An Improved Temporal Formulation of Pupal Transpiration in *Glossina*

S. J. Childs

Department of Mathematics and Applied Mathematics, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa.
tel: +27 51 4013386, email: simonjohnchilds@gmail.com

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Abstract

The temporal aspect of a model of pupal dehydration is improved upon. The observed dependence of transpiration on time is attributed to an alternation between two essential rates, for which the deposition of a thin, pupal skin inside the puparium and its subsequent demise are thought to be responsible. As the temperature varies with time, so the developmental stages, to which the model data pertain, also warp. A one-to-one mapping between the constant-temperature time domain of the data and that of some more general case at hand is obtained by exploiting the puparial-duration formula of Phelps and Burrows (1969) and Hargrove (2004). In this way integration on the constant-temperature time domain is facilitated. The results of the Bursell (1958) investigation into pupal dehydration are otherwise used as a rudimentary data set. These data are generalised to all temperatures and humidities by invoking the property of multiplicative separability, as well as by converting relationships established in terms of steady humidities at one, constant temperature, to alternatives in terms of a calculated, total water loss and vice versa. The resulting, *Glossina morsitans* model is extrapolated to other species using their relative surface areas, their relative protected and unprotected transpiration rates and their different fourth instar excretions (drawing, to a lesser extent, from the data of Buxton and Lewis, 1934). In this way the problem of pupal dehydration is formulated as a series of integrals and the consequent survival can be predicted. The hypothetical effect of a two-day heat wave is investigated and the apparent vulnerability of the sensu strictu pupal stage, in xerophic and mesophilic species, is counter-intuitive. The effect on hygrophilic species appears to be qualitatively different.

Keywords: pupal water loss; transpiration; dehydration; pupal mortality; tsetse; *Glossina*. 
1 Introduction

Early mortality is considered to be the most significant, by far, in any model of tsetse population dynamics (Hargrove, 1990 and 2004) and a cursory inspection of the literature suggests pupal dehydration to be the most challenging aspect of modelling it. While both pupal and teneral mortality are crucial in deciding the viability of any tsetse population, the vastly different dynamics of pupal and teneral transpiration afford each the status of a topic in its own right. Just exactly how significant pupal dehydration is, only becomes clear with the conclusion of a model for teneral dehydration (Childs, 2014). The consequences of pupal dehydration are in no way limited to pupal mortality and the prospects of eclosion alone. Transpiration continues after eclosion up until the moment the teneral fly has its first meal. The ultimate effect of cumulative water loss on a given cohort is therefore likely to be best assessed in terms of the proportion of original larvae which have sufficient reserves to achieve their first feed as teners (the hypothesis of Childs, 2014). Combined dehydration and fat loss are thought to culminate in significant early mortality and while rates of teneral dehydration are generally several times higher than those characterising the pupal stage (Bursell, 1958 and 1959), the pupal rates prevail many times longer. Water loss during the pupal phase can therefore decide the fate of the teneral. Pupal stage mortality is of still greater relevance in the context of control measures, since the pupal stage is neither susceptible to targets, nor aerial spraying.

This research is particularly concerned with modelling the temporal dependence of pupal transpiration rates and, even more especially, the variation in the metabolic time-table to which those rates pertain. The variation of the metabolic rate is ultimately temperature dependent, consequently, so are the temporal domains to which each mode of transpiration applies. The formula of Phelps and Burrows, 1969 (modified by Hargrove, 2004), assumes a significant role in resolving this dependence in the absence of any more detailed information. It facilitates the formulation of both a mapping and its derivative. The model is otherwise based on the investigations of Bursell (1958) and, to a lesser extent, the data in another authoritative work, Buxton and Lewis (1934). One of the problems with the Bursell (1958) investigation is that many of the data are not of much use in the format in which they were presented. Many of the data are for steady humidities at 24.7 °C, a problem that can often be overcome by re-interpreting the data to be a function of calculated, accumulated water loss, or vice versa.

The transpiration-rate data for G. morsitans may be considered to fall into four, essential categories: Temperature-dependent data, humidity-dependent data, history-dependent data and time-dependent data. One observes a certain amount of corroboration between points on the respective curves. Some of this corroboration is demanded, for example, where the data sets intersect, however, other of it comes as a pleasant surprise. The time-dependent data is a case in point. Time-dependent transpiration would appear to be nothing more than an alternation between two basic rates. These two essential modes of transpiration are thought to arise as a result of the protection afforded by the deposition of a thin, relatively impermeable, pupal skin inside the puparium and its subsequent slow, then finally-catastrophic demise. It should, nonetheless, be emphasized that the work is neither concerned, nor reliant on any particular biological explanation for the different rates. Two different rates are simply observed to exist.
It should otherwise be pointed out that the Bursell (1958) data are robust in the sense that they encapsulate a myriad of effects. Apart from the more obvious, such as vapour pressure, the data incorporate the effects of spiracular control, the metabolic oxidation of fat to water and any other responses of the organism to its environment, insofar as that environment may be quantified in terms of humidity and temperature. The data are an empirical record and the nett result of a compendium of effects, including such vagaries as the exchange of heat and fluids with the environment. The data even resolve phenomena such as an excretion and an historical conditioning of the puparium. They therefore circumvent the need for any intricate, mechanistic model, involving fluxes into and out of the organism etc. all of which would be necessary were individual metabolic processes and reactions to be considered in isolation; a strategy otherwise known as microsimulation.

