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THE IMPACT OF PROPOXUR ON ANOPHELES GAMBIAE S.L. AND SOME OTHER ANOPHELINE POPULATIONS, AND ITS RELATIONSHIP WITH SOME PRE-SPRAYING VARIABLES

by

L. Molineaux,[1] G. R. Shidrawi,[2] J. L. Clarke[3]
R. Boulzaguet[4] and K. Dietz[5]

1. INTRODUCTION

Residual spraying with propoxur was used during the intervention phase of the Research Project on the Epidemiology and Control of Malaria in the African Savanna, conducted by the Government of the Federal Republic of Nigeria and the World Health Organization in the Garki district of Kano State in 1970-1975. Under propoxur, the man-biting population of *Anopheles gambiae* remained at a relatively high level, and the effect of the insecticide varied significantly between different villages; the effect varied also between different anopheline species. This paper analyses these variations and some associated variables, and discusses the causal interpretation and the predictive value of the associations observed.

2. MATERIAL AND METHODS

A. gambiae, species A and B, and *A. funestus* are the local vectors; two other species, *A. rufipes* and *A. pharoensis*, were collected in relatively large numbers; the density indices of all species undergo a very wide seasonal variation, and, in most of the study area, are hardly measurable in the dry season. The baseline period included the dry and wet seasons of 1971 and the dry season of 1972; spraying with propoxur covered 165 villages, compact and/or scattered, situated in an area of approximately 875km^2, during the wet seasons of 1972 and 1973, in 3 or 4 rounds each, at intervals of two months, at the dosage of 2 gm techn.propoxur/m^2. The intervention period includes these two wet seasons and the intervening dry season. Intensive entomological evaluation was conducted in eight compact villages, six sprayed and two unsprayed (see Fig.1). During the wet season the following studies were carried out at fortnightly intervals: night-biting collections (NBC, with 2 man-nights indoors and 2 man-nights outdoors each in two stations; collector-baits rotated systematically between indoor and outdoor positions, stations and villages, so as to avoid any bias resulting from differential attraction and/or ability), pyrethrum spray collections (PSC, in 5 to 12 huts), and exit-trap collections (ETC, in 3 huts during the baseline period, in 5 huts during the intervention period). In the dry season, NBC and ETC were performed every five weeks only. The collections were conducted in fixed stations, different for each type of collection. In sprayed villages, the huts used as collection stations were, in principle, sprayed like any other hut; collections were also made in artificial pit-shelters, but these were very unproductive and the relationship of the yield to the populations of anophelines resting outdoors is unknown. There was no systematic larval search. Identification of species A and B of *A. gambiae* was performed only in a fraction of the PSC.

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[1]Medical Officer, WHO/MPD, Geneva, Switzerland.
[2]Entomologist, WHO/EMRO, Alexandria, Egypt.
[3]Entomologist, WHO, Sri Lanka.
[4]Technical Officer, WHO, Kano, Nigeria.
[5]Statistician, WHO/HST, Geneva, Switzerland.

3. RESULTS

3.1 The effect of propoxur on the number of A. gambiae s.l. collected by NBC and its variation between villages

As regards the different species and the various methods applied, only the NBC collections of A. gambiae s.l. produced sufficient numbers for a comparison between the different sprayed villages.

In measuring the effect of propoxur on the NBC, both the pre-spraying data and the data from the unsprayed controls should be taken into account. It was assumed that, without propoxur, the treated villages would have shown in 1972 and 1973 the same proportional changes as the unsprayed controls; under this assumption, the expected total numbers of A. gambiae collected by NBC indoors (IN) and outdoors (OUT) in the wet seasons of 1972 and 1973 in a given village were 0.42 and 1.33 times the 1971 figure. The corresponding factors for the indoors and outdoors collections were calculated separately; Table 1 shows the numbers actually collected, by village and by wet season, and, in the case of the sprayed villages, the ratio of the number observed to the number expected. The numbers collected by night-biting collection (IN, OUT, or TOT) are directly comparable since they represent, for each year and village, the total yield obtained during a period starting with very low densities at the end of the dry season, rising to high or relatively high densities during the wet season and returning finally to very low densities at the end of the wet season, using a fixed number of baits and a fixed sampling frequency. A few collections were missed (see footnote (1) to Table 1), but only at times of low density, so that the total number collected is very little affected. The analysis therefore starts from the actual numbers collected: replacement of the few missing NBCs by interpolation would have no effect on the conclusions. On the average, in the sprayed follow-up units, the density of A. gambiae - as estimated by NBC - was reduced to 10 and 13% of its expected value (wet seasons of 1972 and 1973 respectively). The reduction was greater indoors and lesser outdoors, but there was a relatively strong association between the indoors and outdoors reductions in the same village (see Table 1).

From Table 1 it can be seen that the ratio between observed and expected numbers varies from village to village. The differences are real: in the six sprayed villages the distribution of A. gambiae collected in 1972 and 1973 is significantly different from the one expected on the basis of 1971, related to indoors, outdoors, and total collections (chi-square test, $p < .001$). The differences are also relatively stable: there is a strong positive correlation between the 1972 and the 1973 findings (observed/expected) in the same village (correlation coefficients significantly different from zero at the 5% level for IN, OUT, IN + OUT; rank correlation coefficients significantly different from zero, at the 5% level, for IN, IN + OUT).

While the NBCs in the six sprayed villages produced higher numbers in 1973 than in 1972, the effect of spraying, in terms of reduction of the expected NBC yield, is on the average, only slightly (probably not significantly) smaller in 1973; in individual villages it appears to be smaller in 3 cases, greater in the remaining 3.

3.2 Variables associated with inter-village differences

In looking for variables possibly associated with the significant and relatively stable differences between villages, the following factors were considered: the coverage achieved by the spraying operations, the risk of immigration of vectors, and the baseline entomological observations.

The recorded spraying coverage was very high (99%); it varied very little between villages, the variations in 1972 and 1973 were not significantly correlated. There was no significant association with the variation of the effect of propoxur.

The latitude of sprayed villages and their proximity to unsprayed villages were taken as possible indicators of the risk of immigration of vectors. Neither is regularly associated with the variation of the effect of propoxur, as can be seen from Fig. 1 and Table 1. For instance, the two northernmost villages which are closest to unsprayed villages (Ungwar Bako 4, Rafin Marke 5), are at the extremes of the range of response to propoxur.

Among the baseline entomological observations considered, some relate to the proportion of species A and B in the different villages; most relate to A. gambiae s.l. and in particular to possible indicators of its behaviour.

The ratio of NBC to PSC (man-fed component) yields is possibly an index of exophily (see Discussion), and its potential association with the variation in response to propoxur was investigated. The results are given in Table 2 and Figs. 2 and 3.

