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AN EXAMPLE FROM THE WORLD OF TSETSE FLIES

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Abstract. In biomathematics, communication between mathematicians and biologists is crucial. This matter is illustrated using studies aimed at estimating mortality rates of tsetse flies (*Glossina* spp.). Examples are provided of apparently sound pieces of mathematics which, when applied to real data, provide obviously erroneous results. More serious objections arise when mathematical models make no attempt to address the real world in such a way that they can be tested. Unless models account for the known biology of the problem under investigation, and are challenged with data, the existence and nature of imperfections in the models will likely not be detected.

1. **Introduction.** Biomathematics, the application of mathematical techniques in biology, has shown its value through the solution of otherwise intractable problems. The subject area is set about, however, with numerous pitfalls: three, perhaps linked, dangers spring to my mind. The first arises when a mathematician, keen to arrive at a mathematically elegant solution of a biological problem, disregards important elements of the biology. The second arises when a biologist, through insufficient mathematical knowledge or self-confidence, is not (or feels not to be) in a position to question the mathematics. The third occurs when failure of the two sides to communicate leads to apparently plausible results that are not adequately interrogated by either side.

As an illustration of the problems that can arise in this regard, I consider below the problem of estimating mortality in populations of wild animals – using as my example the tsetse flies (*Glossina* spp) with which animals I have worked for the past four decades. The mathematics involved is quite simple but allows the illustration, nonetheless, of the dangers of accepting at face value the results of a mathematical analysis. More particularly it illustrates the dangers of ignoring the detailed reality of the biology in the mathematical analysis.

2. **Results.** Bearing this in mind I start by going back to square one of the natural history of tsetse flies and look at their unique life cycle. We ignore the details at our peril – because they must, by their nature, contain within their number the elements responsible for the dynamics of the population. This should become clear as we proceed. The following provides an outline: further details of the life-cycle, and background to this presentation, are available online [11].

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Tsetse flies (*Glossina* spp.) are important, indeed the sole, vectors of human and animal trypanosomiasis in Africa. They are also interestingly unique biologically. Both sexes feed only on blood and reproduction is by way of *adenotrophic viviparity* – eggs hatch in the uterus and three larval instars are nourished via a milk gland. Third instar larvae are deposited singly; the larva burrows rapidly into the soil, pupates immediately and remains underground, *without feeding further*, developing into a young adult fly. This makes the tsetse almost unique among blood-sucking insects: others such as horse flies, stable flies, black flies, horn flies, mosquitoes and midges all lay eggs and require a moist environment for the free-living larvae that hatch from these eggs and must find their own food before they themselves pupate.

The implicit requirement for energy and raw materials to be provided by the tsetse mother means that the larva is huge – often weighing more than the female that has just deposited it. Hardly surprisingly the female only produces one such little monster every nine days or so, and the resulting pupa takes 3–7 weeks, depending on temperature, to develop into an adult fly. Moreover, the adult female keeps the physiological load of reproduction within reasonable bounds by "cheating" a little with the offspring that she produces. Thus, while the teneral (*i.e.* unfed) adult that emerges from the puparial case has the full linear dimensions of the mature adult, its mother never provides enough energy, or raw material, to produce a fully mature adult. Instead, the teneral has a poorly developed flight musculature, and lower levels of fat reserves than mature adults. The first 2-3 blood-meals are used to redress these shortfalls (Figure 1). Unsurprisingly, the birth rate in tsetse

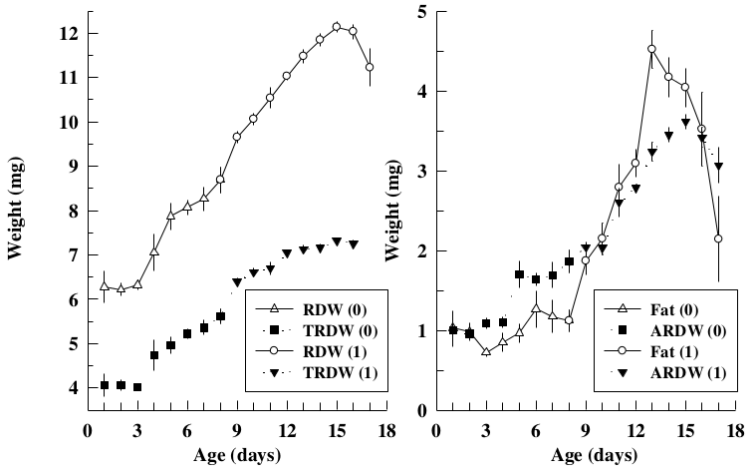

FIGURE 1. Increases with age in fat, and residual (*i.e.*, fat-free) dry weight of the thorax (TRDW), abdomen (ARDW) or whole female tsetse fly (RDW), during her first two ovulations. *G. pallidipes* sampled at Rekomitjie Research Station, Zambezi Valley, Zimbabwe [7].

