

Behaviour of tsetse (Diptera: Glossinidae) during the hot season in Zimbabwe: the interaction of micro-climate and reproductive status

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Abstract

Studies were made of the behaviour of *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood during the hot season (September–November) in Zimbabwe, and attributes of samples of tsetse from refuges, odour-baited traps, targets and mobile baits were compared. Various arrangements of electric nets were used to study tsetse as they entered or left artificial refuges. The peak time of entry into a refuge varied between 0800 h and 1400 h and coincided with the time when the air temperature reached 32°C; the response was stronger if 32°C occurred earlier in the day. The peak time of exit varied between 1500 h and 1700 h, being significantly later on hotter days, but did not show a clear temperature threshold. Micro-meteorological measurements showed that refuges were significantly cooler than the surrounding riverine woodland during the day but warmer at night. There was no significant difference between the air temperatures in leafless mopane woodland and semi-evergreen riverine woodland during the day but at night the riverine woodland was significantly cooler. Combining the micro-meteorological data with the estimated local movements of tsetse suggested that during the hot season, tsetse experienced temperatures 2°C cooler than the daily mean in a Stevenson screen located in mopane woodland. Compared with the catches of tsetse from traps, refuges had higher proportions of *G. m. morsitans*, males, young flies and females in the later stages of reproduction, and it is suggested that during the hot season, samples from refuges were less biased than traps with respect to species and sex composition, age and reproductive status. During the hot season, tsetse populations declined by *c.* 90% and although air temperatures exceeded lethal levels (*c.* 40°C), the refuge-entering responses meant that adult flies probably experienced a maximum of only *c.* 35°C. It is suggested that the decline in numbers is not due to direct mortality effects of temperature on adults but may be due, in part, to a doubling in the rates of reproductive abnormality during the hot season and an increase in adult mortality related to a temperature-dependent decrease in pupal period.

Introduction

Since the 1970s, research into tsetse behaviour has concentrated on the responses to hosts, primarily to develop baits for control and monitoring, in which regard they have been very effective (Green, 1994). The convenience of odour-baited traps has also meant that they have been widely used

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to provide samples for research purposes. Unfortunately, however, samples from odour-baited traps are biased with respect to age (Hargrove, 1991), nutritional condition (Randolph & Rogers, 1981; Hargrove & Packer, 1993; Torr & Hargrove, 1998), species composition (Hargrove, 1991) and pregnancy state (Randolph & Rogers, 1986; Randolph *et al.*, 1991; Hargrove, 1995). These biases complicate the interpretation of the resulting data and have been cited as the source of errors of estimation of such parameters as age distributions (Hargrove, 1991), mortality rates (Hargrove, 1993) and the physiological states of male (Hargrove & Packer, 1993) and pregnant female tsetse (Hargrove, 1999a,b). We need less biased systems on which to base our models of tsetse biology – and as a benchmark to correct the biases in trap, and other, samples of tsetse.

Artificial 'refuges' (Vale, 1971) provide a candidate for such a system for the hottest times of the year in Zimbabwe. When ambient temperatures exceed *c.* 32°C, tsetse enter dark sheltered places such as rot holes in trees (Pilson & Leggate, 1962a,b; Pilson & Pilson, 1967). Samples of flies taken from artificial refuges and traps differ with respect to sex and species ratio (Vale & Phelps, 1978), nutrition (Vale, 1971; Vale & Phelps, 1978; Hargrove & Packer, 1993) and reproductive status (Hargrove, 1999a,b). In each case, refuges appear to sample a greater proportion of those groups under-represented in trap catches. The number of tsetse caught in a refuge is, however, crucially dependent on temperature and there are indications that there is an interaction between physiological status and response to refuges (Vale, 1971; Torr, 1988). We report studies of the interaction of micro-meteorological conditions and the fly's reproductive state on the behaviour of tsetse during the hot season in Zimbabwe, and compare various attributes of tsetse samples from refuges, odour-baited traps and targets, and a mobile electric sampling device.

Materials and methods

Studies on *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood (Diptera: Glossinidae) were carried out in riverine woodland within 3 km of Rekomitjie Research Station, Zambezi Valley, Zimbabwe during September–November 1992. These months are generally the hottest period in the year at Rekomitjie (Gibson & Torr, 1999).

Artificial refuges

Three 'box'-type artificial refuges (Vale, 1971) were placed *c.* 100 m apart in semi-evergreen woodland on the banks of the Chiuyi River near Rekomitjie. Each refuge consisted of an empty 180 l metal drum placed horizontally on the ground with one end removed. The drum was painted black internally and was insulated by a layer of earth. Its entrance was shaded by a thatched box (1 × 1 × 1 m). Flies entering the refuge between 0530 h and 1800 h were caught by a 1 × 1 m electrocuting net (Vale, 1974a), attached to the front of the refuge and sealing its entrance. The net was mounted on a metal hopper sunk into the ground; flies that struck the net were killed or stunned and fell into the hopper. To sample flies leaving a refuge, an identical net was operated between 1400 and 1800 h. Prior to 1400 h it lay horizontally on the ground, allowing flies to enter the refuge. It was then moved, carefully, so as not to disturb flies in the refuge, to the vertical position to seal the

exit and switched on. Flies emerging subsequently from the refuge struck the net and fell into the hopper.

Finally, to sample flies which had entered and remained in the refuge, it was left open between 0500 and 1400 h during which time tsetse were free to enter and leave. At 1400 h the refuge was sealed with a hessian curtain and all the flies present within were collected (Vale, 1971). This is referred to below as the 'standard' refuge catch. The three refuge catching treatments were assigned daily, at random, to the three refuges in a Latin-square design.

Traps and targets

The numbers of tsetse attracted to the odour from an ox were recorded to provide an index of the relative abundance of tsetse. The odour of an ox, placed in a ventilated pit (Vale, 1974b), was exhausted at 2000 l min⁻¹ via a 25 cm diam. ventilation shaft fitted with a 12 V co-axial fan. Flies attracted to the odour were caught on an electric net (1.5 × 1.5 m) placed 1 m downwind of the source. Tsetse orientate imprecisely towards an odour source unless it is marked with a visual stimulus (Vale, 1974a,b). Accordingly, a black cloth square (0.75 × 0.75 m) was sewn to the centre of the net to provide a visual target. The electric net was mounted on a corrugated tray coated with polybutene. Tsetse that struck the net were stunned or killed and fell onto the sticky tray. The electric net was operated between 1500 and 1800 h when tsetse are generally most active at Rekomitjie (Hargrove & Brady, 1992).

Flies were also captured from an 'Epsilon' trap (Muzari & Hargrove, 1996) baited with acetone (500 mg h⁻¹), 1-octen-3-ol (0.4 mg h⁻¹), 4-methyl phenol (0.8 mg h⁻¹) and 3-n-propyl phenol (0.1 mg h⁻¹) (Torr *et al.*, 1997). The trap was operated between 1600 and 1800 h. The collecting cage of the trap consisted of a wire-framed cage covered with fine black polyester netting. The trap and target were operated in the patch of riverine woodland referred to by Hargrove & Packer (1993) next to the Rekomitjie River *c.* 4 km south-west from the site of the refuges.

Mobile bait

An electric net was mounted on the back of a four-wheel drive pick-up as described by Hargrove (1991) and operated along a route *c.* 1–3 km from the traps and refuges. The net was mounted on a metal hopper. The mobile bait was operated continuously between 1600 h and 1800 h.

Collection and ovarian dissection of sampled flies

For methods that were operated continuously, flies were collected at 30-min intervals and transferred to a dark, humid, styrofoam box held in the field. For flies caught from refuges using electric nets, the hopper was fitted with a removable base so that flies could be collected without disturbing flies still present in the refuge.

