4 square before application and

X = the number of individuals of the median class for the 4 x 4 cm square after application (see Table 2)

e.g. reduction of class 8 to 5

$$\text{Difference} = \frac{202-16}{202} \cdot 100$$

$$= 92\ \%$$

TABLE 2 Percentage change in the numbers between abundance classes, used to estimate percentage mortality of blackfly larvae as a result of larvicide applications. A change in the abundance class from a median of 8 before application, to a median of 5 after application, represents a larval mortality of 92 %

Medain class after application	Median class before application									
	1	2	3	4	5	6	7	8	9	10
1	–	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99
2		–	66	83	94	97	99	99	99	99
3			–	50	81	92	97	99	99	99
4				–	62	83	93	97	99	99
5					–	56	82	92	97	98
6						–	59	82	93	97
7							–	56	82	92
8								–	60	80
9									–	52

TABLE 3 Estimates of the abundance of blackfly larvae and pupae on stones in the Orange River on 1 November 1993 in each of 10 abundance classes. Observed (O) values were based on the ten-point visual method, and expected (E) values were based on actual counts. Data are expressed as frequencies in 40 samples. Median values are underlined, and the 95 % confidence limits of the median are indicated by vertical lines

Class	Overall				Highest			
	Larvae		Pupae		Larvae		Pupae	
	O	E	O	E	O	E	O	E
1	–	–	–	–	–	–	–	–
2	–	12	11	40	–	–	1	2
3	–	18	19	–	–	–	9	8
4	9	9	10	–	1	–	17	11
5	21	1	–	–	15	15	11	15
6	10	–	–	–	20	24	2	4
7	–	–	–	–	4	1	–	–
8	–	–	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–	–

TABLE 4 Four independent estimates of the abundance of blackfly larvae on the same 30 stones, expressed as frequencies in each of 10 abundance classes. Estimates were based on the highest (H) larval densities within a 4 x 4 cm quadrat, as well as the overall (O) larval densities on the habitable (usually upper) surface of each stone. Stars (*) indicate investigators with no previous experience in using the visual method. Median values are underlined, and the 95 % confidence limits of the median are indicated by vertical lines

Class	Robert		Morgan		*Rachel		*Gavin	
	H	O	H	O	H	O	H	O
1	–	–	–	–	–	–	–	
2	–	–	–	–	–	–	–	
3	–	–	–	–	–	–	–	
4	–	–	–	–	–	–	3	
5	–	–	1	1	2	1	3	3
6	–	2	–	–	2	5	2	6
7	3	3	2	5	2	3	4	6
8	3	11	7	8	6	8	3	6
9	10	10	7	8	5	7	9	3
10	14	4	13	8	13	6	9	3

RESULTS

Accuracy

The average overall abundance of blackfly larvae at the fourth rapids downstream of Gifkloof Weir was 1 900/m^2 (± 1 100 SD; n = 40), equivalent to Class 2. Overall abundance based on the visual method was estimated as Class 6 (Table 3). The visual method therefore overestimated overall abundance, highlighting the difficulties of visually "smoothing" numbers over the stones (Table 3). When estimates were based on the abundance within the area of highest density, the visual method compared favourably with the enumeration of population densities by direct counting (Table 3). The average population density within the area of highest density was 17 625/m^2 (± 8 228 SD; n = 40). By contrast, the visual method estimated the population density as between 14 375 and 36 250/m^2 (Class 6). Despite the good agreement between actual counts and visual estimates based on the area of highest density, the visual method may give unreliable results when small larvae (first and second instar), which are easily overlooked in the field, are numerous.