is much lower than for the other flies mentioned above, which may lay hundreds of eggs at a time. It is thus obvious that tsetse populations can only survive if they are able, equally, to keep their mortality at low levels. But how low is low, and how will we measure this mortality rate? Early approaches utilised the technique

termed mark-release-recapture (MRR) applied to adult flies. Flies can be caught in the field, marked with dots of coloured oil paint on the dorsal thoracic surface to indicate the time and position of capture, and released into the environment. The proportions of marked flies among subsequent samples can be used to estimate population numbers, and birth and death rates, using well understood mathematical techniques [15, 21, 22].

A serious problem with the MRR method of estimating mortality is that it is hard to differentiate mortality from emigration. A way around that problem is to make the measurements in a closed population, in particular using an island situation – closed to in and out migration. Using such an island it was possible to show that mortality among adult tsetse increases in a regular manner with temperature (Figure 2).

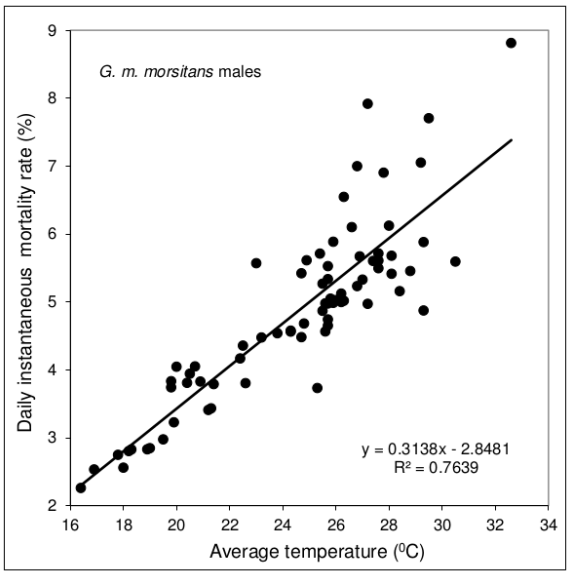

FIGURE 2. The relationship between temperature and mortality in adult tsetse flies, *Glossina morsitans morsitans* Westwood, estimated in a multiple mark-release recapture exercise carried out on Antelope Island, Lake Kariba, Zimbabwe [9, 10].

It is much more difficult to interpret the results of MRR studies in open populations: moreover, the experiments themselves are labour-intensive, costly and time consuming. Accordingly, tsetse biologists have sought other ways of estimating mortality. In particular they have borrowed techniques from demography, and the studies of wild vertebrates [1], and have tried to estimate mortality from the age distribution of samples of animals. An absolute requirement for this approach is an ability to estimate the age of individual animals: for humans this can be simple in societies where individuals know their own ages and divulge them accurately; in other animals various attributes such as size, or dental wear, can be used. None of these would work for tsetse. However, it was noted that the very regular production of larvae by female tsetse results in well-defined changes in the ovarian structure which can be used to provide an estimate of age (Figure 3).

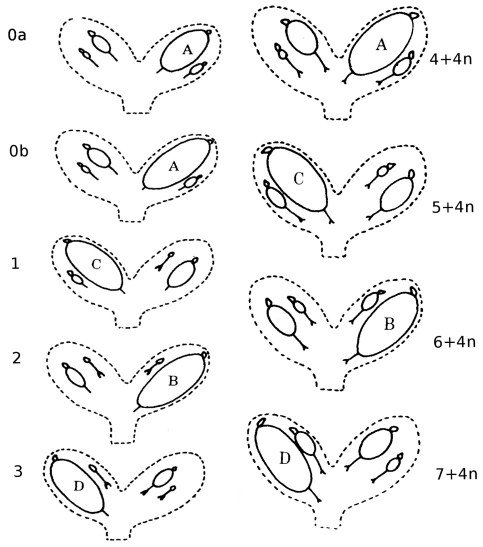

FIGURE 3. Diagrammatic representation of changes in the appearance of tsetse fly ovaries during successive ovulations. Symbols next to each diagram indicate the number of ovulations completed: 0a and 0b indicate females that have not yet ovulated for the first time and where the largest oocyte is shorter, or longer, respectively, than about 0.6 mm [2].