Female flies were dissected within 18 h of collection and assigned to ovarian categories (Challier, 1965). For each fly, the lengths of the uterine inclusion and of the two largest oocytes were recorded. The percentage of pregnancy completed was estimated where possible from the length of the larva *in utero*. If the uterus was empty or contained an egg, or if the larva was broken or lost, the length of the largest oocyte was used unless it had already reached its

mature length. In the latter case, or if the largest oocyte was broken or lost, the length of the second largest oocyte was used. In each case it was assumed that the lengths increased exponentially. It was then necessary only to fix the upper and lower limits in order to estimate the proportion of pregnancy completed. Hargrove (1995) provides a complete description of this process but the following example shows explicitly how it works. Suppose the lengths of the second largest oocyte at the start and end of pregnancy were fixed at s and e respectively. If the length of the second largest oocyte in a sampled fly was x , then the proportion of pregnancy completed (PPR) was estimated by:

$$\text{PPR} = (\ln(x) - \ln(s)) / (\ln(e) - \ln(s)) \quad (1)$$

If a fly had ovarian or uterine products which were shorter, or longer, than the fixed values assumed to correspond to the start, or end, of pregnancy, then the estimated proportion of pregnancy completed would then be either <0 or >1 . In the former case the proportion was reassigned to 0. In the latter case it was reassigned to 1 if there was a larva *in utero*. If, however, the uterus was empty then the fly was placed at the beginning of the next pregnancy and the proportion was reassigned to 0.

Other algorithms (Hargrove, 1999c) were used to detect and record unusual relationships between oocyte and uterine lengths. These can result from reproductive abnormalities arising either by chance or due to stress – naturally or from handling at capture. Since some of the unusual relationships might be due to dissector error, the neutral term ‘anomaly’ is used below where the source of such unusual relationships is unclear. Abortions have been cited as the only major source of reproductive loss (Hargrove, 1999c). An apparent abortion is identified when a fly which has ovulated at least once has an empty uterus and no mature oocyte in the ovaries.

Meteorological measurements

Daily maximum and minimum temperatures were recorded from a mercury thermometer in a Stevenson screen at Rekomitjie Research Station. Measurements were also made of shade temperature, relative humidity and wind speed using an automatic weather station (type WS01, Delta-T devices, Newmarket, UK) at an open site adjacent to deciduous woodland dominated by ‘mopane’ trees (*Colophospermum mopane*: Fabaceae) c. 300 m from Rekomitjie Research Station. The logger was programmed to record hourly means. The same measurements were made at a second weather station in riverine woodland within 100 m of the refuges; hourly measurements of temperature and relative humidity were also made in one of the refuges.

Experimental design and analysis

Data were analysed using GLIM4 (Francis *et al.*, 1993) which fits models using a maximum likelihood method. For experiments where the catches from different treatments were compared, daily catches (n) were transformed to $\log_{10}(n+1)$ for analysis of variance. To analyse experiments where data were classed into different categories (e.g. reproductive status), a model with a Poisson error and log link was specified and the significance of changes in deviance was assessed on the basis of a G -test. To analyse proportions (e.g. percentage of males), a binomial model

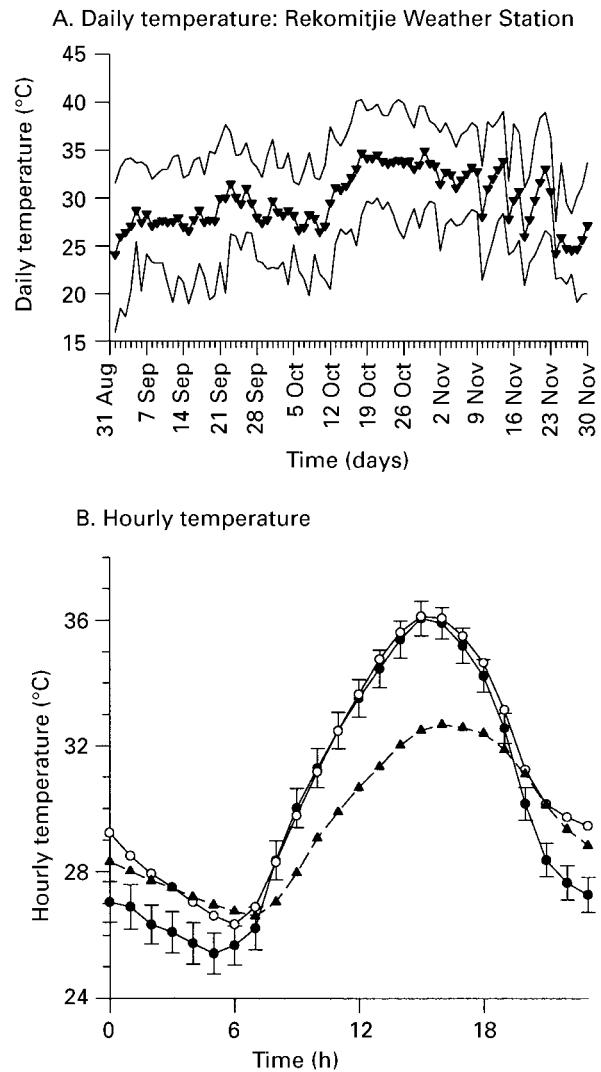


Fig. 1. A, Mean daily air temperatures in September–November 1992 with the range of daily maxima and minima (line only). B, The mean hourly air temperatures in riverine (●) and mopane (○) woodland and in a refuge (▲) located in riverine woodland. Simultaneous temperatures from mopane, riverine and refuges were made over 28 days between September 21 and November 30; temperatures for refuges are the means from measurements made at three different refuges. Error bars (\pm SE) are shown for riverine temperatures only.

with a logit link was used and the significance of changes in deviance was assessed by χ^2 . Means are accompanied by their standard errors unless stated otherwise.

Experiments and results

Micro-meteorological measurements

The mean daily temperatures at Rekomitjie (fig. 1A) showed a general increase between September and early November. Simultaneous measurements of mopane, riverine and refuge temperatures were made over 28 days between September 21 and November 30. Air temperatures at the

Table 1. Regression analysis of catches (n) of tsetse from an odour-baited target or a standard refuge.

Device	Species	<i>a</i>	Day	Temp.	Sex	Observations	<i>r</i> ²
Target	<i>Glossina pallidipes</i>	3.72	-0.024	-0.022	0.25	30	0.88
	F		178.4***	1.17 n.s.	6.3*		
	<i>G. m. morsitans</i>	1.81	-0.017	0.0076	0.14	30	0.85
	F		136.9***	0.2 n.s.	2.8 n.s.		
Refuge	<i>G. pallidipes</i>	-2.99	-0.018	0.14	0.076	20	0.66
	F		17.3**	9.5*	0.3 n.s.		
	<i>G. m. morsitans</i>	-2.927	-0.020	0.13	0.084	20	0.66
	F		17.5**	7.8*	0.35 n.s.		

The full regression model is $Y = a + b.N + c.T + d.S$, where Y is $\text{Log}_{10}(n + 1)$, N is day number (where 1 is 1 September, T is the daily maximum temperature ($^{\circ}\text{C}$) and S is sex (1 = male; 2 = female)). Table shows parameter estimates for full model, their respective F -values and the r^2 for the full model. Asterisks indicate that a parameter is significant at the 0.05 (*), 0.01(**) or 0.001 (***) level of probability by F -test; n.s. indicates not significant.

mopane and riverine sites were not significantly different during the day but at night the riverine habitat was significantly cooler, the greatest difference being at around midnight when the riverine habitat was $c. 2^{\circ}\text{C}$ cooler (fig. 1B). The relative humidity of the two sites and the hourly means varied between 25% ($\pm 3.7\%$) at 1600 h and 54% (± 4.8) at 0600 h and there was no significant difference between the sites. Wind speed was significantly higher during the day at both sites and higher at the mopane site; in the riverine woodland, mean wind-speed ranged between 1.1 m s^{-1} (± 0.2) at 1100 h and 0.2 m s^{-1} at 2100 h compared to 2.6 m s^{-1} and 0.7 m s^{-1} , respectively, in the mopane woodland.

The temperature inside a refuge was significantly cooler than the air temperature during the day but significantly warmer at night (fig. 1B). During the day, the temperature difference peaked at 1500 h when the refuge was $c. 3.6^{\circ}\text{C}$ cooler than the outside air temperature whereas at night, the refuge was up to 1.7°C warmer.

Regression analysis showed that refuge temperatures during daylight hours only were affected significantly by time of day ($P < 0.05$, F -test) and riverine air temperature ($P < 0.001$, F -test) but there was no significant interaction between these. The regression co-efficient for temperature (0.6 ± 0.02) differed significantly from unity, indicating that the difference between the refuge and the surrounding riverine habitat increased with temperature. For example, when the riverine temperature at 1400 h was 32°C , the refuge was 2°C cooler compared to being 5°C cooler when the surrounding woodland was 40°C .