TABLE 5 Comparison between individuals using the visual method of assessing abundance of blackfly larvae on 30 stones. Estimates were based on the highest (H) larval densities within a 4 x 4 cm quadrat, as well as the overall (O) larval population densities on the habitable (usually upper) surface of each stone. (Tukey Multiple Comparisons Test; Zar 1984)

	Robert		Morgan		Rachel	
	H	O	H	O	H	O
Morgan	0,80	0,73	–	–	–	–
Rachel	1,44	0,78	0,64	1,52	–	–
Gavin	2,59	4,24*	1,78	4,98**	1,14	3,45

ns P > 0,05; * P < 0,05; ** P < 0,01

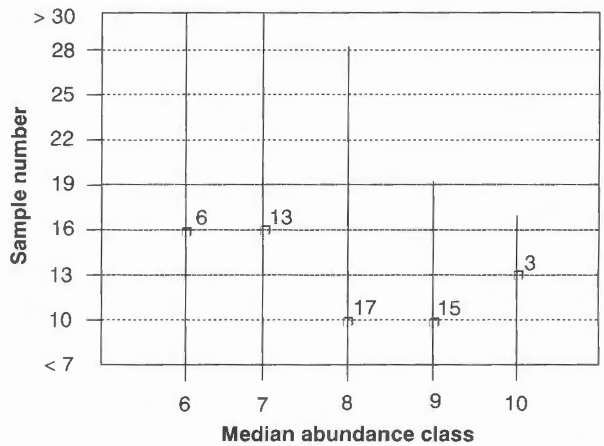

FIG. 5 Minimum number of samples required for estimating the abundance of blackfly larvae using the visual method. Minimum number was defined as the point at which the 95 % confidence limits of the median were within one abundance class on either side of the median. Data are based on weekly estimates of the abundance of blackfly larvae within the area of highest density on 30 stones collected at Gifkloof throughout 1992. Average values and total ranges are indicated. Numbers refer to the sample size

Sample number

The number of samples required for the 95 % confidence limits to be within one class on either side of the median are given in Fig. 5. For population classes equal to or less than 7, the required sample size often exceeded 30, but for the higher classes a sample size

TABLE 6 Estimated abundance of blackfly larvae at various distances up- and downstream of the Upington road bridge, in each of 10 abundance classes, before and after an application of the larvicide temephos (AbateR 200EC). Data are expressed as frequencies on 30 substrates in each of 10 abundance classes. Median values are underlined, and the 95 % confidence limits of the median are indicated by vertical lines. The percentage mortality was determined from Table 2

Site	Control		Site 1		Site 2		Site 3		Site 4		Site 5	
Distance (km)	-16		0,8		4,3		6,2		11,8		15,8	
Class	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	–	–	–	3	–	27	–	12	–	–	–	–
2	–	–	–	1	–	3	–	8	–	–	–	–
3	–	–	–	1	–	–	1	5	–	1	–	–
4	–	–	–	–	–	–	6	2	–	2	–	2
5	1	–	2	2	–	–	3	3	–	6	–	1
6	2	–	1	5	–	–	6	–	3	9	1	8
7	2	3	11	10	2	–	9	–	5	10	4	11
8	10	4	13	6	11	–	5	–	12	2	15	8
9	13	12	2	1	13	–	–	–	8	–	9	–
10	12	11	1	0	4	–	–	–	2	0	1	–
% Mortality	0		56		99		97		82		56	

of 30 is sufficiently accurate for the estimation of relative population density. Except in West Africa, where the aim of the Onchocerciasis Control Programme is to eradicate river blindness in man, control of blackflies would rarely be undertaken at larval population densities less than Class 7. Furthermore, during large-scale control operations, when long distances have to be travelled and population estimates must be made quickly, a sample size of 30 is practically manageable.

Personal bias

When estimates of larval abundance were based on overall densities (i.e. "averaged" for the habitable surface area of each stone), one person consistently underestimated larval abundance, compared with the other three (Tukey Multiple Comparisons Test; $P < 0,05$; Zar 1984:199; Tables 4 and 5). When estimates were based on the area of highest abundance within a 4 x 4 cm quadrat, there was no significant personal bias (Kruskal-Wallis ANOVA, $H = 3,5$; $0,90 < P < 0,95$; Table 4). All four people obtained the same median-abundance value (Class 9), with the 95 % confidence limits within one class on either side of the median (Table 4). The closest estimates were obtained between the two inexperienced members ($q = 0,64$; Tukey Multiple Comparison Test; Zar 1984), and the largest discrepancies were between an experienced and inexperienced member ($q = 2,59$; Table 5).