Tsetse have paired ovaries, with two oocytes in each ovary. In the normal fly, oocytes develop and are ovulated in the strict order: right inner (labelled A), left inner (C), right outer (B) and left outer (D). The relative sizes of the oocytes thus allow us to distinguish between flies that have ovulated 0, 1, 2 or 3 times. The oocytes of a fly that has ovulated 4 times have, however, the same relative sizes as a fly that has not yet ovulated (Figure 3). Category 0 and category 4 can, however, be distinguished because ovulation results in the ovariole bearing a follicular relic of the previous ovulation. A fly that has ovulated 4 times has a relic on each ovariole, while a fly in ovarian category zero has no relic on any ovariole. Similarly, flies in ovarian category 1 can be distinguished from flies in ovarian category 5, 2 from 6 and 3 from 7.

Unfortunately, only one relic is ever seen on an ovariole: further ovulations do not result in changes in the appearance of the relics and it is thus only possible for flies in ovarian categories 4, 5, 6 and 7 to estimate the number of ovulations modulo 4. For example, a fly in ovarian category 4 can have ovulated 4, 8, 12, 16 ... *etc* times and similar statements apply to flies in ovarian categories 5, 6 and 7.

Using the above ovarian dissection technique, female tsetse flies sampled in the field can be assigned to an ovarian category and it is possible thereby to construct a sample age distribution. Under certain regularity conditions, which we will have cause to think about more carefully later, it is possible to estimate mortality from such data. Thus if we assume that a fly survives any given ovulation cycle with probability φ, then the probability that a fly dies in ovarian category zero is $p_0 = (1-\varphi)$ and in category one $p_1 = \varphi^1(1-\varphi)$. In general, the fly dies in category

$i(i \geq 0)$ with probability $p_i = \varphi^i(1-\varphi)$ and this also gives the probability that a randomly chosen fly is in ovarian category i.

Where, as described above, it is not possible to pinpoint exactly the number of ovulations completed by flies in ovarian categories $k = 4, 5, 6$ and 7, we pool the flies in ovarian categories $k+4n$, forming the sums (S_k) of the probabilities for flies which have ovulated $k, k+4, k+8 ...etc.$ times. Thus

$$p_4 = \varphi^4(1-\varphi)$$
$$p_8 = \varphi^8(1-\varphi)$$
$$p_{12} = \varphi^{12}(1-\varphi)$$

and so on. The total probability is thus:

$$\begin{aligned} S_4 &= (1-\varphi)(\varphi^4 + \varphi^8 + \varphi^{12} + ...) \\ &= (1-\varphi)\varphi^4(1 + \varphi^4 + \varphi^8 + ...) \\ &= \frac{\varphi^4(1-\varphi)}{(1-\varphi^4)} \end{aligned}$$

Similar calculations can be carried out for S_5, S_6 and S_7 and, generally:

$$S_i = \frac{\varphi^i(1-\varphi)}{(1-\varphi^4)} \quad \text{for } 4 \leq i \leq 7$$

In estimating the survival probability (φ) we seek a value of φ that maximises the probability of obtaining the particular sample that we actually obtained.

The likelihood (L), for a sample containing n_i flies in each of the i categories, is given by the product (denoted here by the symbol Π) of the probabilities (assumed identical) of sampling each of the individual flies:

$$\begin{aligned} L &= \prod_{i=0}^{3} \varphi^{in_i}(1-\varphi)^{n_i} \prod_{i=4}^{7} \frac{\varphi^{in_i}(1-\varphi)^{n_i}}{(1-\varphi^4)^{n_i}} \\ &= \prod_{i=0}^{7} \varphi^{in_i}(1-\varphi)^{n_i} \prod_{i=4}^{7} \frac{1}{(1-\varphi^4)^{n_i}} \end{aligned}$$