Seasonal changes in tsetse numbers

Catches from an odour-baited target were used as an index of the changing abundance of tsetse. To facilitate comparisons with catches from refuges, only the 15 days when the maximum temperature exceeded 31.5°C were considered. For each species, daily catches were regressed against time and temperature with sex entered as a factor. Catches of both species, from targets and refuges, declined significantly with time (fig. 2, table 1). Odour-baited targets, which presumably catch host-orientating flies, and refuges, which sample resting flies, showed a similar decline in numbers with time, consistent with the idea that tsetse populations declined by $c. 90\%$ over the period of the study.

Only catches from refuges showed a significant increase

with temperature, as might be expected given that the targets catch flies responding to 'host' stimuli, whereas refuges sample flies as they accumulate in cooler resting sites. There was no significant interaction between sex and either time or temperature.

The ratios of catches of female:male, for both species separately, and of *G. pallidipes*:*G. m. morsitans* for both sexes combined were significantly higher in samples from targets than from refuges. The ratios for trap samples were higher yet (table 2). These results accord with those from many other studies (table 2; see Discussion).

Diurnal pattern of entry

The timing of entry of different species and sexes of tsetse into a refuge was analysed using the catches from eight days between 22 September and 22 October 1992; on these days the maximum temperature was $>31.5^{\circ}\text{C}$ and >10 flies of each species and sex were captured over the entire day. The maximum daily temperature ranged between 32 and 41.5°C over this period and between 36.0 and 41.5°C for the eight days of the experiment.

The median time of entry was significantly earlier ($P < 0.05$; F -test) at higher temperatures for both sexes of both species (fig. 3). On days when the maximum temperature was $>39^{\circ}\text{C}$, the median time of entry was 0900–1000 h compared with 1300–1430 when the maximum temperature was $<35^{\circ}\text{C}$. Increasing temperature also altered the pattern of entry. To illustrate this, the total catch of all tsetse caught over 14 days between 22 September and 16 November 1992 was pooled into three different groups according to whether the mean hourly air temperature peaked at $<36^{\circ}\text{C}$, 36 – 39°C or $>39^{\circ}\text{C}$. The catches for each temperature range were pooled into 30 min bins. As the temperature increased, the peak time of entry was not only earlier (as shown above) but also larger and more distinct (fig. 4). The peak time of entry coincided with the time when the ambient air temperature reached 32°C (fig. 4D).

Diurnal pattern of exit

The pattern of exit was also influenced by temperature. The total catches of tsetse from 11 days were allocated to two groups according to whether the maximum daily temperature was $<37^{\circ}\text{C}$ (4 days, 303 flies) or $>37^{\circ}\text{C}$ (7 days, 552 flies).

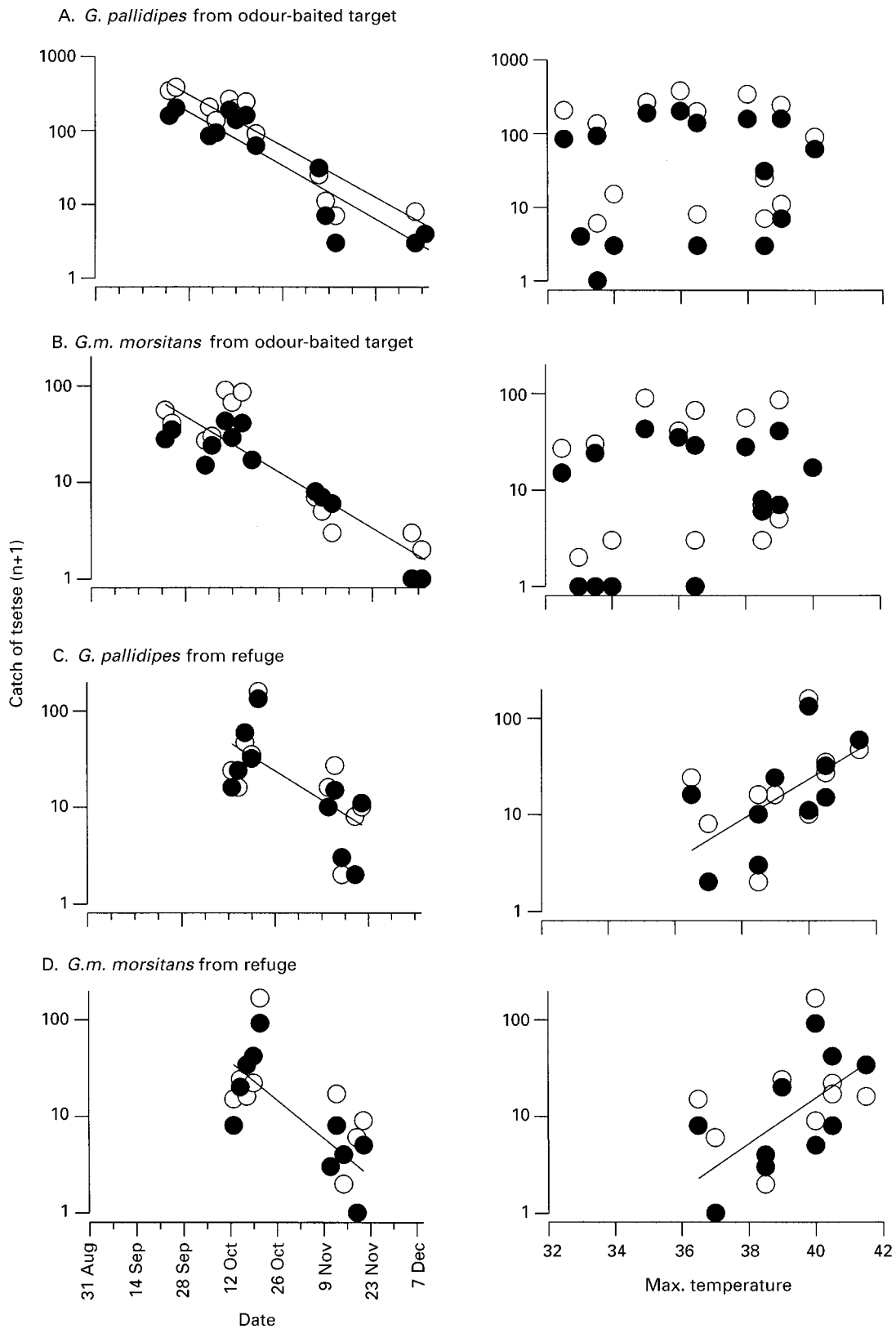


Fig. 2. Scatterplots of male (●) and female (○) tsetse caught from odour-baited targets or standard refuges against either date (left hand column) or daily maximum temperature for days where the temperature was $>31.5^{\circ}\text{C}$. Lines fitted by linear regression; see table 1 for parameter estimates and significance levels.

Table 2. Sex and species ratios in catches of *Glossina m. morsitans* (*G.m.*) and *G. pallidipes* (*G.p.*) from studies using box refuges, odour-baited electric nets and targets, or traps.