Worked example

There was no significant change in larval abundance at the control site before and after larvicide application (Table 6), but at 800 m downstream of the point of larvicide application, the median larval abundance value dropped from Class 8, before application, to Class 7, after application (Table 6). This represents a larval mortality of approximately 56 % (Table 2). At the second treated site (4,3 km downstream), mortality approached 100 %, and larval mortality exceeded 80 % for at least 11,8 km downstream of application. At 15,8 km, mortality dropped to 56 %, representing an effective downstream "carry" (> 80 % mortality) of about 12 km. The changes in larval abundance before and after application were significant ($P < 0,0005$) at all treated sites (One-tailed Mann-Whitney U-Test; Zar 1984).

DISCUSSION

The proposed ten-point visual method of sampling immature blackflies provides a semi-quantitative index of population size, and provides a standardized way of saying "a few" or "a lot". The method assumes that users can distinguish blackfly larvae from other similar-looking fauna, and distinguish pupae from empty pupal cases.

The main advantage of this method over conventional counting methods is that large numbers of samples can be collected quickly. The time taken to assess the abundance of larval blackflies on 30 substrates was about 15 min, depending upon substrate accessibility. The method can be used effectively with minimal equipment and little training, although first-time users should test their estimates against actual (counted) values until they are confident that their estimates are realistic.

In the Onchocerciasis Control Programme in West Africa numbered ribbons are attached to vegetation samples so that the same substrata can be sampled (Kurtak, personal communication 1993). This provides detailed information on larvicidal efficacy in different parts of the river. In this study no attempt was made to sample the same substrata before and after larvicide application. This was because *S. chutteri* prefer the stones-in-current biotope, and tend to release their hold when substrata are returned to the water. Even if they did remain attached, it would be difficult to relocate stones in turbid rapids. On the other hand, some species which prefer marginal vegetation, such as *S. damnosum* s.l., tend to remain attached when substrata are returned to the water, and are therefore suited for "post-sampling".

The proposed method could be adapted for estimating the relative abundance of different-sized blackflies, and organisms such as limpets, leeches, turbellaria and caddisfly larvae, which tend to remain attached to substrata when disturbed. The method could not be used for animals which let go easily, such as mayfly and stonefly nymphs. One disadvantage of the proposed method is that it is limited to loose substrates (stones and trailing vegetation) no deeper than about arm's length.

Samples taken from the edge of large and inaccessible rivers may give biased estimates of blackfly abundance. Where rapids consist of boulders too large to be lifted by hand, an underwater viewing device may be used to assess populations, providing the water is sufficiently clear. I have found that a plastic tube with a perspex plate fitted on one end is useful for examining benthic fauna. Alternatively, underwater cameras may be used to photograph the stream bed (Depner & Charnetski 1978). Failing these methods, a rough idea of blackfly abundance may be obtained by scraping a white cloth or canvas gardening glove over the surface of boulders, and assessing the numbers of larvae which attach to the material (Stoeckl, personal communication 1991). However, in rivers where natural substrates are inaccessible, artificial substrates may be the only reliable option for sampling immature blackflies.

A further disadvantage of the proposed method is that small larvae (first and second instar) may easily be overlooked in the field. The method therefore provides unreliable results when small larvae are abundant. The method should not be used for estimating overall population densities, and should be limited to estimating abundance within a small (4 x 4 cm) area. Despite the limitations, the visual method is practical, simple and quick, and is suited for use in blackfly-control programmes where rapid assessment of larval and pupal abundance is required.

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