

Mortality estimates from ovarian age distributions of the tsetse fly *Glossina pallidipes* Austen sampled in Zimbabwe suggest the need for new analytical approaches

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Abstract

Mortality estimates are central to understanding tsetse fly population dynamics, but are difficult to acquire from wild populations. They can be obtained from age distribution data but, with limited data, it is unclear whether the assumptions required to make the estimates are satisfied and, if not, how violations affect the estimates. We evaluate the assumptions required for existing mortality estimation techniques using long-term longitudinal ovarian dissection data from 144,106 female tsetse, *Glossina pallidipes* Austen, captured in Zimbabwe between 1988 and 1999. At the end of the hot-dry season each year, mean ovarian ages peaked, and maximum-likelihood mortality estimates declined to low levels, contrary to mark-recapture estimates, suggesting violations of the assumptions underlying the estimation technique. We demonstrate that age distributions are seldom stable for *G. pallidipes* at our study site, and hypothesize that this is a consequence of a disproportionate increase in the mortality of pupae and young adults at the hottest times of the year. Assumptions of age-independent mortality and capture probability are also violated, the latter bias varying with capture method and with pregnancy and nutritional status. As a consequence, mortality estimates obtained from ovarian dissection data are unreliable. To overcome these problems we suggest simulating female tsetse populations, using dynamical modelling techniques that make no assumptions about the stability of the age distribution.

Keywords: *Glossina pallidipes*, tsetse, mortality estimates, ovarian dissection, stable age distribution

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Introduction

Tsetse fly mortality rates are particularly important in influencing, directly, the population dynamics of the insects (Hargrove, 2004) and, indirectly, the trypanosomes that they

transmit (Rogers, 1988). Since laboratory animals and wild populations can differ greatly in their life expectancies it is essential, though more difficult, to obtain data from free-ranging field populations (Seber, 1982). Mark-recapture techniques (Jolly, 1965; Seber, 1965) applied to closed tsetse populations have been used to estimate birth and death rates (Hargrove & Williams, 1998) but, in open populations, it is difficult to differentiate rates of birth from immigration, and death from emigration (Jackson, 1941, 1944). Mark-recapture experiments are also often protracted, labour intensive and financially and logistically challenging: biologists have accordingly sought

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simpler approaches to the estimation of mortality using age distribution data.

Carey *et al.* (2012a, b) have suggested that insect age distributions and, thereby, population mortality rates, can be estimated by following the time course of survival, in the laboratory, of insects sampled in the field. This novel technique has not been applied to tsetse flies. Ovarian dissection provides, however, an accurate, more rapid, less labour intensive and cheaper alternative. The technique is used to determine the number of times females have ovulated and, since ovulation occurs at regular intervals, these data can be used to determine tsetse age distributions (Saunders, 1960, 1962; Challier, 1965) and, in principle, fly mortality rates (Rogers *et al.*, 1984; Van Sickle & Phelps, 1988; Hargrove, 1993; Jarry *et al.*, 1996, 1999).

Estimates of adult female mortality from ovarian age distribution data have, however, often been inconsistent with mark-recapture estimates on the same tsetse population (Hargrove, 1993) and have sometimes been counter-intuitive in suggesting, for example, that mortality rates reach a minimum at the end of the hot-dry season in Zimbabwe when populations appear to be under maximum stress and to be declining in numbers (Hargrove, 2004). It is our hypothesis that these problems stem not from our failure to estimate the age distribution but, rather, from violations of the assumptions that underlie existing estimation procedures.

Techniques for estimating mortality from ovarian dissection data rely on three major assumptions (Van Sickle & Phelps, 1988). First, that capture probability is not dependent on the age of the fly. Second, that the population under study has a stable age distribution. Last, that adult mortality is not a function of age. To evaluate age-independence of capture probability, flies can be caught using different sampling methods. If the observed ovarian age distribution differs significantly between sampling methods then at the most one sampling method is age-independent (Hargrove, 1990, 1991). To evaluate whether the age distribution is stable, the ovarian category distribution should remain constant over time for a given sampling method (Williams *et al.*, 1990). Checking the first two assumptions is uncommon, however, in part because the data are generally too sparse: checking the age independence of mortality is even more difficult and only one field study explicitly addresses this issue (Hargrove, 1990, Hargrove *et al.*, 2011).

The current analysis uses data from a long-term longitudinal sampling study launched in late 1988 at Rekomitjie Research Station, Zambezi Valley, Zimbabwe. Over an 11-year period three different sampling systems were used to capture 293,215 female *G. pallidipes*, 144,106 of which were dissected and assigned to their ovarian category, making it the most extensive study of its kind. The aim is to determine from these data whether sampling is age-independent and whether flies have a stable age distribution. We explore differences across three different sampling techniques and over time, to determine selection biases inherent in each of these techniques. These biases have clear implications for mortality estimation and help elucidate reasons for violations of the required assumptions. We find clear evidence of age-dependent capture probability and non-stable age distributions. These results indicate that standard methods of estimation from ovarian dissection data produce unreliable mortality estimates and illustrate the need for new estimation methodologies.

Methods

Capture methods

Tsetse were captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe between September 1988 and December 1999 as part of a long-term monitoring of tsetse population levels, age structure, trypanosome infection rates and physiological status, as well as in support of other experimental work carried out at Rekomitjie.

Capture and processing methods are fully described and illustrated in the Supplementary Materials. Briefly, flies were captured using: (i) traps. Stationary mechanical traps, such as those described by Hargrove *et al.* (1995), or electric nets (Vale, 1974), baited with host odour; (ii) a vehicle-mounted electric target (VET) (Hargrove, 1999c), which consisted of a 1 × 1 m electric net (Vale, 1974) mounted on the back of an open pickup; (iii) artificial refuges (Vale, 1971) where tsetse flies accumulate when temperatures exceed about 32°C: refuges were accordingly only operated in the hot months September–December.