	Device	Odour	<i>d</i>	Catch		Ratio (females/males)		Sp. ratio <i>G. p./G. m.</i>	Date ^{Ref}
				<i>G. p.</i>	<i>G. m.</i>	<i>G. p.</i>	<i>G. m.</i>		
1	Refuge	–	10	649	499	1.1 (0.1)	1.3 (0.1)	1.3 (0.1)	10/92 ¹
2	Target	1 ox	15	3081	695	1.7 (0.1)	1.7 (0.1)	4.4 (0.2)	“
3	Trap	AOP	19	2347	218	2.0 (0.1)	2.9 (0.5)	10.8 (0.8)	“
4	Refuge	–	18 ^a	270	36	1.2 (0.1)	1.6 (0.5)	7.5 (1.3)	10–12/68 ²
5	“	–	26 ^b	2496	302	1.2 (0.1)	1.3 (0.2)	8.3 (0.5)	“
6	“	–	15 ^c	3923	393	1.3 (0.1)	0.6 (0.1)	10.0 (0.5)	“
7	“	–	30	1056	125	1.2 (0.1)	1.1 (0.2)	8.5 (0.8)	9/68 ²
8	“	–	31	8736	934	1.2 (0.1)	0.7 (0.1)	9.4 (0.3)	10/68 ²
9	“	–	30	4256	477	1.3 (0.1)	1.3 (0.1)	8.9 (0.4)	11/68 ²
10	“	–	16	387	25	1.2 (0.1)	1.3 (0.1)	15.5 (3.2)	12/68 ²
11	“	–	26	439	279	1.0 (0.1)	1.0 (0.1)	1.6 (0.1)	11–12/68 ²
12	Refuge	–	10	19,000	3671	1.2 (0.1)	1.3 (0.1)	5.2 (0.2)	10/73 ³
13	El. nets	1 ox	10	19,779	1929	2.0 (0.1)	1.4 (0.1)	10.3 (0.2)	“
14	Theory			195,412	26,247	2.7 (0.1)	2.3 (0.1)	7.5 (0.1)	“
15	Theory			22,032	7804	1.3 (0.1)	1.8 (0.1)	2.8 (0.1)	10/73 ⁴
16	Refuge	–		20,277	8800	1.8 (0.1)	1.6 (0.1)	2.3 (0.1)	9–12/74 ⁵
17	Trap/net	1–6 ox??		81,379	14,311	2.5 (0.1)	1.4 (0.1)	5.7 (0.1)	“
18	Trap	0 t	11	2437	431	2.1 (0.1)	0.9 (0.1)	5.7 (0.3)	9–12/75 ⁶
19	“	0.5 t	11	9836	1121	2.2 (0.1)	2.0 (0.1)	8.8 (0.3)	“
20	“	3.5 t	6	14,829	1398	2.2 (0.1)	2.2 (0.1)	10.6 (0.3)	“
21	“	6.5 t	6	19,164	1554	1.7 (0.1)	2.4 (0.1)	12.3 (0.3)	“
22	“	9.5 t	6	25,336	1675	1.8 (0.1)	2.6 (0.1)	15.1 (0.3)	“
23	“	11.5 t	12	34,438	2253	1.8 (0.1)	2.7 (0.1)	15.3 (0.3)	“
24	Refuge	–		13,459	5690	1.5 (0.1)	1.5 (0.1)	2.4 (0.1)	10–12/76 ⁷
25	Pits	13 t		308,240	13,711	2.8 (0.1)	1.8 (0.1)	22.5 (0.2)	10–11/76 ⁷

Devices, unbaited or baited with ox odour or a blend of acetone, octenol and phenols (AOP), were operated over *d* days during the months and years indicated. All samples were taken in riverine fringe vegetation during the hot season. The samples marked with ^a, ^b or ^c were taken on days when the maximum temperatures were 32–35, 35.1–38 or 38.1–42.5°C respectively. All catches are totals over the periods shown. The variance of sex and species ratios (*r*, where $r = a/b$) were calculated from $\text{var}(r) = r^2(\alpha^2/a^2 + \beta^2/b^2)$ where α and β are the variances of *a* and *b* which were approximated by $\text{var}(n) \approx n$ when $n > 10$. The standard error of each ratio is the square root of the variance; these values are shown in parentheses.

References: ¹Present study; ²Vale (1971); ³Phelps & Vale (1978); ⁴Hargrove (1981); ⁵Vale & Hargrove (1975); ⁶Hargrove & Vale (1978); ⁷Hargrove & Vale (1979)

For each species and sex the timing of exit was significantly ($P < 0.05$, G test) earlier on cooler days (fig. 5). However, the probability of a fly leaving was more dependent on time than temperature. Of the tsetse present at 1400 h, 17.5% ($n = 781$) left the refuge between 1400 and 1500 h. A much greater proportion (59.9%, $n = 401$) of those present at 1600 h left between 1600 and 1700 h but the temperature within the refuge actually increased slightly ($P < 0.05$, *F*-test) by 0.1°C h⁻¹ (± 0.03) between 1400 h and 1700 h. The leaving response does not therefore seem to be a response to falling temperature within the refuge, but is more a response to the fly's internal timing (Huyton & Brady, 1975).

Behaviour around refuges

The catches from the 'standard' refuge and that from the electric net which captured departing flies, both sampled flies which were present in the refuge at 1400 h. The mean catches from these two systems should thus be the same. This category of fly would also have been captured by the electric net which caught flies as they entered the refuge

between dawn and 1400 h. The 'standard' catch was not significantly lower than catches of flies entering or leaving the refuge (table 3; $P > 0.05$) consistent with the idea that most flies entering a refuge in the morning, landed and remained there until the afternoon peak of activity. However, the catch of male *G. pallidipes* from the external surface of an electric net was about half that from the other methods, suggesting that the electric net reduced the probability of this group of flies entering the refuge (Griffiths & Brady, 1994). It is also possible that some of the flies which were stunned, as they entered the refuge, escaped before they were collected. There is less likelihood of such escapees with departing flies since, even if they recover after the first contact with the electric net, they are still inside the refuge.

Ovarian dissections

Dissection success rates

Four dissectors each dissected 526, 800, 740 and 743 female tsetse during the study. Flies from epsilon traps and

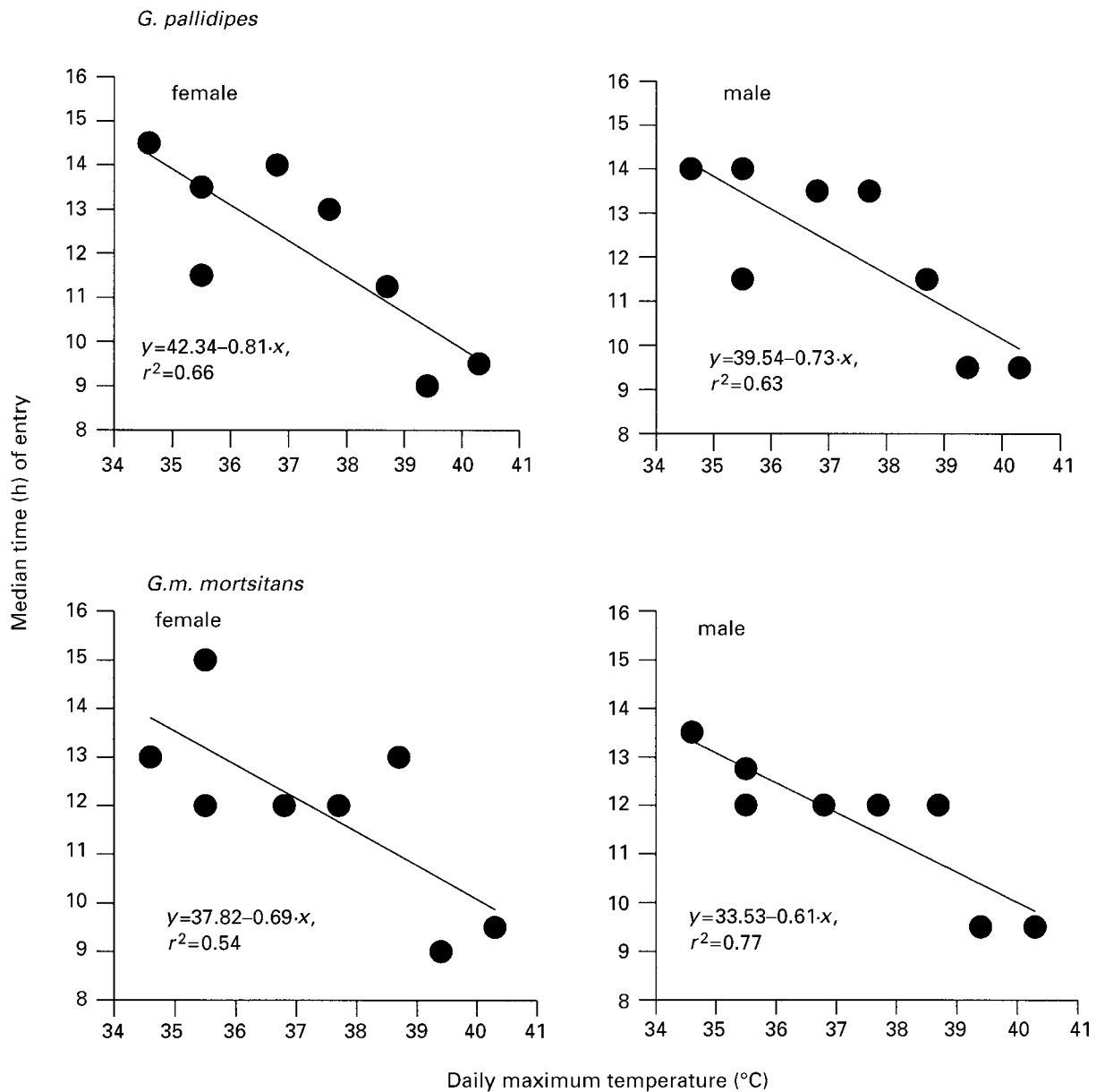


Fig. 3. Scatterplot of median time of arrival versus daily maximum temperature for different species and sexes of tsetse. Ordinate shows time of day. Each estimate of median time of arrival is based on a sample of at least 10 flies.

the standard refuge capture were all alive at the time of dissection; 98.5% of the 1170 tsetse from these devices were assigned to an ovarian age category and 99.1% to their pregnancy stage. The 11 failures were due to accidental breakage of the ovaries during dissection. For the mobile bait, however, where the flies were caught on electric nets, the success rates were only 85 and 89% respectively. This was due largely to the fact that the majority of these flies was killed at capture and then began to decay; others were desiccated and partially destroyed if they became stuck in the electrocuting grid.