With respect to the pre-spraying NBC/PSC ratio the observed variation between villages, is relatively wide and significantly associated with the variation in the response to propoxur: the higher the pre-spraying ratio, the higher the residual NBC yield (observed/expected) after spraying; the association holds for the indoors NBC or the average NBC (it does not hold for the outdoors NBC alone). The PSC was expressed in (females/hut) times (proportion of fed females positive for human blood in the precipitin test); the ratio of the PSC yield in females/hut to the PSC yield in fed females/sleeper did not in fact vary significantly between times or places.[1]

If we use the total PSC yield (females/hut) instead of its man-fed component only, the correlation between the pre-spraying NBC/PSC ratio and the residual (observed/expected) NBC remains significant but becomes somewhat weaker; the man-fed proportion of the PSC yield showed only moderate variation: 0.61 (49/80) in village 3, Sugungum, 0.88 to 0.98 (in samples of 51 to 132) in the others; by itself, it showed no correlation with the residual NBC.

Some other pre-spraying variables may reflect vector behaviour: the ratios between NBC (OUT) and NBC (IN), between ETC and PSC, between fed and gravid in the PSC and in the ETC; between males and females in the PSC; the biting hours. Their variation between villages was compared to the variation in post-spraying NBC (observed/expected); there was a significant association only in the case of the biting hours, as shown in Table 3 and Fig.4: the later the median biting hour, the greater the post-spraying NBC (Observed/expected).

The absolute post-spraying NBC is significantly associated with the pre-spraying NBC, but the relative (observed/expected) post-spraying NBC is not.

The observed pre-spraying distribution Species A and B of A. gambiae s.l., captured by PSC, and its relationship with the residual NBC under propoxur, by village, are shown in Table 4. This information is not available for all villages, for 1971 it applies only to the month of August, and some of the ratios are based on relatively small numbers. With these restrictions in mind it appears that, the higher the pre-spraying proportion of species B, the higher the residual NBC (observed/expected); however, the association is usually not statistically significant. The proportion of species B may vary relatively little between villages (in 1970, the variation was not statistically significant, by the chi-square test) in comparison with the residual NBC.

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[1] Project IR 0172, Entomology baseline report

The village characteristics which we have tried to relate to the response to propoxur, may obviously be interdependent; the pre-spraying NBC (IN + OUT)/PSC ratio is, in fact, strongly associated with the pre-spraying median biting hour (r = + 0.90; R = + 0.90, both significantly different from zero at the 5% level), not, however, with the pre-spraying proportion of species B (r = +0.21 and R = +0.13, using the cytotaxonomic results of 1970; r = + 0.13 and R = + 0.68, using the cytotaxonomic results of 1971).

3.3 Pre-spraying NBC/PSC ratio, median biting hour and response to propoxur: comparison between species

As already mentioned, four anopheline species were collected in relatively large numbers: A. gambiae s.l., A. funestus, A. pharoensis and A. rufipes; of these, only the first three were collected in large numbers by NBC. Table 5 shows their pre-spraying NBC/PSC ratios and their residual densities under propoxur. It should be noted that we used, for convenience, the total numbers collected by all sampling methods during the whole baseline and intervention periods, respectively. This permits comparisons between species, even though the NBC/PSC ratio is biased upwards by the inclusion of the dry season (when PSC is more productive than NBC), and even though the two periods differ in their seasonal composition so that the "expected" is here more arbitrary as compared to Table 1.

At least for the three species biting man in relatively large numbers, there is a clear positive association between the pre-spraying NBC/PSC ratio (1.17; 0.82; 43.34 for A. gambiae, A. funestus, A. pharoensis, respectively) and the residual density under propoxur (0.07; 0.02; 0.79).

The median biting hour (IN + OUT), in the wet season of 1971 (baseline), in all villages combined, was:

for A. gambiae s.l.	01 : 55
for A. funestus	03 : 18
for A. pharoensis	21 : 55

Thus, among these three species, there is a negative association between the pre-spraying median biting hour and the residual NBC under propoxur, while between villages the association was positive (see above).

3.4 NBC/PSC ratio : variation over time

In the course of the wet season and the early dry season of 1971 (baseline), the NBC/PSC ratio of A. gambiae s.l. increased progressively as shown in Table 6 and Fig. 5. There was no corresponding systematic change in the ETC/PSC ratio, in the fed/gravid ratio in either ETC or PSC, or in the man-fed proportion of the PSC yield. The latter was studied only in September, October and November, i.e. the second half of the period considered.

The NBC/PSC ratios of the two untreated villages, studied in detail in three successive years, are shown in Table 7. The ratios vary from year to year; within a given year, the ratios are higher in village 2 than in village 1; the NBC (IN)/PSC ratio was less variable than the NBC (IN + OUT)/PSC ratio; replacing the total PSC by its man-fed component reduced the variation somewhat.

4. DISCUSSION

4.1 Sampling error

Sampling error in the pre-spraying NBC and/or PSC would, by itself, produce variation in the NBC/PSC ratio. However, if the variation in the ratio were due to error in the PSC, no association would be expected with the residual NBC (observed/expected); if the variation in the ratio were due to error in the NBC, a negative association would be expected with the residual NBC (observed/expected) since over-estimation of the pre-spraying NBC (and of the NBC/PSC ratio) would over-estimate the effect of the insecticide, and vice-versa.

4.2 Resting Behaviour

The NBC/PSC ratio must reflect both the resting behaviour of an anopheline mosquito and the relative bias of the two sampling methods. In the case of A. gambiae s.l. in the West African savanna, a relatively high ratio (i.e. higher than expected under the hypothesis of complete endophily) has often been noted and attributed either to the relative bias of the sampling methods (Garrett-Jones and Shidrawi 1969) or to relative exophily (Hamon et al 1959). The observation that the prespraying NBC/PSC ratio of A. gambiae s.l. in a given village is positively associated with its residual (observed/expected) NBC under propoxur would at least in part be explained by exophily. The positive association of the pre-spraying NBC/PSC ratio of a given man-biting species with its residual (observed/expected) NBC under propoxur again suggests that the ratio is an indicator of exophily; this seems obvious in the extreme case of A. pharoensis, but may also be true for the species of which large numbers are collected indoors (A. gambiae s.l., A. funestus). It seems also more plausible to explain differences of the NBC/PSC ratios between species by differences in the degree of exophily rather than by species-specific in the relative bias of the two sampling methods.

The NBC/PSC ratio of A. gambiae s.l. increases progressively in the course of the wet season. In theory, this could be due to progressive increase in anthropophily (a decreasing contribution of animal-fed mosquitos to the PSC), to a decrease in the time spent indoors, to a decrease in the proportion resting indoors after feeding, or to a change in the relative bias of the two sampling methods. There is no systematic change in the results of the precipitin test, which rules out the first explanation; there is also no systematic change in the ETC/PSC ratio, or in the fed/gravid ratio in either ETC or PSC, which rules out the second explanation. Of the last two explanations, a progressive increase in the proportion resting outdoors, possibly related to the simultaneous marked increase in vegetation (Foll et al, 1965; Hamon, 1963) is more plausible than a systematic change in the relative bias of the two sampling methods.