To minimize wing fray, resulting from flies buzzing in trap cages, traps were cleared every 30 min. With this frequency of clearing it was not feasible to run the traps all day over the entire period of the study. Instead, traps and the VET were operated for the last 2–3 h in the afternoon to cover the evening peak of tsetse flight activity. Changes in trap catch levels of *G. pallidipes* were calculated as the geometric mean of the catches during these late afternoon periods. This was not ideal because the distribution of trap catches shows a diurnal rhythm which changes with season/temperature at Rekomitjie (Hargrove & Brady, 1992). In order to gauge the level of bias consequent on the trapping regime, all-day catches were made on 50 days between 1991 and 1994 and the proportion of the catch found in the last 3 h of the day was calculated for different months of the year.

Flies captured using the VET were generally dead on collection and were given priority for dissection to ensure minimal degeneration of tissue; 98.7% were dissected within 24 h of collection. Flies from traps and refuges were living at the time of capture; cages were immediately wrapped in a moist black cloth and transferred to a polystyrene box, and then to an insectary and kept alive at 25°C until dissection.

Tsetse life cycle and ovarian development: age estimation using ovarian dissection

An understanding of the results presented in this paper requires knowledge of the peculiarities of the tsetse life cycle, and of qualitative and quantitative aspects of ovarian development and, finally, of details of the ovarian dissection technique for estimating the age of female tsetse. These matters are fully described in the Supplementary Materials.

Estimating mortality from ovarian dissection data

In the past, mortality in adult female tsetse has been estimated from ovarian age distributions of sampled flies using the following assumptions: (i) mortality is independent of age for all flies other than those in ovarian category 0, which are excluded from such analyses because they are obviously underrepresented in trap catches; (ii) capture probability is similarly independent of age for these older age categories; (iii) the population has, at least, a stable age distribution

and, sometimes, is also stationary – that is, has zero growth rate. If these assumptions are valid, then it is a straightforward matter to estimate the mortality from the rate of decline of numbers with age category, using either non-linear least squares (Rogers *et al.*, 1984; Van Sickle & Phelps, 1988) or, as used here, a maximum-likelihood approach (Hargrove, 1993).

Simulation studies

We investigated the effect of perturbing a stable age distribution, through increasing the mortality of young flies, on the mortality of older flies – as estimated from ovarian age data. We simulated a tsetse population using daily age categories and daily time steps over an entire year. We initially assumed a 27-day pupal duration, a pupal mortality of 1.08% per day, a 9-day interlarval period and, initially, a mortality of 3% per day for every adult. With these choices the population was stationary. We then perturbed the age distribution by increasing the mortality, only among adult flies in ovarian category 0, to 10% per day on every day for a 2-month period. We then used the maximum-likelihood method to estimate the mortality, in flies older than ovarian category 0, before, during and after the period of raised mortality among ovarian category 0 flies.

Statistical analyses and methods

Data were analysed using Microsoft Excel and Stata 11 (StataCorp, College Station, Texas). Error bars in all figures indicate 95% confidence intervals (95% CI). The simulation was carried out in Excel, using also the Solver function to estimate mortality.

Results

Changes in catches, mean age and estimated mortality for females caught in traps

Over the 11 years of the study 293,215 female *G. pallidipes* were captured: 241,270 of these were from odour-baited traps, 20,014 from the VET and 31,931 from refuges. Ovarian dissections were performed on 144,106 of the captured flies. Mean daily catches from traps maintained a relatively stable level between late 1988 and 1992 (fig. 1A). Catches then crashed by more than an order of magnitude in a single month, following a sustained heat wave when, between 16 October and 4 November 1992, the lowest daily maximum temperature (T_{\max}) was 38°C and the average T_{\max} over the 20 days was 40.2°C. Catches recovered during 1993 but then declined again in 1994 and 1995. The mean T_{\max} in November 1994 was 37.8°C, and in October 1995 it was 38.1°C (fig. 1D) – the highest recorded temperatures for these months at Rekomitjje since record collection began in 1959. Moreover, the rainfall for the 1994/95 season was only 308 mm, the lowest rainfall recorded since record collection began and only 44% of the average seasonal rainfall of 693 mm for the period 1959–2013.

Mean ovarian ages invariably peaked at the end of the year, when temperatures were high, and often following a serious population decline (fig. 1B): there tended to be smaller peaks in mean ovarian category, and troughs in estimated mortality, mid-year when maximum temperatures took their lowest values (fig. 1C, D). We hypothesized that the rapid fluctuations in mean ovarian category, and thus in estimated mortality, reflect the instability of the population age distribution. For consecutive months of the study we compared the ovarian

category distributions of flies captured in odour-baited traps. Among the 126 such comparisons that were possible, 93 (74%) differed at a significance cut-off of 0.05 (χ^2 tests), whereas we would only expect, by chance, about 6 or 7 to be statistically significant at a cut-off of 0.05 if there were a stable age distribution. Significant differences in age distribution from month to month were seen for all times of year, not just the hot-dry season.

Diurnal distribution of trap catches

The proportion of all-day trap catches taken in the last 3 h of daylight varied from a high of 61% (95% CI 55–67%) on 5 days in May 1993 to a low of 42% (95% CI 36–49%) on 14 days during November 1992, with proportions for July–August 1991, September 1992, March–April 1993 and October 1994 all lying between these extremes. The proportion thus varied by at most a factor of 1.5 so that, while late hot-season catches will be biased downwards due to a greater proportion of fly activity taking place in the morning, this effect will be small in relation to the major declines in catches seen at this time of the year. Catch levels are anyway not used here in estimating mortality, serving instead only to provide a rough idea of annual, and longer-term, variation in population levels.