The success rates for the flies caught on electric nets at refuge entrances were even lower – 80 and 83% respectively.

This failure rate was due to the fact that flies entering refuges often contained high levels of residual blood meal (Hargrove & Packer, 1993; Hargrove, 1999a,b) and such flies, when killed, decay particularly rapidly.

Anomalies in ovarian dissection records

The percentages of anomalies (see Materials and methods) found in the records of the four dissectors were 8.7, 3.8, 7.0 and 15.2 ($P < 0.01$, G test for the difference). Since the flies were assigned more or less at random to the dissectors, this result suggests that operator error needs to be considered as an important factor in this part of the study.

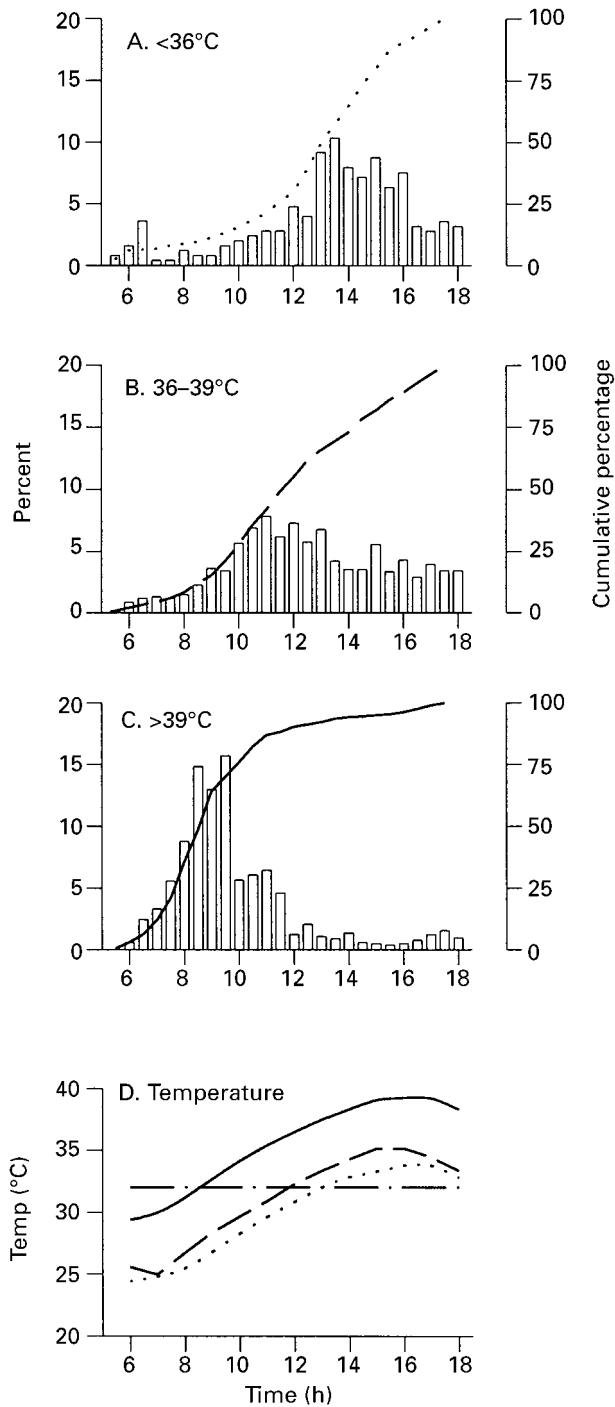


Fig. 4. Frequency distribution of the timing of tsetse entering a refuge on days where the maximum temperature was $<36^{\circ}\text{C}$ (A), $36\text{--}39^{\circ}\text{C}$ (B) or $>39^{\circ}\text{C}$ (C); lines on graphs show the percentages plotted cumulatively. D shows the mean air temperature on days when the maximum was $<36^{\circ}$ (lower dotted curve), $36\text{--}39^{\circ}$ (middle broken curve) and $>39^{\circ}$ (upper solid curve); horizontal (dash-dot) line shows 32°C .

The levels of anomalies found by each dissector were, however, consistently higher than those found by the same

dissector at other times of the year. During the cooler months of June and August 1992 for instance, the percentage anomalies found by the four dissectors were 4.1, 0.3, 2.4 and 6.4% – all markedly lower than the levels found during October. Presumably the true levels of abortion and other reproductive abnormality increased as a result of the particularly high temperatures in October. The analyses below exclude flies that showed reproductive anomalies.

Effect of age and reproductive status

Dissections were carried out on 1187 *G. pallidipes* and 574 *G. m. morsitans* females captured from refuges on five days between 14 and 22 October. To assess the effect of age and reproductive status on the time of entering and departing a refuge, the catch of tsetse from each day was allocated into two equally sized groups such that the entry or exit times of one group were all earlier than the second group.

For *G. pallidipes*, there was no significant effect of age or pregnancy state (as measured by uterine content) on the timing of entry or exit (fig. 6) from refuges. For *G. m. morsitans*, too, there was no significant effect of reproductive status on the timing of entry or exit and the pooled percentages of *G. m. morsitans* ($n = 181$) with a uterus which was either empty or contained an egg or first, second or third instar larva were 25, 32, 13, 20 and 9% respectively which is similar to the results for *G. pallidipes* (fig. 6). Too few *G. m. morsitans* were caught to determine whether ovarian age had an effect on the timing of exit, or of entry and exit, from a refuge.

Reproductive status of tsetse caught using different methods

Tests for differences in the distributions of the ovarian ages and pregnancy stages of flies caught at refuges using different methods showed that these were either not significant, or were negligible anyway in absolute terms. These results are consistent with the evidence from overall catch levels (see above) that most flies that enter the refuge in the morning tend to stay there until at least 1400 h.

There were larger differences between the distributions of ovarian ages of *G. pallidipes* caught using refuges, traps and the mobile bait ($P < 0.01$ for each of the three possible contrasts, fig. 7A). There was no significant difference between dissectors in the ovarian age distributions they found for flies caught using these different methods. The trap caught high proportions of flies in ovarian categories 4–7 and few flies (*c.* 3%) in category zero. There were insufficient *G. m. morsitans* caught in traps to carry out meaningful statistical analyses on these samples, but there was no significant difference between the ovarian ages of the samples of this species caught in refuges and on the mobile bait (fig. 7C; $P > 0.05$, G test).

For *G. pallidipes*, the distributions of percentage pregnancy differed significantly between refuge and the mobile bait and between refuge and trap (fig. 7B, D; $P < 0.01$, G test, in each case). The difference between trap and the mobile bait just failed to reach significance at the 0.05 level. The refuge sample approached most closely the quasi-uniform distribution expected from a random sample. The mobile bait sample differed in the paucity of flies in the last stages of pregnancy; this was also true of the trap sample, which showed an additional bias in favour of flies at the start of pregnancy.