4.3 Predictive value of NBC/PSC ratio

If the NBC/PSC ratio is indeed an indicator of exophily and a predictor of the effect of a residual insecticide, it would be interesting to know whether it applies to other anopheline species, environments, insecticides. So far, a comparison has been made with observations in Kisumu, Kenya, where fenitrothion produced a much greater reduction in A. gambiae s.l. than the one produced by propoxur in Garki[1]; the recorded spraying coverage was equally high in both places; the entomological sampling methods were essentially the same. In Kisumu, the pre-spraying NBC (IN/PSC ratio is available from six locations for five months: it was 0.37 on the average, and varied from 0.24 to 0.47 between the individual localities (vs 0.32 to 2.7 in Garki, see Table 2, using the total PSC as in Kisumu). The ratio of persons to huts was two in both places, so that the lower NBC/PSC ratio in Kisumu suggests that the local A. gambiae s.l. is more endophilic; this could at least in part explain the difference of result. In Kisumu, under fenitrothion, there was no significant correlation between the residual NBC of a given village and its pre-spraying NBC/PSC ratio, but the numbers of mosquitos collected after spraying were very small, and the variation of the pre-spraying NBC/PSC ratio was also very small.

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[1] Project IR 0403, Monthly reports

If the NBC/PSC ratio is a qualitative predictor of the effect of a residual insecticide, it would be interesting to know its value for a quantitative prediction also. This will obviously depend on the importance of the other factors, such as the model applied for this factor, the quality of the spraying operations and the measurement error. If an exponential model is fitted to the data included in Fig.2, the following relationship is obtained:

$$y = 1 - \exp(-0.058\ x)$$

where y is the residual NBC and x the pre-spraying NBC(IN)/PSC (man-fed) ratio. The fitting was made by wėighing the points by their pre-spraying NBC(IN)/PSC ratio. The corresponding curve has been included in Fig.2. According to the model, the residual density (observed/expected) increases with the pre-spraying NBC/PSC ratio, from zero for a ratio of zero (i.e. when the pre-spraying NBC is zero, the residual NBC is also zero) to a maximum of 1, when the ratio tends to infinity (i.e. when the pre-spraying PSC is negligible in relation to the NBC, the latter is not affected by spraying). In Kisumu the average pre-spraying man-fed proportion in the PSC was 0.95, the average pre-spraying NBC(IN)/PSC (man-fed) was 0.37/0.95 = 0.39, and the residual NBC (IN, observed/expected), expected from the above model, was 1 - exp(0.058)(0.39) = 0.022. The residual NBC (IN, observed/expected), estimated from the actual observations of the last baseline year and the first intervention year by the same method as in Garki was 0.007.

4.4 Degree of error (prediction)

In practice, the spontaneous changes estimated in the comparison villages and used in the above "predictions" are only known a posteriori and therefore not available for a prediction in the true sense. If they are ignored, the best estimate of the expected NBC, in the absence of insecticide, is the pre-spraying NBC in the same village; the correlation between pre-spraying characteristics (NBC/PSC ratio or median biting hour) and residual NBC (observed/expected, where "expected" is redefined as the uncorrected pre-spraying value) in a particular year (1972, 1973) is not affected because it involves only the multiplication of the residual NBC by a constant, which does not affect its correlation coefficient, or rank correlation coefficient with any other variable; however, a large error is introduced in the prediction of the actual NBC from the NBC/PSC ratio, or, for that matter from any other predictor: the prediction would, by definition, be the same for 1972 and 1973, while the outcome is very different (by a factor of 1859/458 = 4.1, on the average, see Table 1); this is a simple illustration of the relatively large error that may be involved in making absolute predictions.

4.5 Median biting hour

The median biting hour may be determined in part by the distance from the breeding sites if the mosquito re-feeds on the night of oviposition, which is probably usual for *A. gambiae* s.l. in Garki (35 specimens collected by NBC at different times were classified by the condition of their ovariolar sacs; all had uncontracted sacs). Based on the comparison between villages with respect to *A. gambiae* s.l., it may be suggested to use the median biting hour in predicting the effect of a residual insecticide as it determines the potential exposure time; but this hypothesis is probably too simple, in view of the fast action of propoxur and of the comparison between species. The median biting hour of *A. gambiae* s.l. is strongly related to the NBC/PSC ratio; it may be determined by the same genetic and environmental factors (see below) and may be only indirectly related to the response to a residual insecticide; this response probably varies, not with the exposure time for exposed mosquitos, but with the proportion of mosquitos not exposed at all.

4.6 Species composition, A. gambiae

In this study the pre-spraying proportion of species A and B was a less reliable predictor of the effect of propoxur than the pre-spraying NBC/PSC ratio. Also the variation, between villages, of the A/B ratio, does not appear important enough to explain their variation in the residual NBC under propoxur - the two pre-spraying years gave inconsistent results.

The relationship between other cytotaxonomic observations (inversion polymorphism), and response to the residual insecticide, has not yet been analysed.

4.7 Genetic and environmental factors

The resting behaviour of a mosquito is the result of genetic factors, environmental factors, and chance. Detailed genetic and environmental studies would be required to understand and predict behaviour. However, to predict response to a residual insecticide it may be preferable and, when only limited resources are available also, more reliable, to measure behaviour itself; this apporach integrates the effects of genetic and environmental factors. A ratio between numbers collected by different standard sampling methods, e.g. the NBC/PSC ratio, may be a useful indicator of behaviour; even though it is an indirect and imperfectly understood indicator, it may have some predictive value.

4.8 Test of hypothesis

There are obvious limitations to the interpretation of associations demonstrated retrospectively. It may be argued that the hypothesis of the predictive value of the NBC/PSC ratio has, in fact, been tested prospectively: on the one hand the study was explicitly designed in a way allowing the detection of differences between villages, including differences in their response to the insecticide; on the other hand, the NBC/PSC ratio has been used previously as an indicator of exophily (Hamon et al 1959). Admittedly, this is not a prospective approach, but a true experiment is not feasible since the pre-spraying degrees of exophily and/or NBC/PSC ratio do not lend themselves to manipulation. It may, however, be desirable to include the testing of the hypothesis in projects designed for the evaluation of insecticides.

5. SUMMARY

A significant and stable difference in the residual night-biting collection (NBC, observed/expected) of A. gambiae s.l. has been observed between different villages of the West African savanna, sprayed for two years with propoxur. The residual mosquito density of a given village was positively associated with some of its pre-spraying characteristics: the NBC/PSC ratio, the median biting hour and, possibly, the proportion of species B; it was not significantly associated either with several other pre-spraying characteristics such as absolute density, ratios between NBC (IN) and NBC (OUT), between ETC and PSC, between fed and gravid in the PSC or the ETC, between males and females in the PSC, or with variations in recorded coverage, or with latitude or distance from unsprayed villages.