Mortality estimates using data from various capture methods

The VET capture system, used regularly only until 1993, produced samples with a different age pattern from those captured in traps. For the months of January–August the mean age was always lower for flies caught using the VET than for trapped flies: for the hot months of September–December, however, this difference disappeared (fig. 2). The mean age of flies from artificial refuges, operated only in the hot season, were similar to those from traps. Given the similarity of this pattern across the years, we pooled the data for the years 1988–1993 in order to provide a clearer picture of the changes with season for the different sampling systems.

Over the period when both traps and the VET were used regularly, estimated mortalities during the first 8-months of the year were always higher for the VET data than for the trap (fig. 3). Mortality estimates, obtained from samples taken using all three capture systems, decreased over the last 3–4 (hottest) months of the year and, during these hot months, there was no significant difference between the mortality estimates derived from trap, VET or refuge samples.

Ovarian category distribution as a function of capture method and season

For data pooled over the entire period of the study, there were significant differences between the distributions of the ovarian categories in catches of *G. pallidipes* from different sampling systems ($P < 0.001$, χ^2), most strikingly in the proportions of flies in ovarian category 0 (table 1): for the VET this proportion was three times as high as for the trap and twice as high as for the refuge catches.

For further analysis the data were aggregated on the following (approximate) ‘seasons’: hot-wet (December–February), warm-dry (March–May), cool-dry (June–August) and hot-dry (September–November) (fig. 4). A number of striking features then emerge. Between December and August the proportion of ovarian category 0 females of *G.*

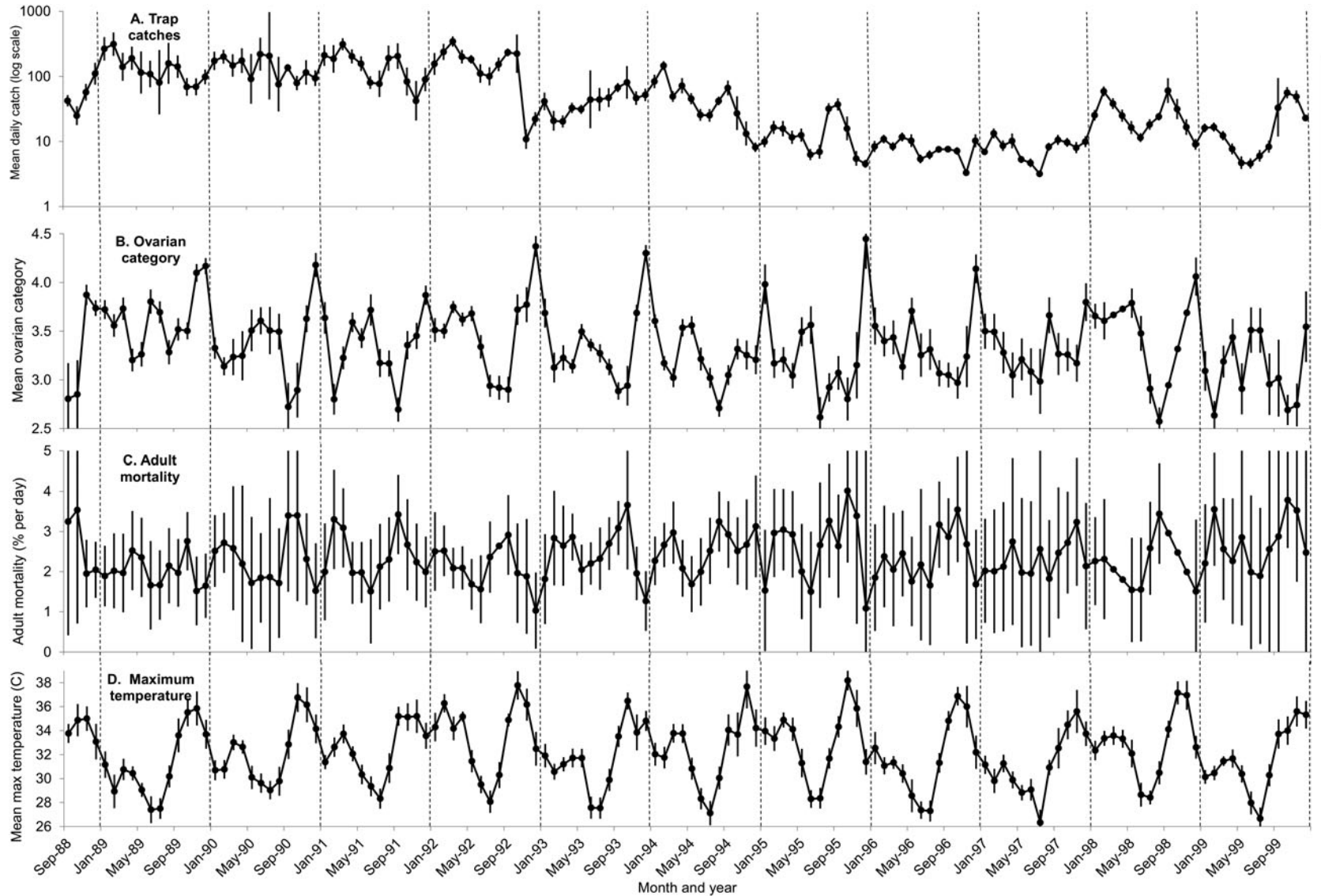


Fig. 1. Trap catches, mean ovarian category and adult mortality for female *G. pallidipes* trapped at Rekomitjie Research Station, Zambezi Valley, Zimbabwe: September 1988–December 1999. (A) Mean catch per odour-baited trap per afternoon capture session. (B) Mean ovarian categories of all flies caught in odour-baited traps. (C) Maximum-likelihood estimates of mortality, (D) Mean monthly maximum temperature measured in a Stevenson Screen at Rekomitjie Research Station.