We seek the value of φ that produces the largest value of L or, equivalently, of $\log L$. To do this we differentiate $\log L$ with respect to φ, set the derivative equal to zero and solve for φ in the usual manner. Thus:

$$\log L = \sum_{i=0}^{7}(in_i \log \varphi + n_i \log(1-\varphi)) - \sum_{i=4}^{7} n_i \log(1-\varphi^4)$$

$$\frac{\partial \log L}{\partial \varphi} = \frac{1}{\varphi}\sum_{i=0}^{7} in_i - \frac{1}{1-\varphi}\sum_{i=0}^{7} n_i + \frac{4\varphi^3}{1-\varphi^4}\sum_{i=4}^{7} n_i$$

Setting this equal to zero and multiplying through by $\varphi(1-\varphi^4)$, noting that $(1-\varphi^4) = (1-\varphi^2)(1+\varphi^2) = (1-\varphi)(1+\varphi)(1+\varphi^2)$, gives:

$$(1-\varphi^4)\sum_{i=0}^{7} in_i - \varphi(1+\varphi)(1+\varphi^2)\sum_{i=0}^{7} n_i + 4\varphi^4 \sum_{i=4}^{7} n_i = 0$$

For the maximum likelihood solution we must therefore solve

$$x_1(\varphi + \varphi^2 + \varphi^3 + \varphi^4) - x_2(1-\varphi^4) - 4x_3\varphi^4 = 0 \tag{1}$$

where

$$x_1 = \sum_{i=0}^{7} n_i$$

$$x_2 = \sum_{i=0}^{7} i n_i$$

$$x_3 = \sum_{i=4}^{7} n_i$$

Figure 4 shows distributions of ovarian categories of samples of tsetse captured in the field in Zimbabwe at two different times of the year, using two different sampling methods: i. A Vehicle-mounted Electric Target (VET): this consists of an electric net [23] mounted on the back of an open truck, driven through tsetse habitat. Flies react to the visual stimulus and are electrocuted when they intercept the electric net [8]. ii. Stationary odour-baited traps. Tsetse that are in flight intercept the odour plumes and are trapped using a mechanical device [24].

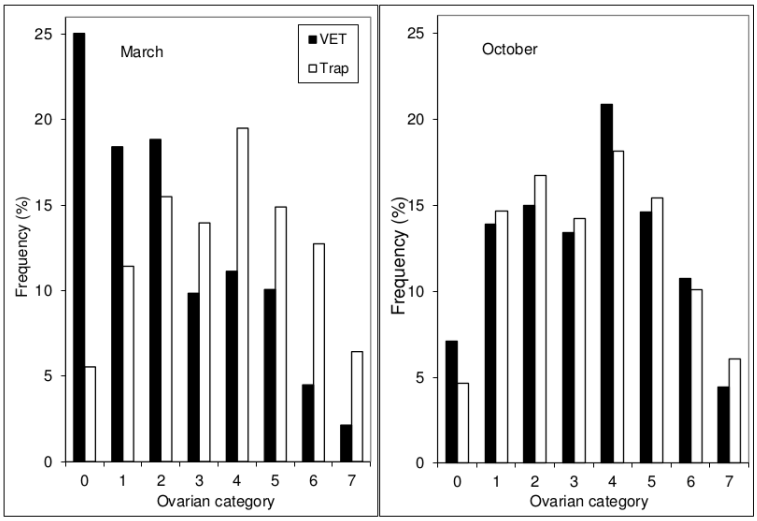

FIGURE 4. Ovarian category distributions for female *G. pallidipes* captured in traps or on the VET. Rekomitjie Research Station, Zambezi Valley, Zimbabwe, September 1988 – December 1993

When Equation (1) was applied to the data for flies caught in March, using the VET, the resulting survival per ovulation cycle was $\varphi = 68.2\%$, a mortality of $\mu = 1 - \varphi \cong 30\%$ (Table 1). When, however, the same procedure was applied to the trap data – collected from exactly the same area over exactly the same time period – the mortality was only about 14%, less than half of the value estimated from the VET sample ($P < 0.05$ for the difference).

Inspection of Figure 4 suggests that the source of this difference lies primarily in the discrepancy between the proportions of ovarian category zero flies, flies that have not yet ovulated, produced by the two sampling methods: this category appears to be significantly under-represented in the trap sample. This problem has been

TABLE 1. Estimates of mortality per ovulation for female *G. pallidipes* captured using either a VET or an odour baited trap. Flies captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, in the months indicated between September 1988 and December 1993.