In comparison between the species A. gambiae s.l., A. funestus and A. pharoensis, the residual NBC (observed/expected) was positively associated with the pre-spraying NBC/PSC ratio, and negatively associated with the median biting hour.

The pre-spraying NBC/PSC ratio thus appears to be a predictor of the variation, between villages or species, in the reduction of the NBC by residual spraying. It could reasonably predict the difference between the response of A. gambiae s.l. to propoxur in Garki and to fenitrothion in Kisumu and may assist in forecasting the effect of a residual insecticide. The limitations of any absolute prediction must, though, be kept in mind, especially in view of unpredictable spontaneous changes of the underlying situation.

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RESUME

Une différence significative et stable dans la collecte de spécimens piquant la nuit (CSPN, observée/prévue) d'A. gambiae s.l. a été observée entre divers villages de la savane d'Afrique occidentale traités pendant deux ans par pulvérisations de propoxur. On a mis en évidence une assocaition positive entre, d'une part, la densité anophélienne résiduelle d'un village donné et, d'autre part, certaines de ses caractéristiques antérieures au traitement: rapport CSPN/CPP*, heure médiane de piqûre et, peut-être, proportion de l'espèce B; par contre, on n'a pas découvert d'association significative avec plusieurs autres caractéristiques antérieures au traitement (densité absolue, rapports entre CSPN (air libre) et CSPN (habitations), entre CPS** et CPP, entre spécimens gorgés et gravides dans la CPP ou la CPS, entre mâles et femelles dans la CPP) ni avec des variations dans la couverture insecticide, avec la latitude ou avec la distance par rapport aux villages non traités.

Dans la comparaison entre les espèces A. gambiae s.l., A. funestus et A. pharoensis la CSPN résiduelle (observée/prévue) était positivement associée avec le rapport CSPN/CPP antérieur au traitement, et négativement associée avec l'heure médiane de piqûre.

Le rapport CSPN/CPP antérieur au traitement paraît ainsi constituer un prédicteur de la variation, entre villages ou espèces, de la réduction de la CSPN du fait de pulvérisations d'insecticides à effet rémanent. Il aurait assez bien permis de prédire la différence entre la réponse d' A. gambiae s.l. au propoxur à Garki et au fénitrothion à Kisumu. Il peut donc aider à prédire l'effet d'un insecticide à effet rémanent, mais il ne faut pas perdre de vue les limitations inhérentes à toute prédiction absolue, en particulier eu égard aux modifications spontanées imprévisibles de la situation initiale.

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* CPP : collecte après pulvérisation de pyrèthre.

** CPS : collecte dans piège à la sortie

Table 1 The number [1] of A.gambiae s.l. collected by night-biting collection (NBC), by wet season and by village, and, in the sprayed villages, the ratio of that number to number expected [2]

Village	Treatment	1971(21/6 to 7/11)			1972(22/5 to 22/10)			1973(18/6 to 4/11)		
		IN	OUT	TOT	IN	OUT	TOT	IN	OUT	TOT
1, Kwaru	none	359	275	634	95	138	233	386	689	1,075
2, Ajura	none	495	344	839	187	204	391	438	445	883
Subtotal	none	854	619	1,473	282	342	624	824	1,134	1,958
3, Sugungum	propoxur in 1972 & 1973	2,439	2,939	5,378	61 (.076)[3]	186 (.11)	247 (.11)	289 (.12)	804 (.15)	1,093 (.15)
4, Ungwar Bako	propoxur in 1972 & 1973	335	442	777	4 (.036)	12 (.049)	16 (.049)	15 (.046)	35 (.043)	50 (.048)
6, Matsari	propoxur in 1972 & 1973	943	522	1,465	11 (.035)	42 (.15)	53 (.085)	44 (.048)	153 (.16)	197 (.10)
8, Jaya	propoxur in 1972 & 1973	670	678	1,348	19 (.086)	37 (.099)	56 (.098)	79 (.12)	239 (.19)	318 (.18)
5, Rafin Marke	propoxur in 1972 & 1973	288	153	441	16 (.17)	38 (.45)	54 (.29)	42 (.15)	85 (.30)	127 (.22)
7, Nasakar	propoxur in 1972 & 1973	495	933	1,428	5 (.031)	27 (.052)	32 (.053)	10 (.021)	64 (.037)	74 (.039)
Subtotal	propoxur in 1972 & 1973	5,170	5,667	10,837	116 (.068)	342 (.11)	458 (.10)	479 (.096)	1,380 (.13)	1,859 (.13)

(1) the actual numbers collected are shown; the number of collections was nearly, but not exactly constant for each village and wet season; in 1971, villages 1-3 had 10 NBC's, villages 4-8 had 9; in 1972, village 4 had 9, the others 10; in 1973, village 4 had 8, the others 10 (see text, 3.1)

(2) the number expected was computed on the assumption that villages 3-8 would, in the absence of propoxur, have undergone the same natural change as villages 1-2; e.g. the expected no. IN, in village 3, in 1972 = 2,439 x (282/854) = 805

(3) ratio of the observed to the expected, i.e. 61/805 = 0.076

Table 2 The pre-spraying ratio of the man-biting rate of A.gambiae s.l. to its man-fed indoor resting density, in the wet season of 1971, in various villages, and the residual NBC (observed/expected), under propoxur, in the wet seasons of 1972 and 1973.

Village	NBC(IN)/man-nights / (PSC/huts)(HBI)(1) in 1971	NBC(IN), obs./exp.(2) in 1972	NBC(IN), obs./exp.(2) in 1973	NBC(IN + OUT)/man-nights / (PSC/huts) (HBI) in 1971	NBC(IN+OUT) obs/exp(2) in 1972	NBC(IN+OUT) obs/exp(2) in 1973
5 Rafin Marke	$\frac{288/36}{(115/39)(50/51)} = 2.8$	.17	.15	$\frac{441/72}{(115/39)(50/51)} = 2.1$	.29	.22
8 Jaya	$\frac{670/36}{(810/81)(104/117)} = 2.1$	.086	.12	$\frac{1,348/72}{(810/81)(104/117)} = 2.1$	.098	.18
3 Sugungum	$\frac{2,439/40}{(5,066/69)(49/80)} = 1.4$	.076	.12	$\frac{5,378/80}{(5,066/69)(49/80)} = 1.5$	.11	.15
6 Matsari	$\frac{943/36}{(1,422/66)(123/132)} = 1.3$	.035	.048	$\frac{1,465/72}{(1,422/66)(123/132)} = 1.0$	.085	.10
4 Ungwar Bako	$\frac{335/36}{(1,125/79)(119/132)} = 0.72$	.036	.046	$\frac{777/72}{(1,125/79)(119/132)} = 0.84$	.049	.048
7 Nasakar	$\frac{495/36}{(2,019/47)(99/112)} = 0.36$	.031	.021	$\frac{1,428/72}{(2,019/47)(99/112)} = 0.52$	.053	.039