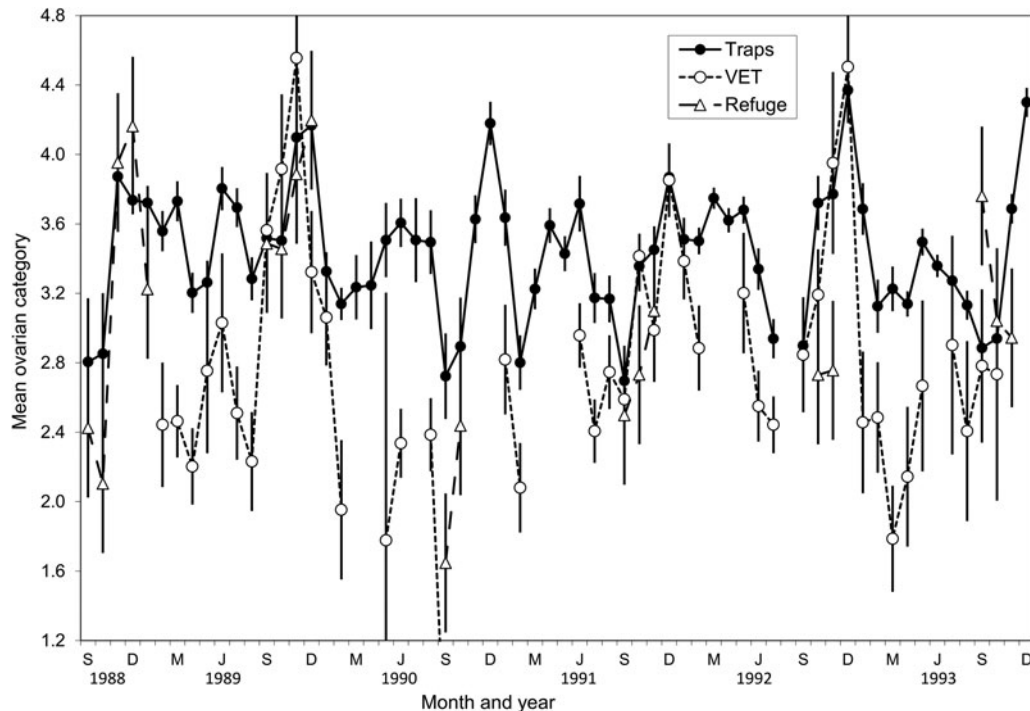


Fig. 2. Mean ovarian categories of female *G. pallidipes*, captured using three methods, as a function of month of capture.

pallidipes was about four times as high in flies from the VET as in those from traps (fig. 4). Similarly, for the VET between January and September, frequencies declined, within each seasonal grouping, as the ovarian category increased between 0 and 3. For trap catches, conversely, category 0 always had the lowest proportion of flies and there was never even a convincing decline in numbers between categories 1 and 3. In the hot-dry months of September–November, there were significant declines in the proportions of category 0 flies caught from the VET: for all three capture systems there were fewer flies in ovarian category 0 than in ovarian category 1 during these months.

Mean ovarian category as a function of season and capture method

Mean ovarian categories of *G. pallidipes* caught using traps, refuges or the VET, and pooled for each month over all years, provide a synopsis of seasonal changes and how these means differ with capture method (fig. 5A). For flies from traps, mean ovarian category peaks at about 4.2 at the end of the year, dropping sharply to 3.2 by the end of February. There is then an increase in mean ovarian category until May/June and a decline thereafter until August/September and the onset of the hot-dry season.

Samples from the VET showed a different pattern of changes with season. The decline in mean age at the beginning of the year was markedly more precipitous, moving from a value of about 3.8 down to 2.2 before increasing, again more sharply than seen in the traps samples, to a peak in June. For every month between January and September the mean ages of flies from the VET were significantly lower than those from the traps. Thereafter, however, the mean ages increased more rapidly than in traps, such that for

October–December the mean ages of flies from traps differed little from those captured from the VET or in artificial refuges (fig. 5A).

Lower mean ages of *G. pallidipes* from the VET, during the first two-thirds of each year, is largely, but not entirely, due to the higher proportions in these samples of flies in ovarian category 0. When category 0 flies are excluded from the analysis, the difference between the mean ages of flies from the VET and from traps is greatly reduced (fig. 5B). Between January and July, however, the mean ages of flies from the VET were still significantly lower than those from traps.

Mortality estimates using data pooled over 12-month periods

A possible way of overcoming the obvious instability of tsetse age distributions, and the rapid changes seen during the hot-dry season, is to pool catches over longer than the (arbitrary) monthly periods used above. Mortality estimates obtained using trap data pooled over successive 12-month periods varied between 2.0 and 2.8% per day (fig. 6A). However, when the identical procedure was applied to the VET data the estimated mortalities were higher for every year in the period 1989–1993 when the VET was regularly used. Moreover, there was no obvious sensible change with temperature (fig. 6B): neither the linear nor quadratic coefficients for the fitted polynomial were statistically significant ($P > 0.1$ in both cases) so there was no reason to suggest any increase in mortality with increasing temperature. Indeed the hottest year (1992), which was characterized by the biggest drop in trap catches throughout the study, was associated with the lowest estimated mortality. In the following year (1993) when trap catches increased consistently across the whole year (fig. 1C),