Capture system	Month	Categories included	Mortality (95% confidence interval)
VET	March	0 - 7	29.7% (23.3% - 36.1%)
Trap	March	0 - 7	14.2% (6.7% -21.6%)
VET	March	1 - 7	31.8% (22.8% - 40.7%)
Trap	March	1 - 7	19.7% (13.9% - 25.5%)
VET	October	1 - 7	19.6% (13.2% - 26.0%)
Trap	October	1 - 7	22.9% (18.7% - 27.2%)

identified by previous workers [25] and category zero flies have accordingly often been excluded from analyses. When, however, the analyses were repeated, excluding the data for category zero, the two sampling systems still gave rise to significantly different results, about 32% and 20%, respectively (Table 1).

The picture becomes further confused when we look at the data for October. Now it appears that category zero flies are seriously under-represented in the data from both sampling systems. Mortality estimates, obtained as above and excluding the data for category zero flies, were very similar for the samples from both VET and traps, about 20 and 23%, respectively ($P > 0.05$ for the difference).

These results involve several difficulties and contradictions. In the first place we must ask which, if either, estimates of mortality seem reasonable – those derived using trap catches, or those from the VET? The March data seem to suggest that the VET data are providing a better answer because the trap data must under-estimate the true mortality as a consequence of under-sampling younger age classes. But when we consider the October data the age distributions, and thus the mortality estimates, do not differ significantly between the two sampling systems. Have the age-dependent sampling biases of the two systems changed with season? Can we now believe either estimate?

Moreover even the relatively lower mortality levels in March than in October are counter intuitive. The mean daily temperature at Rekomitjie Research Station during the years of the study was $27.0°C$ in March and $29.8°C$ in October. Given the mark-recapture results (Figure 2) we should expect mortality to be markedly higher in October: instead the trap results suggest no difference and the VET results suggest a decline in mortality during the hottest time of the year.

In order to understand what is going on we need to go back to the assumptions underpinning the use of age distributions for the estimation of mortality and see whether these assumptions are justified in the case of tsetse. Where the assumptions are violated it is then of interest to see how the violations affect the results, and which violations have the most serious consequences.

In order to estimate mortality, by applying Equation 1 to age distributions of samples from the field, we need to assume (at least) that: i. All age classes are equally sampled. ii. Mortality is independent of age. iii. The age distribution is stable.

i. All age classes are equally sampled

The results discussed above have already indicated that at least one of the sampling systems (the VET or trap) must provide samples that are biased with respect to age. Indeed, by looking at the relative proportions of flies of each age category, from each sampling system, it is clear that as flies get older they become progressively more likely to be captured in traps compared to the VET (Figure 5). As a consequence, mortality estimates obtained using trap samples under-estimate the true value. As expected, when MRR estimates of mortality for an island population of tsetse were compared with estimates from ovarian dissection data, for flies captured in traps, the latter were always significantly lower [6].

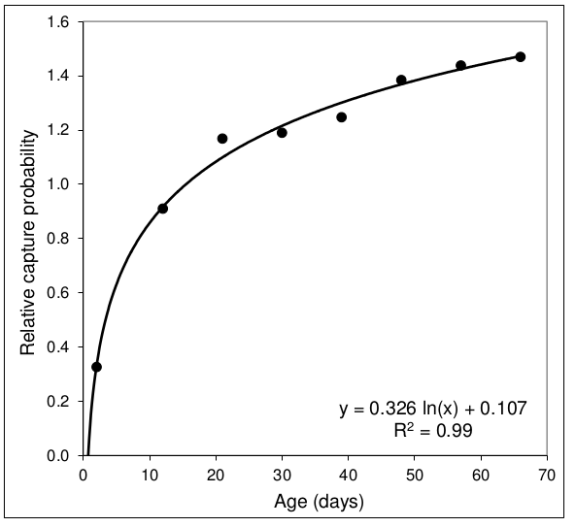

FIGURE 5. Ratio of the proportion of flies of different estimated ages captured in a trap compared to a VET.