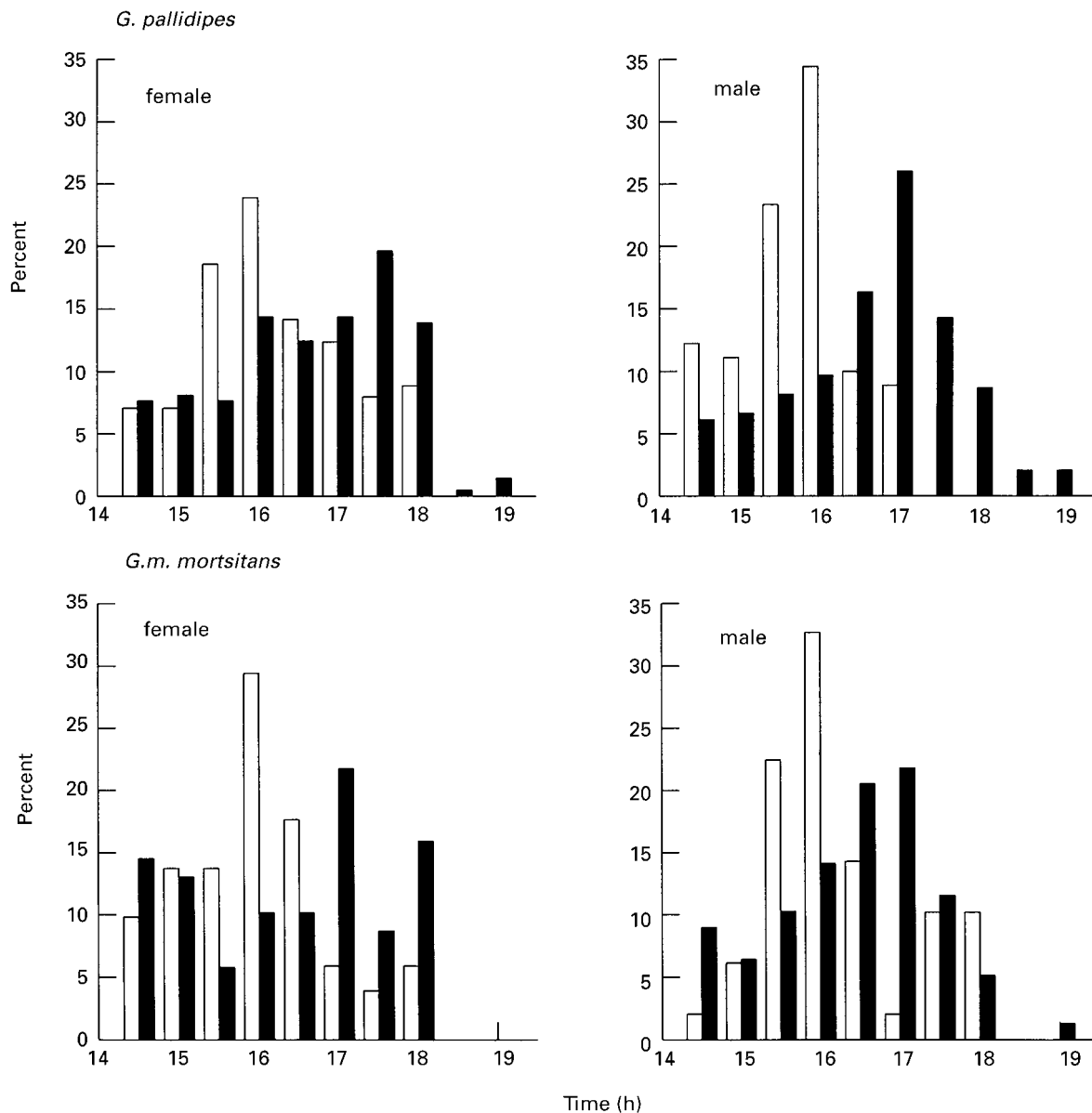


Fig. 5. Frequency distribution of the timing of tsetse leaving a refuge when daily maximum was $<37^{\circ}\text{C}$ (open bars) or $>37^{\circ}\text{C}$ (solid bars). Respective mean temperatures in the refuges were 32.9°C and 34.8°C at 1500 h compared with 33.1°C and 34.9°C respectively at 1700 h.

Differences in the distribution of percentage pregnancy for the mobile bait and refuge samples of *G. m. morsitans* just failed to reach significance ($P = 0.06$, G Test). The mobile bait samples showed marked peaks for percentage pregnancy at c. 10, 50 and 90%. In contrast, the refuge samples of this species showed only the last of these three peaks (fig. 7D). Trap samples for *G. m. morsitans* were too small for meaningful comparisons with other methods.

Flies with empty uteri can be placed either at the end of one pregnancy or at the beginning of the next (Randolph *et al.*, 1991). What was unusual about such flies in this study, however, was that the oocyte lengths were often much

greater than expected of a fly at the beginning of pregnancy. Indeed 50/149 (34%) were so large that they were marked by the checking algorithm as 'anomalies'. It seemed that many of these flies should have ovulated but had not done so. It seems likely that the high proportion of such flies, which are almost never seen at other times of the year (Hargrove, 1999c), is related to increased stress at high temperatures. On the other hand, all of this type of anomalous fly, and 95% (141/149) of all parous flies with empty uteri, were caught in refuges. Since refuges sample classes of flies which are rarely found by other techniques it is also possible that such flies do occur at cooler times of the year but are rarely caught.

Table 3. Detransformed mean catches of tsetse (transformed in brackets) collected from refuges by various sampling methods designed to catch flies as they rested, entered or exited the refuge, their respective transformed standard errors (SE) and the probability (*P*) that transformed means in the same column are significantly different.

Sampling method	Sampling period	Behaviour	<i>Glossina pallidipes</i>		<i>G. m. morsitans</i>	
			Male	Female	Male	Female
Standard	1400	Resting	18.8 (1.30)	20.2 (1.33)	11.9 (1.11)	12.9 (1.14)
Electric net – external surface	0600–1400	Entering	9.3 (1.01)	20.7 (1.34)	10.7 (1.07)	9.5 (1.02)
Electric net – internal surface	1400–1800	Exiting	19.7 (1.32)	23.9 (1.40)	7.9 (0.95)	10.0 (1.04)
S.E.			0.12	0.09	0.09	0.11
<i>P</i>			0.19	0.85	0.41	0.68

Discussion

Catch composition

Sex and species ratios

The present results support earlier findings (table 2) that the sex and species ratios in samples of tsetse from refuges differ consistently from those caught using stationary baits. For *G. pallidipes*, 12 independent estimates of the sex ratio in refuge samples, made at various times over a 25-year period at Rekomitjie Research Station, provided values in the range 1.0–1.8; and eight of these were in the range 1.2–1.3. The ratios for *G. m. morsitans* were almost as consistent, bearing in mind the smaller sample size on which they are based; eight out of 12 sex ratios were in the range 1.3–1.6. In contrast, samples from odour-baited targets, nets or traps gave ratios in the range 1.7–2.8 for *G. pallidipes* and were generally >2.0 for *G. m. morsitans*.

The species ratio among catches from refuges was more variable and there is a suggestion of a shift over the years in favour of *G. m. morsitans*. Thus, in 1967 and 1968 the ratios were generally ≥ 7.5 , in 1973–1976 they were 5.2, 2.3 and 2.4 respectively and in the present study they had fallen to 1.3. There may be variation between sites, which were spread over a 5 km range, but samples in the current study, both from refuges and electric nets, had much lower proportions of *G. pallidipes* than in Vale & Phelps' (1978) study. This evidence is consistent with the idea that there has been a substantial decline in the proportion of *G. pallidipes* in the Rekomitjie area over the last 25 years.

The interpretation of species ratios in catches from odour-baited electric nets and traps is complicated by the fact that the ratio changes with odour concentration (table 2, rows 18–23). This may reflect the greater mobility of *G. pallidipes* and/or a stronger response to odour than in *G. m. morsitans* (Hargrove *et al.*, 1995). Since trap efficiencies also tend to be higher for *G. pallidipes* than *G. m. morsitans*, whereas there is little difference for electric nets (Hargrove & Vale, 1979), ratios of *G. pallidipes*:*G. m. morsitans* tend to be higher in catches from traps.

To assess which among the various samples best reflects the actual population structure, one needs to estimate the true population profile. Phelps & Vale's (1978) theoretical estimates, based on mark–recapture and removal trapping

techniques (table 2, row 14), generally indicated sex ratios in excess of 2.0. They were thus always higher than the ratios calculated from refuge and electric net catches, but closer to the latter.

Hargrove (1981) argued that Phelps & Vale's (1978) population estimates were not valid since the area sampled was not closed to immigration and emigration. A model which took movement into account gave much lower population estimates (row 15); it predicted a sex ratio of 1.3 in *G. pallidipes*, close to the ratio of 1.2 actually found in refuges in Phelps & Vale's (1978) study and in many others (table 2). To this extent it appears that refuges give a representative sample of the sexes in *G. pallidipes*. In contrast, stationary odour-baited electric nets appear to over-estimate the proportion of females in the *G. pallidipes* population (table 2, row 13); by extension (see above) trap samples are therefore even more biased in favour of females. The results of Hargrove & Vale (1978, 1980) suggest that the concentration of odour used with a trap does not greatly affect the observed sex ratio of either species; samples are strongly biased in favour of females regardless of concentration.