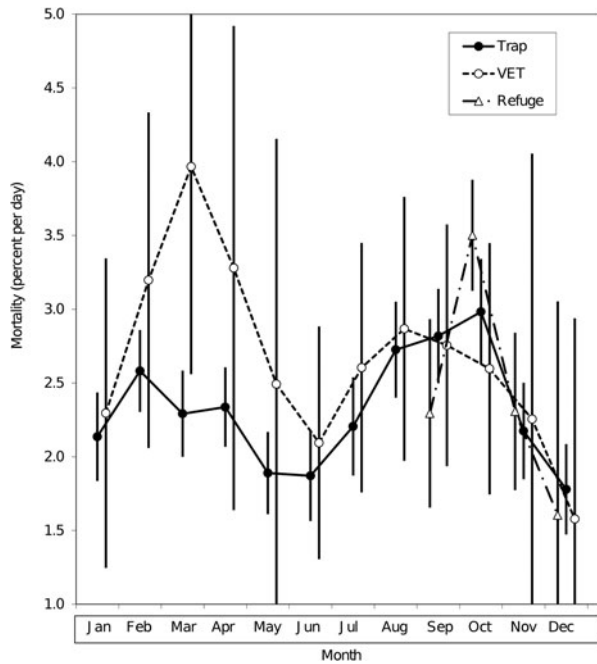


Fig. 3. Mortality in adult female *G. pallidipes* estimated, using maximum-likelihood, from ovarian dissection of samples of flies caught using traps, refuges and the VET. Data for February 1989–October 1993 when both traps and VET were in operation.

the estimated mortality increased, rather than decreased (fig. 6A).

Simulation studies

We used simulation to investigate the effect of perturbing a stable age distribution by increasing mortality only among adult flies in ovarian category 0. When the age distribution was stable, for the months labelled August and September (fig 7) the estimated mortality returned the input value of 3% used in the simulation. However, when mortality among ovarian category 0 flies was increased to 10% per day during October and November, simulation confirmed that this perturbation led to a decrease in the mortality estimated from the age distribution of flies in ovarian category >0 (fig. 7). When ovarian category 0 mortality was restored to 3% per day it took two-months for the estimated mortality for older flies to return to close to the input value of 3%. Moreover, the average mortality estimated using data pooled over all 12 months, i.e., across periods when the age distribution was, or was not, perturbed in this fashion, was 2.9%, lower than the constant input value applied in the simulation. The simulation results are thus in accord with the findings from the field-collected ovarian age data in suggesting that perturbations of the age distribution can seriously affect mortality estimates derived from ovarian age distributions.

Discussion

Mortality estimates as a function of season

From the end of 1988 until catches collapsed at the end of 1992 the population of female *G. pallidipes* was fairly stable and

Table 1. The ovarian categories of female *G. pallidipes* captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe using different sampling methods between September 1988 and December 1999.

Ovarian category	Refuge	Trap	VET	Total
0	1170	8116	2196	11,482
	9.3	6.7	19.9	8.0
1	1922	17,598	1786	21,306
	15.3	14.6	16.2	14.8
2	2022	18,872	1547	22,441
	16.1	15.7	14.0	15.6
3	1685	15,491	1146	18,322
	13.5	12.9	10.4	12.7
4	2221	21,263	1662	25,416
	17.7	17.6	15.1	17.4
5	1774	18,452	1291	21,517
	14.2	15.3	11.7	14.9
6	1137	13,314	920	15,371
	9.1	11.0	8.3	10.7
7	594	7451	476	8521
	4.7	6.2	4.3	5.9
Total	12,525	120,557	11,024	144,106

$P < 0.001$ for the difference between methods of the distribution of females between ovarian categories.

the average value of the estimated mortality was 2.2% per day. This is not inherently unreasonable, given that this adult mortality, combined with approximately the same mortality rate among pupae, would result in an approximately stable population (Hargrove, 2004). On the other hand, seasonal variation in mortality among adult female *G. pallidipes*, as estimated from trap catches (fig. 1A), makes little sense. Given that mark-recapture studies show that adult mortality increases with temperature (Hargrove, 2001a, b), one expects high mortality rates in December, when the mean T_{max} always exceeded 31°C, and was greater than 33°C for 8 of the 12 years in this study. In each year of the study, however, the mean ovarian age peaked, and mortality rates took their lowest values, in December. Furthermore, the problem is not limited to estimates derived from trap samples: for flies captured using the VET there is a general decline in estimated mortality from August, through the hot season, to December when the lowest mortalities are estimated to occur (fig. 3) with corresponding increases in mean age over this period, whether or not category 0 flies are included in the analysis (fig. 5A, B).

High mean ages and low mortalities in December are particularly difficult to explain when, as in most years but particularly in 1992, 1994 and 1995, the peak in mean age immediately follows periods of major decline in tsetse catches. This would suggest increased, rather than decreased, mortality (fig. 1A). Increasing mean age could arise from an increased input of older flies, and/or disproportionate increases in mortality among young adults, larvae or pupae. The former scenario seems unlikely to play any important part in the current situation: significant increases in the numbers of older flies could only arise from immigration, and this would tend to increase rather than decrease the population. Trap catches around this time of year are decreasing, indicating a population decline.

An increase in mortality among all adult flies as the hot season commences, with disproportionately increased mortality rate among pupae and/or newly emerged flies is, however, consistent with the following results from this study and published work: (i) decreasing proportions, over the hot season, of

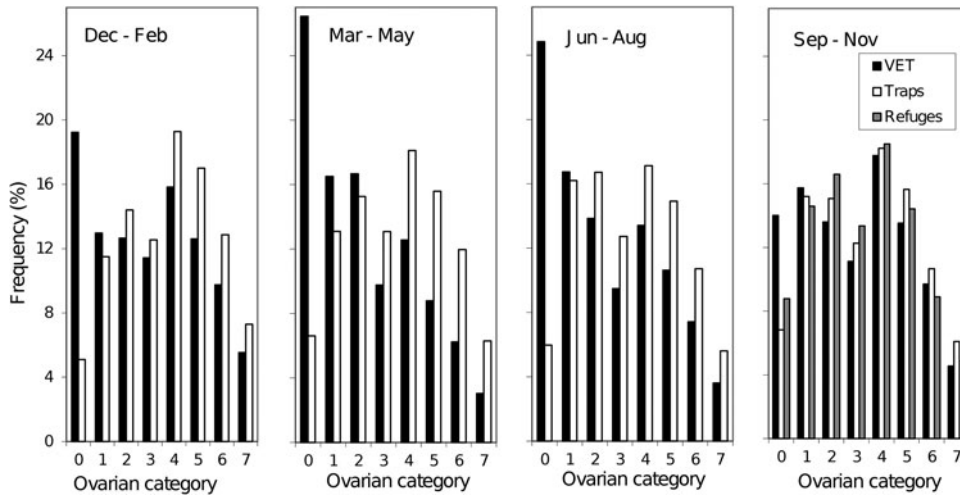


Fig. 4. Ovarian category distributions for *G. pallidipes* captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, September 1988–December 1993. Data pooled on 3-month periods over all years. Total sample sizes: VET 10,207; traps 69,857; refuges 5250.