NBC(IN), 1971 vs 1972: r = +0.91 * (3), R = +0.94 *
NBC(IN), 1971 vs 1973: r = +0.90 *, R = +0.94 *

NBC(IN+OUT), 1971 vs 1972: r = +0.72, R = +0.89*
NBC(IN+OUT), 1971 vs 1973: r = +0.97 **, R = +1.00**

(1) HBI = human blood index (no.pos. for man/no examined, in the PSC)
(2) from table 1
(3) r = correlation coefficient, R = Spearman's rank correlation coefficient
*,** : significantly different from zero, at the 5% and 1% levels, respectively

Table 3 The pre-spraying median biting hour of <u>A.gambiae</u> s.l., and the residual NBC, under propoxur, by village

Village	Median biting hour (1) in 1971 (3)	NBC(IN + OUT), obs./exp. (2) in 1972 (3)	in 1973 (3)
3, Sugungum	01 : 40	.11	.15
4, Ungwar Bako	01 : 58	.049	.048
6, Matsari	02 : 06	.085	.10
8, Jaya	02 : 24	.098	.18
5, Rafin Marke	03 : 09	.29	.22
7, Nasakar	01 : 36	.053	.039

r = + 0.86 * R = + 0.54

r = + 0.76 R = + 0.83

(1) By interpolation from the distribution of bites by two hour periods, NBC (IN + OUT)

(2) From table 1

(3) Wet season, see table 1

*: significantly different from zero at the 5% level

Table 4 The pre-spraying proportion of species B among the A.gambiae s.l., collected by PSC and the residual NBC (A.gambiae s.l., observed/expected), under propoxur, by village.

Village	prop. B July - October 1970 (1)		prop. B, in August 1971 (2)		NBC(IN + OUT), obs./exp. 1972 (3)	NBC(IN + OUT), obs./exp. 1973 (3)
3, Sugungum	-		0.87	(558/640)	.11	.15
4, Ungwar Bako	0.84	(83/99)	0.66	(19/29)	.049	.048
6, Matsari	0.90	(70/78)	0.76	(105/139)	.085	.10
8, Jaya	0.94	(116/123)	0.69	(63/91)	.098	.18
5, Rafin Marke	0.90	(87/97)	-	(4/6)	.29	.22
7, Nasakar	0.93	(62/67)	0.63	(99/156)	.053	.039

r = + 0.82*, R = + 0.80

r = + 0.58, R = + 0.70

r = + 0.09, R = + 0.43

r = + 0.35, R = + 0.13

(1) from Shidrawi (ref. (1))

(2) from Coluzzi et al (ref. (2))

(3) wet season, see table 1

* significantly different from zero at the 5% level

Table 5 The pre-spraying NBC/PSC ratio of different anopheline species and their residual density under propoxur

Period	Villages	Collections	Variable	Species			
				A.gambiae s.l.	A.funestus	A.rufipes	A.pharoensis
Baseline (B) 26/10/70 to 21/5/72	all	NBC (1,592 man-nights) PSC (3,070 hut-collections)	no coll^{d} by NBC	12,948	2,648	10	809
			no coll^{d} by PSC	21,363	6,190	992	36
			NBC (no/man-nights) / PSC (no fem/huts	1.17	0.82	0.02	43.34
	Controls (C)	all	number, N(B,C)	6,551	1,542	131	94
	To be sprayed (S)	all	number, N(B,S)	30,194	8,444	1,044	780
Intervention (I) 22/5/72 to 4/11/73	Controls (C)	all	number, N(I,C)	8,407	532	210	79
			N(I,C)/N(B;C) (1)	1.28	0.35	1.60	0.84
	Sprayed (S)	all	number, N(I,S)	2,525	48	152	519
			N(I,S)/N(B,S) (2)	0.08	0.01	0.15	0.67
			N(I,S)/N(B,S) / N(I,C)/N(B,C) (3)	0.07	0.02	0.09	0.79

(1) The change expected in the absence of spraying

(2) The change observed in the presence of spraying

(3) observed/expected

Table 6 The NBC/PSC ratios of A.gambiae s.l., per 4 weeks period, during the wet season of 1971 (1)

4 weeks period	NBC(IN)/man-nights / PSC/huts	NBC(IN + OUT)/man-nights / PSC/huts
21/6 to 18/7	$\frac{93/24}{227/34}$ = 0.58	$\frac{171/48}{227/34}$ = 0.53
19/7 to 15/8	$\frac{953/24}{2,552/36}$ = 0.56	$\frac{1,765/48}{2,552/36}$ = 0.52
16/8 to 12/9	$\frac{1,357/24}{2,602/33}$ = 0.72	$\frac{2,822/48}{2,602/33}$ = 0.75
13/9 to 10/10	$\frac{798/24}{1,342/35}$ = 0.87	$\frac{1,821/48}{1,342/35}$ = 0.99
11/10 to 7/11	$\frac{92/24}{121/34}$ = 1.08	$\frac{272/48}{121/34}$ = 1.59