It was, however, possible to estimate the degree of this age-dependent sampling bias – using functions such as those shown in Figure 5 – and to adjust for the bias. Whereas the adjusted estimates were closer to the MRR results the adjustment made only a modest difference. This violation is therefore not the only, and probably not the most important, problem.

ii. Mortality is independent of age

MRR studies on an island have been used to show that mortality in adult tsetse in the field is not independent of age [5, 12] (Figure 6). Mortality was highest in flies that had just emerged, declined rapidly during the first week of life, and then increased quite slowly, in females, with time since release as newly emerged adults. In other words tsetse exhibit "ageing". Again, however, the violation of the assumption of age-independent mortality should not, of itself, cause a serious problem. Firstly, the normal procedure of excluding category zero flies from the estimation procedure thereby excludes the young flies with the highest mortality. Secondly, the ageing process is slow in females (much more rapid in males) so that over the first 60 days of life there is not a large change in the mortality. By this age most flies will have died and the contribution of the remainder will be modest.

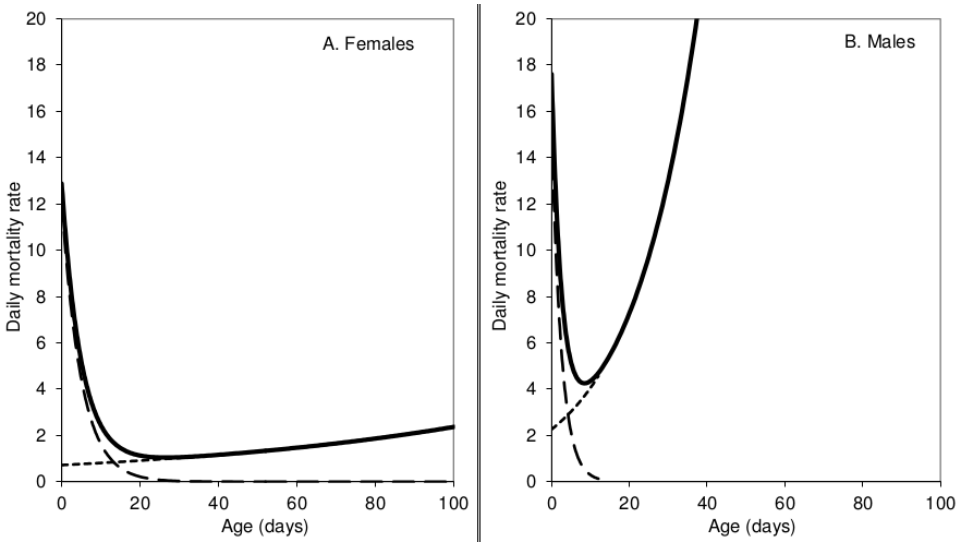

FIGURE 6. Daily mortality rate as a function of age in female and male *G. m. morsitans*, estimated by mark-recapture on Redcliff Island, Lake Kariba, Zimbabwe. The dotted and dashed lines indicate that the mortality can be expressed as the sum of two exponential functions [12].

iii. The age distribution is stable

Where differential mortality among different age groups may be more important is in destabilising the age distribution – and it is here that we begin to see the explanations for the confusing results shown in Figure 4 and Table 1. We begin by considering the cause of the change, between March and October, in the relative frequency of category zero flies in trap and VET catches. One possibility is that age-dependent sampling biases change with season. In March, in this scenario, flies are caught with higher probability on the VET than in the trap, but this difference largely disappears in October. This possibility was checked by making plots of the type shown in Figure 5 and these plots indicated no change with season. At all times of the year the probability of category zero flies being captured in a trap was always markedly lower than from the VET.

An alternative explanation for the much reduced proportion of category zero flies in the October catches from the VET is simply that there is a massive increase in the mortality of young flies at the hottest time of the year. In this scenario the VET fails to catch large proportions of category zero flies for the simple reason that there has been a massive decline in the proportion of these flies in the population.

There are good reasons, from the knowledge of the biology of tsetse, to support such a possibility. It was noted above (Figure 1) that newly emerged adults have minimal fat levels, and that this situation is remedied by the product of the first two or three blood meals. But there's the rub – because, in order to get the first blood meal, the fly must find a host and safely feed on it. And this must be done using a poorly developed flight musculature and before the limited fat reserves are exhausted. As a consequence teneral flies are at increased risk, both of starvation and, for the related reason, that they are known to attempt to feed off high-risk hosts

– such as humans – which older flies actively avoid [23]. It is thus not surprising that the mortality among these flies is higher than for all older flies (Figure 6).