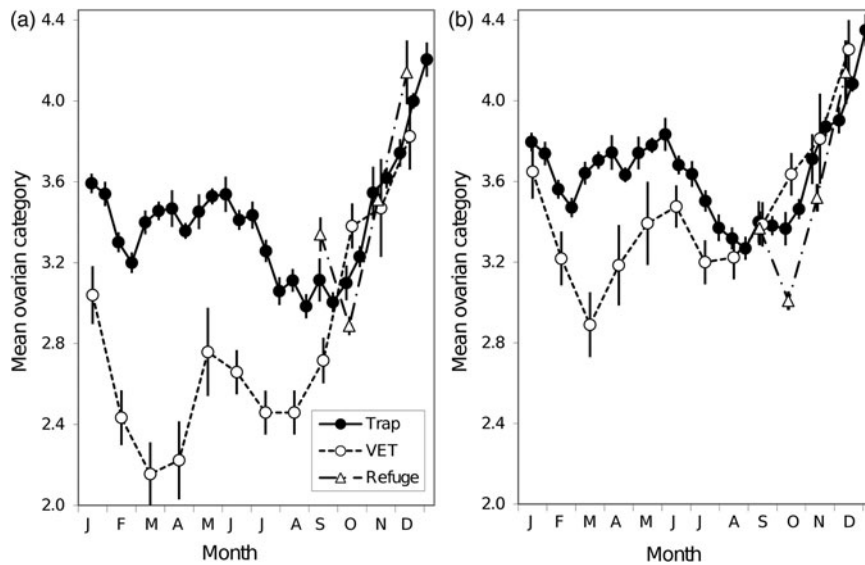


Fig. 5. Mean ovarian categories of female *G. pallidipes*, captured using three methods, as a function of month of capture. (A) Means of all ovarian categories. (B) Means for ovarian categories 1–7. Samples pooled over all years for each capture method. Within each year samples were pooled on fortnightly for trap catches; all other samples were pooled on monthly intervals.

flies in ovarian category 0 regardless of the sampling system used (fig. 4); (ii) a consequent shift during this season in the age distribution, towards older flies, and thus an increase in the mean age (fig. 5); (iii) results from mark-recapture studies, indicating an increase in adult mortality as temperatures increase (Hargrove, 2001a, b); (iv) a decrease in the total adult population and thereby a decrease in trap catches at this time (fig. 1A); (v) a reduction during the hot season in the difference between the age distributions (fig. 4), and mortality rates (fig. 3A), estimated using different sampling systems, because the younger age classes, particularly but not exclusively ovarian category 0 flies, that are sampled more efficiently by the VET than by traps, are largely missing from the

population at the hottest times of the year; (vi) increased levels of parasitized puparia found in the population as the dry season commences (Hargrove & Langley, 1990, 1993); (vii) significantly increased losses among newly emerged, and smaller, flies at the hottest times of the year (Jackson, 1945, 1948; Bursell & Glasgow, 1960; Glasgow, 1961; Phelps & Clarke, 1974; Dransfield *et al.*, 1989). These results are consistent with the idea that decreases in adult mortality towards the end of the hot-dry season, as estimated from age distribution data using current techniques, are spurious – consequent on rapid changes in age distribution resulting from large increases of mortality among immature and young adult tsetse in the hot-dry season.

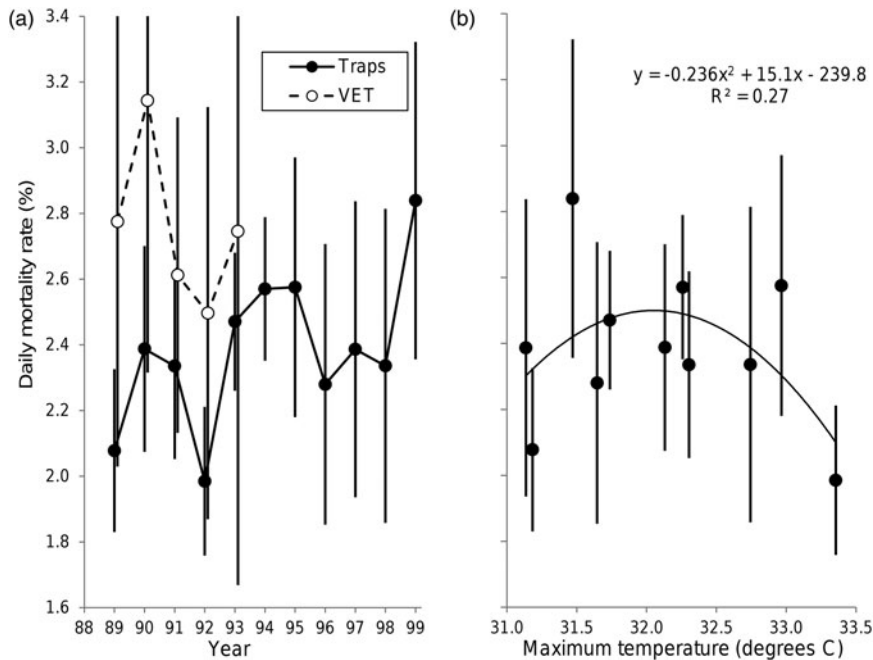


Fig. 6. Maximum-likelihood estimates of mortality in adult female *G. pallidipes* using ovarian dissection data pooled over yearly intervals. (A) Mortality as a function of year for flies captured using odour-baited traps or the VET. (B) Mortality as a function of the mean annual maximum temperature.