For *G. m. morsitans* there was little difference, in Vale & Phelps' (1978) study, between the sex ratios of 1.3 and 1.4 found in refuge and electric net samples respectively. Hargrove's (1981) theoretical estimate of a sex ratio of 1.8 in *G. m. morsitans* was closer to these observed values than Phelps & Vale's (1978) theoretical values of 2.3.

Vale & Phelps' (1978) data show a ratio of *G. pallidipes*:*G. m. morsitans* in electric net catches which was double that found in refuges, with Vale & Phelps' (1978) theoretical values lying somewhere in between. Hargrove (1981) estimated a much lower proportion of *G. pallidipes* from the same data; he also argued that violations of the assumptions involved in Phelps & Vale's (1978) theoretical analysis would affect most seriously their estimates for *G. pallidipes*, since this species is more mobile than *G. morsitans*. If this is correct, the true species ratio lies closer to that reflected by refuges than by those from electric nets. The marked changes in species ratio with odour concentration is further evidence that the use of odour-baited devices is likely to give unreliable estimates of this statistic.

The conclusion from these data is that the sex ratio for both species is *c.* 1.3 and this has varied little over the years.

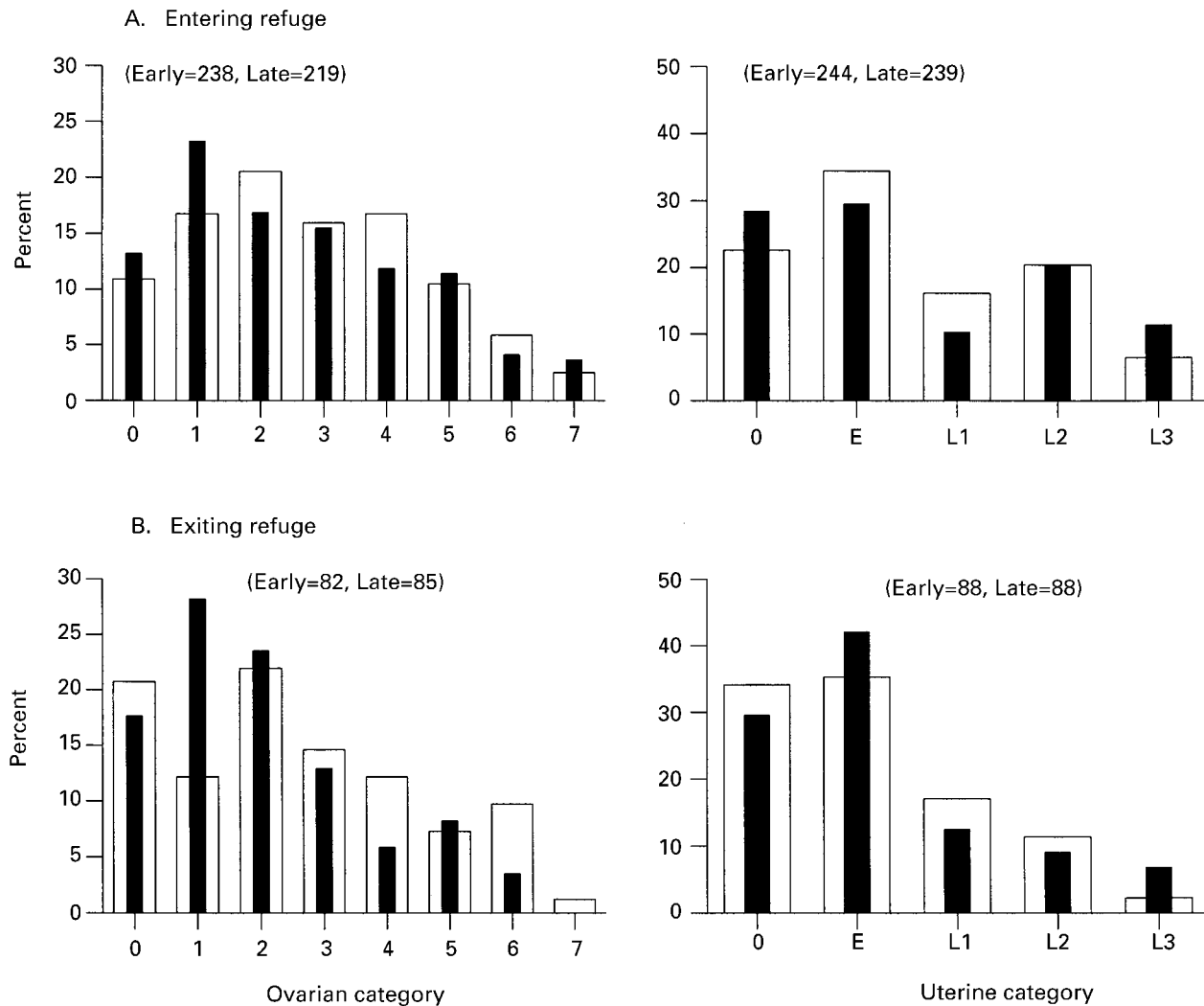


Fig. 6. Frequency distribution of the ovarian categories and uterine contents of *Glossina pallidipes* caught as they entered (A) or departed from (B) a refuge. Numbers of flies dissected that entered early or late are shown as open (\square) or solid (\blacksquare) bars respectively. For uterine contents, 0 denotes an empty uterus and E, L1, L2 and L3 denote egg and first, second, or third stage larvae respectively.

In contrast, the ratio of *G. pallidipes*:*G. m. morsitans* has probably declined over the past few decades. The results presented in table 2 refer, however, only to one vegetation type in one season. Sex and species ratios in other habitats and at different times of the year (Hargrove & Vale, 1980) can show quite different pictures and there is no way of knowing the biases in any of the estimates.

Reproductive status

Flies from refuges, traps and the mobile bait showed marked differences in their reproductive status. Not only was there a higher proportion of refuge flies in ovarian category 0 but there was also a more uniform distribution of flies from various stages in the reproductive cycle; flies from traps and mobile baits showed a paucity of flies in the late stages of pregnancy. The reproductive status of flies caught from traps in the present study is similar to that from several

previous studies of tsetse attracted to natural and artificial baits (Hargrove, 1995; Torr & Hargrove, 1998) and accords with Brady & Gibson's (1983) laboratory study showing that there is a marked reduction in activity in the 48 h preceding larviposition followed by a peak of activity in the 24 h following larviposition.

The biases of refuges, traps and mobile baits

The above differences in the catch composition from baits and refuges suggest that refuges are less-biased with respect to age, sex and species composition and pregnancy status. This is not surprising given that during exceptionally hot periods the ambient temperature exceeds 39–40°C which is the lethal limit for several species of tsetse (Potts, 1933; Nash, 1935). Thus to survive these episodes, all tsetse must move to microhabitats where the temperature is lower.