These realities also explain the higher proportions of category zero flies in VET than in trap samples (Figure 4). With their weak flight muscles the young flies cannot afford to take the risk of making energetically expensive, spontaneous flights which might not result in the location of an odour trail from a potential host. They will be more likely to conserve energy, only flying if they see, or smell, a host as it passes by.

This energy crisis for young flies is exacerbated at extremes of temperature because flies then use increased amounts of fat during pupal development. At low temperatures fat is used at low rates – but the pupal duration increases rapidly as the temperature declines: at high temperatures pupal durations are reduced, but the rate of fat utilisation increases rapidly. The net result of these interactions is that flies experiencing temperatures in the region of 27°C during pupal development emerge with the largest amounts of fat (Figure 7). This is approximately the temperature observed in March at Rekomitjie. In October, when the temperatures are close to 30°C the fat levels are much reduced in newly emerged flies. Moreover, flies at this time of the year are also at their smallest and it has been shown that small flies have particularly low fat levels and that there is strong selection against them at the hottest times of the year. Thus, small male *G. m. morsitans* in Zimbabwe, and small female *G. pallidipes* in Kenya, suffered losses of up to 75% and 45%, respectively, at the hottest times of the year [20, 4].

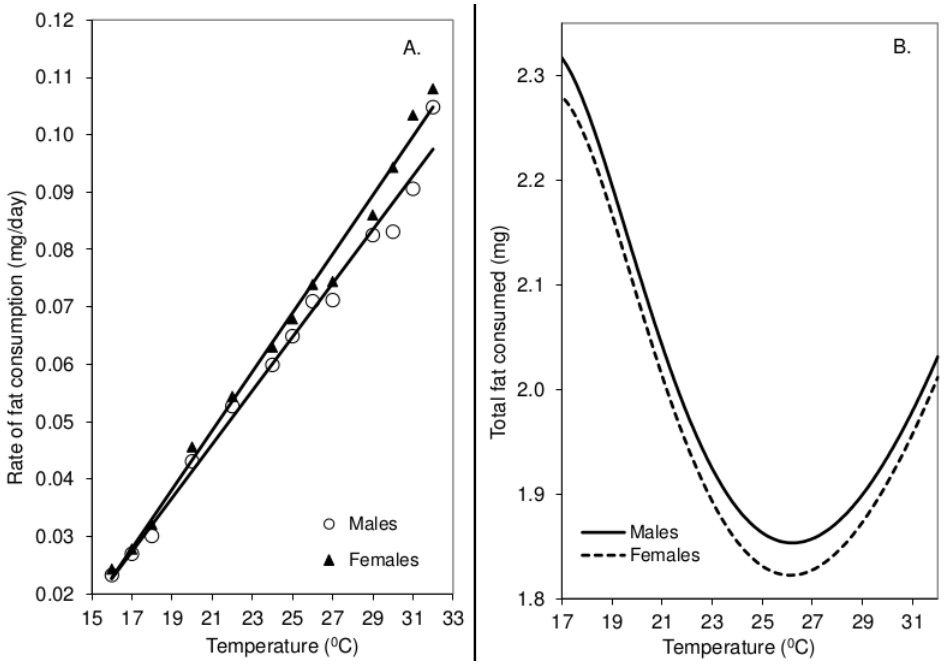

FIGURE 7. Effects of temperature on fat consumption rates (left) and total fat consumed during puparial development in laboratory male and female *G. m. morsitans* [18, 19]

It is thus entirely to be expected that there will be severely elevated mortality in very young flies at the hottest times of the year. This will have the effect of destabilising the age distribution and a 'wave' of reduced frequency in particular ovarian categories will pass through the population in following months (Figure 8). Thus the decline in the proportions of category zero flies that starts in August-September is followed by a decline, between October and December, in the proportions of flies in categories 1-3.

Conversely, when temperatures decline with the onset of the rains in December, the proportion of young flies starts to increase, and keeps increasing until the end of the rains in April. Now a wave of increasing frequency of category zero flies passes through the population. It is reasonable to think, therefore, that tsetse populations at Rekomitjie seldom, if ever, have a stable age distribution [25]. It is this violation of the assumptions which probably has the most serious implications and means that the naïve estimation of mortality from age distributions is liable to produce seriously misleading results.