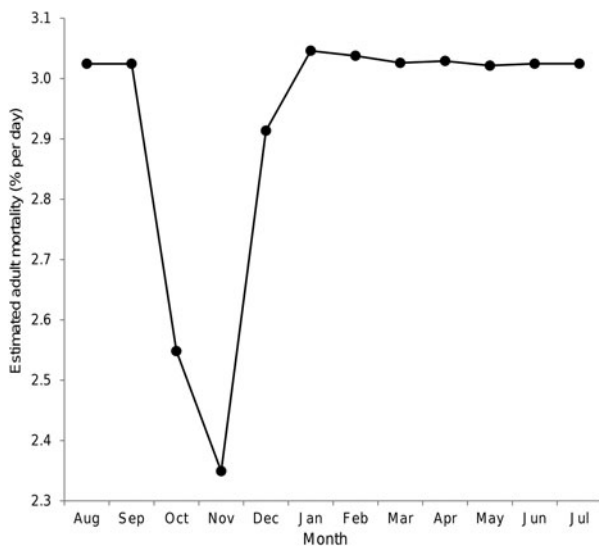


Fig. 7. Mortality in adult female tsetse of ovarian age >0 , estimated by applying the maximum-likelihood method to ovarian age distributions produced by simulation. In all months, the mortality for flies in ovarian age >0 was 3% per day. The mortality for flies in ovarian age 0 was 30% per day for the months labelled October and November: otherwise the mortality was 3% per day as for the older flies. See text for further details of the simulation.

Mortality estimates from samples pooled over 12-month periods

The question then arises about whether it would be possible to estimate mortality from age distributions if data

were pooled over periods longer than the one-month periods used here and elsewhere in the tsetse literature. Pooling catches over 12-month periods will undoubtedly tend to balance births and deaths over the whole period and lead to greater stability in the age distribution, viewed at the larger time scale. As is evident from [fig. 6](#), however, the resulting mean annual mortality estimates do not appear to change in any sensible way either with maximum temperature, or with observed rates of change in trap catches. Moreover, the pooling exercise does not remove the problem that the mortality estimated changes markedly with capture method – and there is no *a priori* reason to suppose that either is giving the correct estimate. We are anyway less interested in changes in mortality between years than we are in how mortality changes with shorter-term meteorological conditions, and the pooling procedure eliminates any possibility of investigating such issues.

Factors determining the differential effect of age on patterns of mortality and capture probability

The above results arise as a direct consequence of the unique tsetse life history. Adult females produce single larvae with all of the energy and raw material required to produce an adult fly. However, while the teneral fly that emerges from the puparium has the linear dimensions of a mature adult, it has low fat levels and underdeveloped flight musculature (Hargrove, 1999a, b). It is thus at increased risk of starving and, at the same time, is poorly equipped to make the flights necessary to get its first blood meal. Unsurprisingly, young flies have the highest mortality in the field – even when meteorological conditions are favourable (Hargrove *et al.*, 2011). At high temperatures these effects are exacerbated because the proportion of fat used during the pupal phase

increases as temperatures rise above about 26°C (Phelps, 1973). Moreover, fly size diminishes in the hot season and fat levels at emergence are relatively lower in smaller flies. Low fat levels and poorly developed flight musculature also mean that young tsetse are ill-equipped to make the spontaneous flights required to find stationary hosts, thus explaining the low proportions of females in ovarian category 0 among flies captured in stationary traps.

What can ovarian age data tell us about mortality rates in tsetse?

It is difficult to escape the conclusion that the application of classical analysis of tsetse ovarian age data is providing, in our study, a seriously misleading picture of mortality in adult female *G. pallidipes*. It thus seems likely that there are serious violations of the assumptions underpinning the estimation procedure. Two questions then arise: (i) Which are the most important violations? (ii) Can we nonetheless obtain mortality estimates from tsetse age distribution data that is not subject to the type of errors seen here?

Examination of assumptions required for estimating mortality from ovarian age data in tsetse

Age distribution is stable

Van Sickle & Phelps (1988) argued that it was unlikely that tsetse populations, at least at Rekomitjie, ever had a stable age distribution. Our finding that ovarian category distributions differed in 74% of pairs of consecutive months tested provides strong support for their hypothesis. Williams *et al.* (1990) used a simulation model to show how increased mortality in particular age groups leads to a wave of disturbance in the age distribution, which passes through the population with time. The rapid decline during the very hot months, in catches of female *G. pallidipes* in ovarian category 0, both from traps and the VET (fig. 4), is consistent with a disproportionate increase in mortality in recently emerged adults, and/or among pupae, at this time. This would produce exactly the type of wave described by Williams *et al.* (1990) and would be expected to destabilize the age distribution for some months.

Samples are unbiased with respect to age

During the hot season (September–December), mortality and mean age estimates for female *G. pallidipes* do not differ significantly with capture method (figs 3, 5A, B). From January–August, however, the mean age of flies captured on the VET is much lower (fig. 5A, B), and the mortality estimates significantly higher (fig. 3), than when flies are sampled using odour-baited traps. Clearly, over these months, at least one of the methods is providing a biased age distribution, regardless of whether category 0 flies are excluded from the analysis. Given that, for *G. pallidipes* caught in traps between January and August, the proportions of flies in ovarian categories 1–3 hardly declines with age (fig. 4) it seems reasonable to concur with previous suggestions that the probability of capturing a female tsetse in a trap increases with the age of the fly (Hargrove, 1991, 1993).