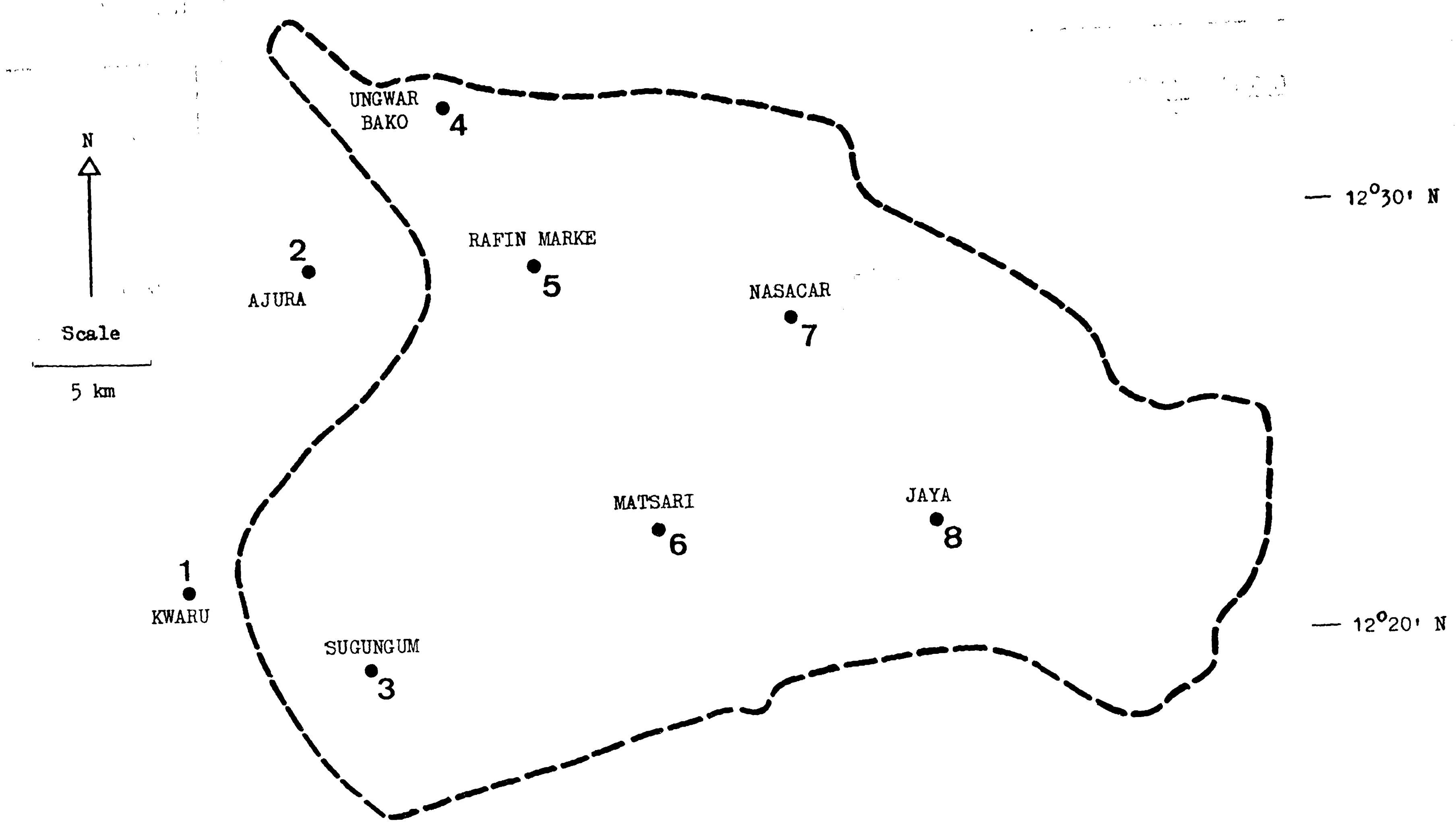
(1) in the villages 1, 2, 3, i.e. those examined every fortnight by NBC and PSC, throughout the period of 20 weeks; see table 1, footnote (1)

Table 7 The NBC/PSC ratio of <u>A.gambiae</u> s.l., in two untreated villages, in the wet season, in three successive years

Village	Year	$\frac{\text{NBC(IN)/man-nights}}{\text{PSC/huts}}$	$\frac{\text{NBC(IN)/man-nights}}{\text{(PSC/huts)(HBI)}^{(1)}}$	$\frac{\text{NBC(IN+OUT)/man-nights}}{\text{PSC/huts}}$	$\frac{\text{NBC(IN)/man-nights}}{\text{(PSC/huts)(HBI)}^{(1)}}$
1 Kwaru	1971	$\frac{359/40}{636/40} = 0.56$	$\frac{359/40}{(636/40)(97/103)} = 0.60$	$\frac{634/80}{636/40} = 0.50$	$\frac{634/80}{(636/40)(97/103)} = 0.53$
	1972	$\frac{95/40}{151/52} = 0.82$	-	$\frac{233/80}{151/52} = 1.0$	-
	1973	$\frac{386/40}{605/45} = 0.72$	$\frac{386/40}{(605/45)(202/210)} = 0.75$	$\frac{1,075/80}{605/45} = 1.0$	$\frac{1,075/80}{(605/45)(202/210)} = 1.0$
2 Ajura	1971	$\frac{495/40}{1,142/63} = 0.68$	$\frac{495/40}{(1,142/63)(81/91)} = 0.77$	$\frac{839/80}{1,142/63} = 0.58$	$\frac{839/80}{(1,142/63)(81/91)} = 0.65$
	1972	$\frac{187/40}{323/74} = 1.1$	-	$\frac{391/80}{323/74} = 1.1$	-
	1973	$\frac{438/40}{548/69} = 1.4$	$\frac{438/40}{(548/69)(121/125)} = 1.4$	$\frac{883/80}{548/69} = 1.4$	$\frac{883/80}{(548/69)(121/125)} = 1.4$

(1) HBI = human blood index (no. pos. for man/no.examined, in the PSC)

Fig. 1 The area within which all (165) villages were treated with propoxur (broken line), and the 8 villages followed by night-biting collection