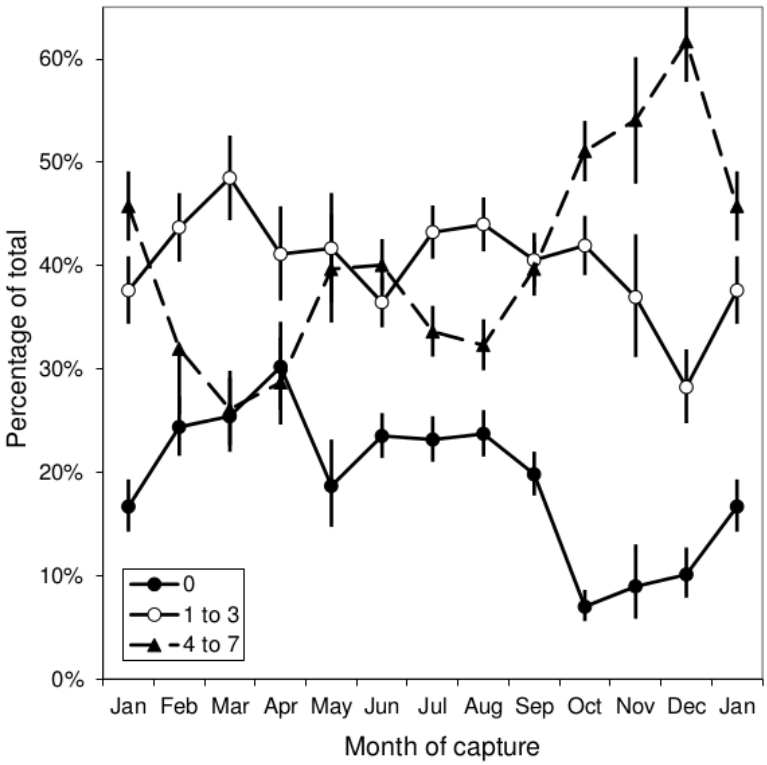

FIGURE 8. Age distributions of female *G. pallidipes* as a function of the month of capture. Ovarian categories pooled as 0, 1 - 3 and 4 - 7. Flies captured using the VET at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, September 1988 – December 1993.

Whereas violation of the assumption of the existence of stable age distributions has been identified as the principle reason for the apparent errors in the estimates of mortality, it should be mentioned that the estimates presented above make the

even stronger assumption that the population is stationary, *i.e.* that the population growth rate (λ) is zero. If an independently estimated value of λ is available this can be used to adjust the mortality estimate appropriately [25]. Such adjustments did not, however, remove the discrepancy between mortality as estimated by mark-recapture and from ovarian age distributions.

Jarry and co-workers went a step further in developing a matrix model which was used to estimate both population growth rates and age-dependent adult mortality rates from ovarian age distributions [13, 14]. When applied to field data, however, their results suggested that, once tsetse had survived category zero, the mortality rate decreased with age. This is quite the opposite of what should be expected on general grounds, and contrary both to laboratory evidence [3, 16, 17] and to the direct mark-recapture estimates from an island population of tsetse (Figure 6). Again, the problem seems to be that the approach required the existence of a stable age distribution – although it also required independent knowledge of the mortality rates in the pupal stages, and these estimates were of questionable accuracy.

3. **Conclusion.** The Rekomitjie studies, and those of Jarry and co-workers, provide good examples of apparently sound pieces of mathematics which, when applied to real data, provide obviously erroneous results. The studies did, however, have the saving grace that they *were* applied to data – so that the problems could be identified. Far more serious objections arise when, as happens too frequently, mathematical models make no attempt to address the real world in such a way that they can be tested. One must, of course, acknowledge the imperfection of all models: but, unless the model both accounts for the known biology of the problem under investigation, and is also challenged with data, the existence and nature of any imperfections will likely not be detected.

All of this leaves unanswered, however, quite how we should address the problem of estimating the mortality of adult female tsetse from ovarian dissection data. Current efforts at SACEMA are aimed at developing more complete models that use information on changes in population levels with season and attempt to estimate the levels of losses in young flies. The new models make no assumptions about the way in which age and mortality rates are related and, most importantly, make no assumption about the stability of the age distribution.

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