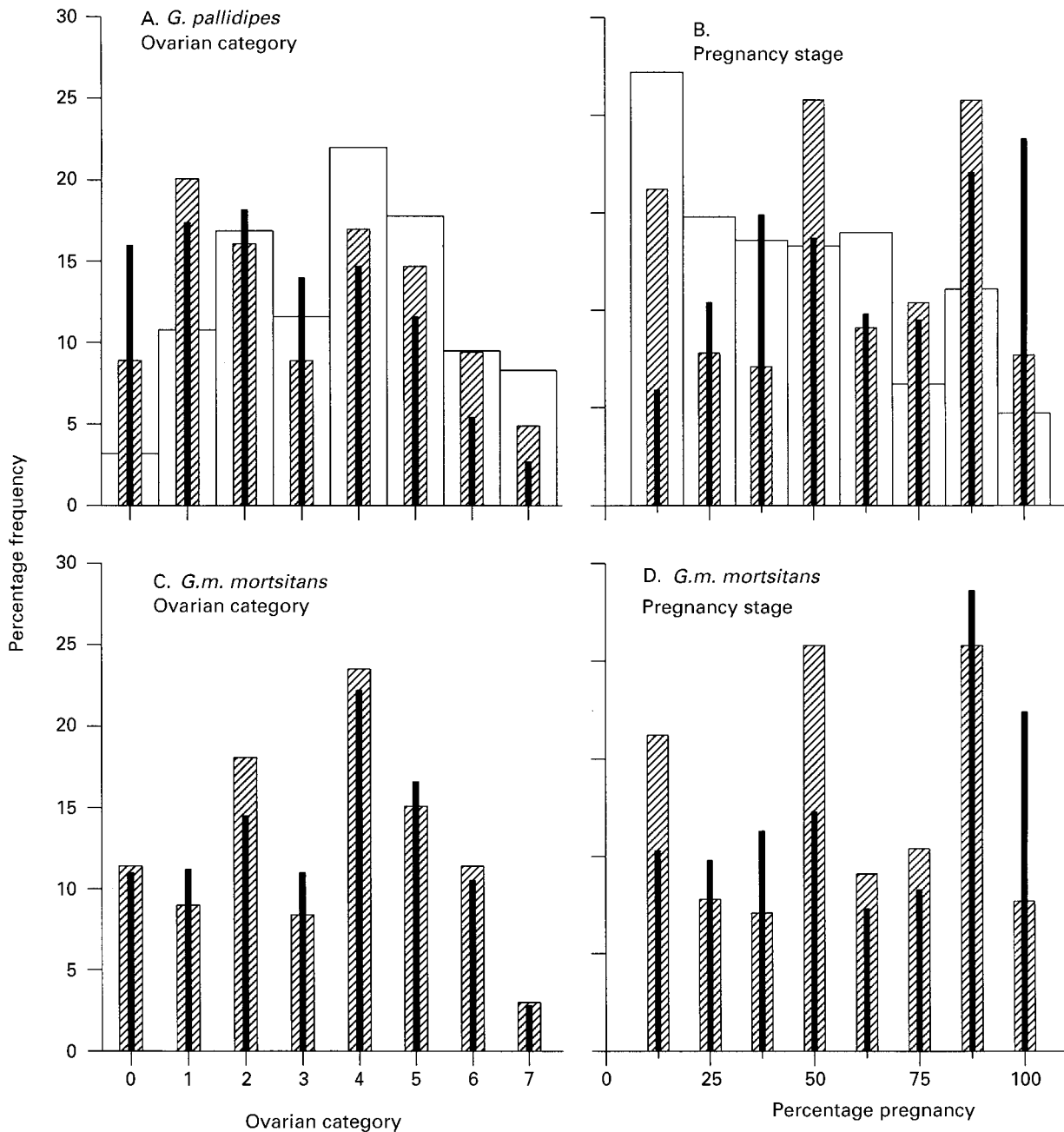


Fig. 7. Frequency distribution of the ovarian categories (A, C) and percentage pregnancy completed (B, D) of tsetse caught from an Epsilon trap (□), the mobile bait (▨) or refuges (■).

There is no such imperative for flies to visit stationary traps. Flies must clearly feed, but they can do so at less regular intervals and do not necessarily need to expend the energy required to locate a stationary bait unless they urgently require food. Females which have recently deposited a larva, and those which require blood to nourish the larva at the stage of its most rapid growth, are obvious examples of flies which are in this straitened circumstance. This is consistent with the observation that peaks in the distribution of percentage pregnancy from trap samples are

seen immediately after larviposition and c. 48 h before this event.

Samples from mobile baits may be expected to lie somewhere between these extremes. Less effort is required to visit such a bait since, it is assumed, the bait passes places where tsetse are resting. The data for *G. pallidipes* support this idea in that the distribution of percentage pregnancy is closer to uniform than that from the trap. Similarly, the distribution of ovarian ages of the mobile bait samples lies between that from refuges and the trap sample.

Behaviour around refuges

Our results agree with those of Vale (1971) in that refuge catches were positively correlated with temperature. They differ in that Vale found that most tsetse occupied the refuge at 1000–1400 h, and left after 1600 h; we found that tsetse entered the refuge at all hours, with the peak period of entry varying between 0900 and 1400 h according to temperature. The peak was bigger and earlier on hotter days and coincided with the time when the ambient temperature exceeded 32°C. Leaving was also correlated with temperature, being later on hotter days.

At temperatures below *c.* 32°C, tsetse rest on the boles and branches of trees (Pilson & Leggate, 1962b; Pilson & Pilson, 1967); they are presumably activated as the temperature reaches 32°C. They enter refuges that are cooler than the outside air, but tsetse caught on electric nets as they entered could not yet have detected this fact. Part of the refuge-orientated response must therefore be a visual response to dark targets. Once flies entered refuges they experienced a steady increase in temperature until 1600 h. Indeed, for flies in refuges, the temperature did not fall markedly until around sunset whereas the ambient temperature outside the refuges declines rapidly from 1600 h onwards. These features suggest that the refuge-orientated response comprises some combination of temperature-mediated activation, a visual response to a dark (black?) target and some form of activity modulation, presumably controlled by the fly's endogenous activity rhythm (Huyton & Brady, 1975; Brady & Crump, 1978; Hargrove & Brady, 1992).

Temperature had a greater effect on the entry response of tsetse into refuges than on the leaving response. This difference is not surprising when we consider the temperature patterns during warmer periods of the hot season (fig. 1B). On days where the maximum ambient temperature was >37°C, the mean temperature was still *c.* 37°C at 1800 h and the temperature in the refuge *c.* 34°C. On such days, tsetse entering a refuge at 32°C in the morning will not be exposed to temperatures below this until the following morning. Thus, if the temperature threshold for leaving a refuge was, like the entry response, set at 32°C, tsetse would become 'trapped' in the relatively warm refuges during the night. A temperature-dependent entry response combined with an endogenously-controlled leaving response would result instead in tsetse optimizing their use of the cooler microhabitats.

The present data can be used to provide a rough estimate of a fly's environmental temperature by assuming that during the hot season: (i) tsetse rest at night in the riverine areas (Pilson & Pilson, 1967); and (ii) during the day they are either at the air temperature unless that is >32°C in which case they enter a refuge. The results (fig. 1B) show that during hot periods tsetse can reduce their mean environmental temperature by *c.* 2°C and at the hottest parts of the hottest days by *c.* 4°C. These results accord with Hargrove & Coates' (1990) suggestion that, during the hot season at least, Zambezi valley tsetse live at temperatures 2–6°C below that indicated by a Stevenson screen.

The reduction in temperature is due not only to tsetse entering refuges during the day but also to the lower ambient temperature of riverine woodland during the night (fig. 1B). The nocturnal difference in temperature between the mopane and riverine habitats is due to the development

of stable atmospheric conditions at night. This reduces atmospheric turbulence and allows cooler air to accumulate in the lower-lying riverine areas. During the day however, unstable atmospheric conditions develop and the associated atmospheric turbulence ensures that there is a large-scale mixing of air between different habitats and thus the riverine and mopane woodlands have similar temperatures (F.X. Meixner, personal communication).

Population changes during the hot season: real or a sampling artefact?

During the 1992 hot season in Zimbabwe, there was a progressive increase in temperature and a decrease in the catches of tsetse from both odour-baits and refuges. Consequently, inferring any causal change between catch and temperature is confounded by the systematic change in temperature and tsetse numbers during the course of the hot season. If, however, we consider only those days where the temperature exceeded 31.5°C, then the catch of tsetse from an odour-baited target declined during the course of the hot season but showed no significant correlation with daily temperature. Catches of tsetse from refuges on the other hand, showed both a significant decline with time and a significant increase with temperature. The marked similarity in the temporal change in catch numbers from devices sampling different fractions of the tsetse population suggests that the tsetse population is declining at this time of year rather than exhibiting a seasonal change in availability.

During the hot season, the ambient temperature frequently exceeded the lethal limit for tsetse. The present data suggest, however, that tsetse in refuges experienced a maximum of 35°C during these hot episodes and thus it seems unlikely that the high temperatures killed adult flies directly.

For the hottest parts of October 1992, Hargrove's (1995) algorithm predicts that the inter-larval period was just 6 days, compared to the more typical 8–9 days at cooler times of year. Typically, tsetse are thought to feed every 2–3 days and the production of a pupa requires three (Gaston & Randolph, 1993), or perhaps more (Langley & Stafford, 1990), meals obtained over 8–9 days. The shorter pupal period during the hot season suggests that either tsetse must feed more frequently or their pupae are produced with fewer meals; either of these processes could increase mortality. On the one hand, if each feeding attempt is accompanied by a mortality risk (Randolph *et al.*, 1992; Hargrove & Williams, 1995) then an increased feeding rate will increase adult mortality. In addition, if pupae are produced with fewer meals, then the pupae and resultant adults are likely to be smaller and subject to a lower rate of survival (Phelps & Clarke, 1974).

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