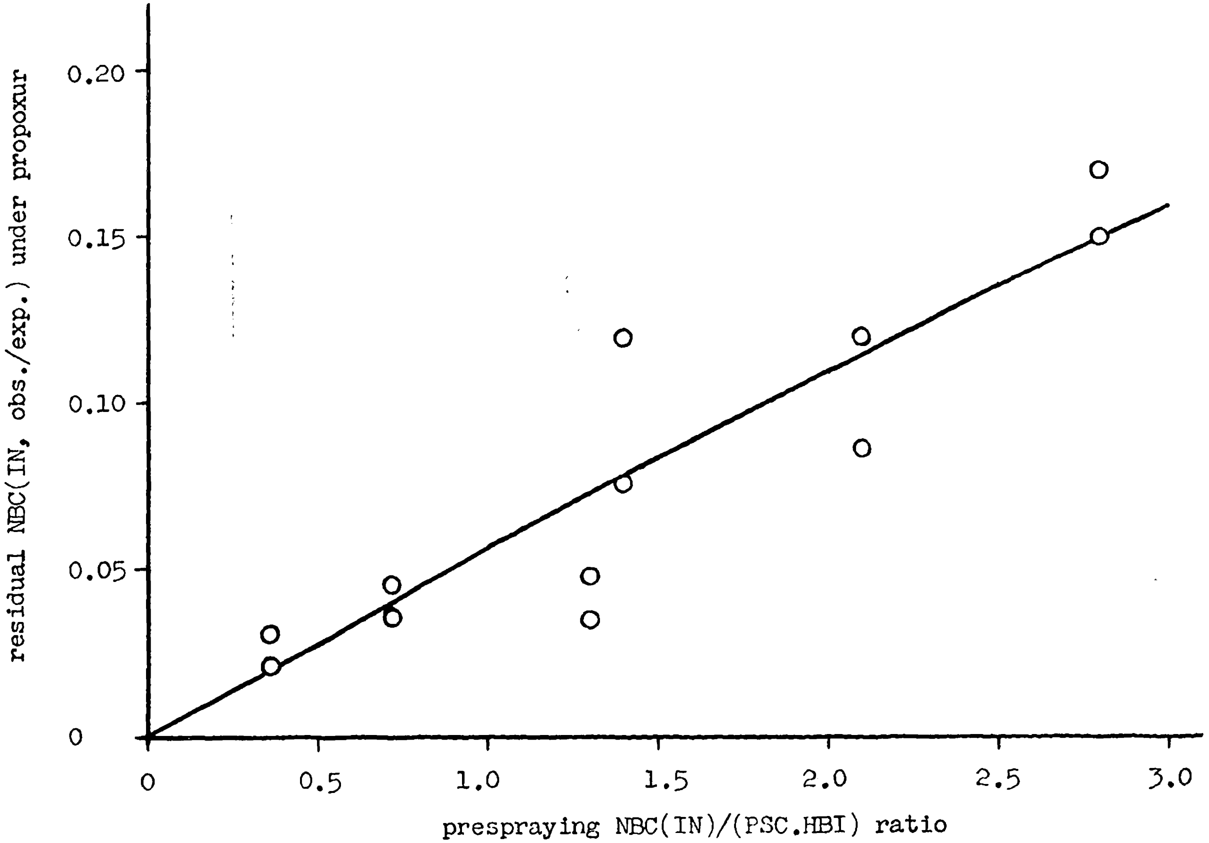


Fig.2 The prespraying (1971) NBC(IN)/(PSC.HBI) ratio of A.gambiae s.l., and its residual NBC (IN), under propoxur (1972, 1973), by village (see tables 1,2). The curve represents $y = 1 - \exp(-0.058\ x)$(see text 4.2).

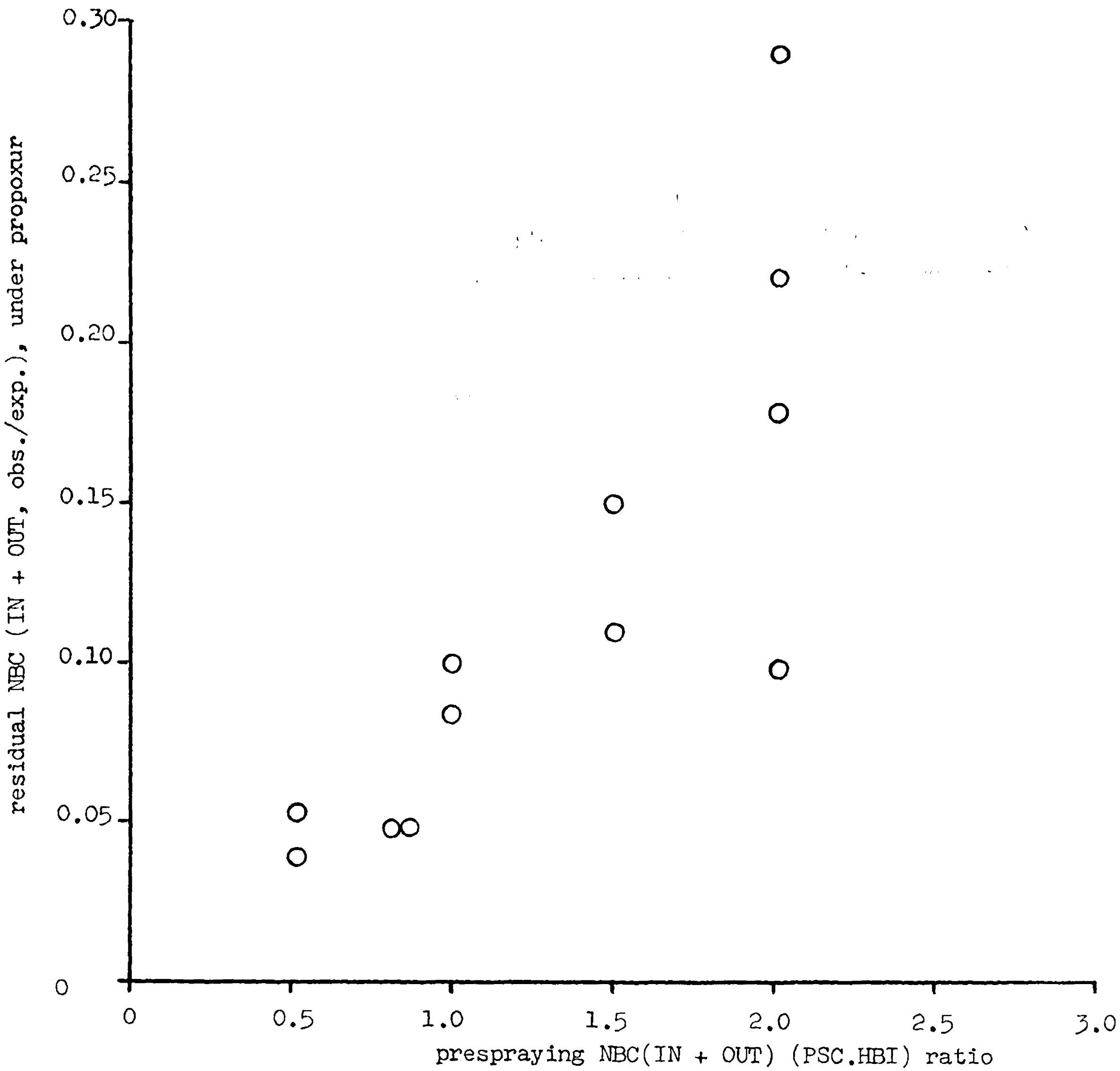


fig.3 The prespraying (1971) NBC (IN + OUT)/(PSC.HBI) ratio of <u>A.gambiae</u> s.l., and its residual NBC (In + OUT), under propoxur (1972, 1973) by village (see tables 1,2)