If age-dependent sampling bias were the major source of error in mortality estimates, however, it should be a simple matter to estimate the bias, correct for it, and thereby arrive at acceptable estimates of mortality. When this was attempted

previously, however, it was found that the resulting adjustments were small and that mortality estimates from ovarian dissection data were still consistently lower than those from mark-recapture (Hargrove, 1993). Moreover, whereas catches from the VET are plainly less biased against young female *G. pallidipes* (fig. 4), mortality estimates based on catches from this sampling method also decline over the last half of the year (fig. 3). It appears, therefore, that age-dependent sampling bias is definitely not the only, and probably not even the major, source of the problem.

Although we cannot exclude the possibility that the capture/mark process increased the mortality among marked flies, and may have contributed to the discrepancy between the mark-recapture and ovarian dissection estimates, the important point that we are making with the present study is not simply that ovarian dissection methods are underestimating mortality. We are saying, more importantly, that there is an unrealistic relationship between temperature and mortality estimates emanating from ovarian dissection data: estimated mortality decreases rather than increases with increasing temperature. Mark-recapture estimates of mortality show more reasonable relationships (Hargrove, 2004).

Samples unbiased with respect to physiological status

Allied to age-dependent sampling biases are biases relative to pregnancy and nutritional status. Van Sickle & Phelps (1988) and Hargrove (1999a) found that trap samples of female *G. pallidipes* were biased in favour of flies at the start of the pregnancy cycle. Present results (not shown) support those conclusions, but the bias changes little with ovarian category so there is little reason to suppose that this will add to the age selection bias.

The nutritional status of flies sampled is also a function of the sampling method. For example, at any given stage of pregnancy, *G. pallidipes* from traps have significantly lower fat levels than those from refuges and it has been argued that the traps are specifically selecting flies with the lowest nutritional state in the population (Hargrove, 1999a, b). Since, however, this bias may be expected to be similar in all ovarian categories, except among the youngest flies, the bias may have minor effects on the estimates of mortality, particularly if ovarian category 0 flies are excluded from the estimation procedure.

Adult mortality is independent of age

As demonstrated, both in the laboratory (Curtis & Jordan, 1968; Jordan & Curtis, 1968, 1972) and in the field (Hargrove, 1990), mortality in adult female tsetse is not independent of age. Age-specific mortality in female tsetse is a U-shaped function, modelled as the sum of two exponential terms, with raised mortality in flies in their first few days of life and an ageing effect setting in following a prolonged period of low natural mortality (Hargrove *et al.*, 2011). Available field data suggest, however, that the increase in mortality rate with age can be fairly slow: in an island population of *G. morsitans morsitans*, adult female mortality only changed from about 1% per day at age 10 days to a little more than 2% per day at 100 days (Hargrove *et al.*, 2011). If, as has been the practice in several studies, ovarian category 0 flies are excluded from analyses, the modest changes with age in the mortality in older flies are thus unlikely to cause major errors in the estimation of the average adult mortality over this age range.

How should we estimate adult female tsetse mortality from age distribution data?

This study has highlighted the fact that current techniques for estimating adult tsetse mortality from ovarian dissection data can produce misleading results. The problem may be particularly acute at Rekomitjie, where the seasonal changes in temperature are large. Elsewhere the problems may not be as severe. Rogers *et al.* (1984), working in Ivory Coast for example, found that adult mortality in *G. palpalis palpalis*, estimated from ovarian dissection data, changed in a sensible manner with the observed temperature, host density and trap catch levels. Perhaps in their situation the age distribution was stable throughout the study. Nonetheless, we do need a more general technique catering for situations, such as those observed at Rekomitjie, where there are clearly marked departures from a stable age distribution.

An entirely new approach to the estimation of insect population age structure has been suggested by Carey *et al.* (Carey *et al.*, 2008, 2012a, b; Carey, 2011; Novoseltsev *et al.*, 2012) who have used the times-to-death, of field-collected insects raised in the laboratory, to estimate shifts in the age structure of the population sampled. Apart from the practical difficulties of applying this method to tsetse, the present study suggests that such an approach would not overcome the essential problem observed here, which is not an inability to measure fly age distributions using ovarian dissection data but, rather, the absence of a stable age distribution in the study population. This problem precludes the simple estimation of mortality even from a sample with perfectly known age distribution. In addition, Carey's approach would not overcome the problem of obtaining a sample from a tsetse population that is unbiased with respect to age.

In an attempt to overcome such problems we have developed a dynamical model of female tsetse populations which makes no assumptions about the stability of the age distribution, using population levels as well as age distribution data to fit mortalities. The model shares features with the matrix approach of Jarry *et al.* (1996, 1999), but we built into the model extra options, capturing established features of tsetse biology: (i) age-dependent mortality rates; (ii) age-dependent capture probabilities; (iii) temperature-dependent mortality rates; (iv) temperature-dependent rates of ovulation; (v) temperature-dependent rates of pupal development; (vi) density-dependent mortality at the pupal stage (Ackley, Liu & Hargrove, in preparation).

Implications for the estimation of mortality in wild animal populations from age distribution data

The present study is unusual in being able to draw on very large data sets, collected over 11 years, and also to reference much other information on the tsetse species studied. For many studies of wild animal populations such rich information is most often not available. The dangers inherent in such situations can be illustrated by imagining that we only had available the age distribution data shown in fig. 4, for flies caught in odour-baited traps. Without further information, naïve estimates of mortality derived from such data appear at face value to be well behaved (fig. 1C) and sit in a believable range of 1–3.5% per day, with modest errors. A more careful analysis shows, however, that the results are anything but reasonable.

Supplementary material

The supplementary material for this article can be found at <http://www.journals.cambridge.org/BER>.

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