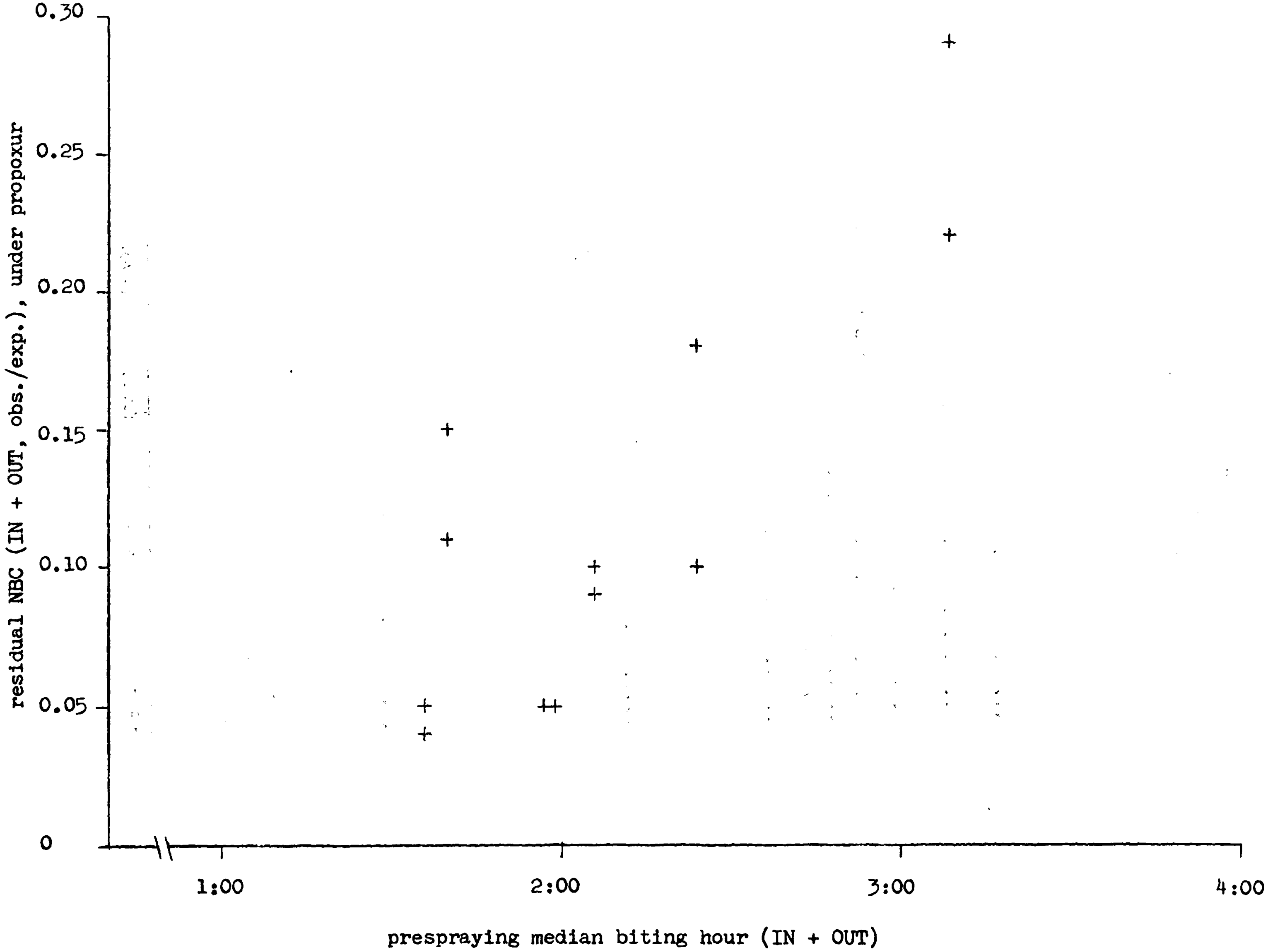


fig. 4 The prespraying (1971) median biting hour (NBC, IN + OUT) of A.gambiae, s.l., and its residual NBC(IN + OUT), under propoxur (1972, 1973), by village (see tables 1, 3)

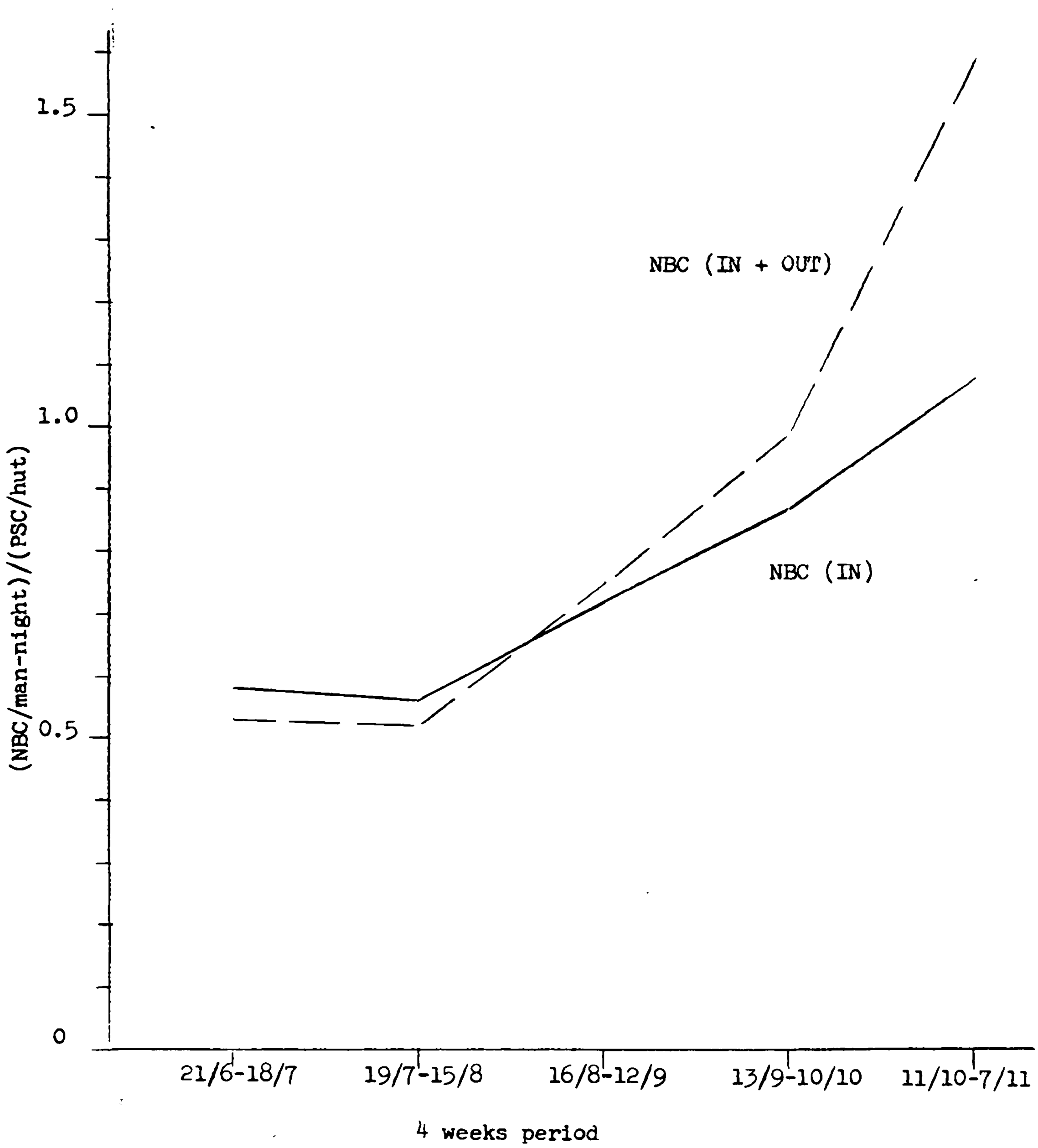


Fig. 5 The NBC/PSC ratios of A.gambiae s.l., per 4 weeks period, during the wet season of 1971 (see table 6)