The main challenges to exploiting the data for the purposes of a model are in six, very specific respects: The humidity and temperature dependence of transpiration, the historical conditioning of sensu strictu pupal transpiration, the temporal dependence of transpiration, the variation of the metabolic time-table with temperature, extrapolating the *G. morsitans*-based model to the rest of the *Glossina* genus and linking total water loss to observed pupal emergence. In the latter instance, the challenge could be described more specifically as utilizing the dependence of survival on humidity at 24 °C, when only the total water loss is known. A similar problem pertains to the historical conditioning undergone prior to and at the beginning of the sensu strictu pupal stage. The final formulation, hence the solution to the problem, is predicated on six important assumptions. Two others are taken for granted, in addition to those explicitly stated and explored. The first is that the Bursell (1958) investigation is comprehensive, to the extent that it encapsulates all salient aspects of pupal water loss. The second is that there is no transpiration at dewpoint. The problem is then reduced to a series of integrals which can be performed on the original, constant-temperature, time-domain of Bursell (1958). Although these integrals are extremely simple, they are both numerous and voluminous and there are issues pertaining to differentiability and continuity. Since a high degree of accuracy from the data, itself, is not expected and the model is not intractably large, expedience takes precedence over taste and the midpoint rule is the preferred integration technique. A least squares fit, Newton’s method and an half interval search are the only other numerical techniques employed.

The aims and broader applications of this work are, in order of priority, the completion of the most challenging compartment of an early mortality model, pupal habitat assessment and a better comprehension of the tsetse pupa’s biology, particularly the Bursell (1958) endeavour. The main causes of early mortality could be summed up as dehydration, fat loss, predation and parasitism. Most of the experimental work needed for a model of early stage mortality has long been complete, notwithstanding that Bursell (1959) would appear to have a small amount of data outstanding insofar as teneral water loss’ dependence on temperature is concerned and Bursell (1960) and Phelps (1973) lack a few data points pertaining to teneral fat consumption’s dependence on activity. Rogers and Randolph (1990) present a limited amount of data linking predation and parasitism to the density at pupal sites and corroborated by Du Toit (1954).

So far as habitat assessment is concerned, this author is by no means the first to postulate the existence of localised and highly confined sites in which some species larviposit. Du Toit (1954) concluded that such sites existed and attributed the successful extirpation of *G. pal-
lidipes from KwaZulu-Natal to focusing their efforts on the Mkhuze region. Although he did
not specifically attribute the existence of the pupal sites to soil humidity, there can be no doubt
as to the motive for the Bursell (1958 and 1959) investigations and Glasgow (1963) identified
river terraces as being of key importance in the control of tsetse. Rogers and Robinson (2004)
found that cold cloud duration was far and away the most frequently occurring variable in the
top five for determining the distribution of both the fusca and palpalis groups, using satellite
imagery. Normalized difference vegetation index (NDVI) ranked second by a significant mar-
gin in those two groups and only just beat cold cloud duration for the morsitans group. It is
not too great a stretch of the imagination to entertain the possibility that cold cloud duration
and NDVI translate directly into soil humidity, as might elevation in the context of river basins,
vleis and low-lying, coastal areas, through which rivers typically meander before terminating
in estuaries. They also found that rainfall was even more relevant when it came to abundance,
as opposed to distribution.

Habitat assessment and tsetse biology are, of course, intricately entwined. That the third and
fourth instar larva should be more vulnerable to dehydration is already apparent from the
Bursell (1958) data. Appearances can, however, be deceptive and just how vulnerable the
organism is, is something which should not be considered in terms of individual stages in iso-
lation. Certainly there are surprises in store so far as the mesophilic and xerophilic species are
concerned. Making a distinction between the hygrophilic, mesophilic and xerophilic species
on the basis of their response to a hypothetical heat wave proves to be an interesting exercise.
The implications of this research for habitat assessment only become properly apparent in the
context of a sequel, the teneral model of Childs (2014). It could partly explain why South
Africa’s sympatric, G. austeni-G. brevipalpis population persists to this very day.

2 The Dependence of Transpiration on Temperature, Hu-
midity and Historical Water Loss

The same transpiration dependences on temperature, humidity and historical water loss are
used as were derived in Childs (2009) and they are explicitly stated in the next section. What
follows is a brief summary of the premises on which they are based.

Bursell (1958) obtained one set of water loss data for variable temperature, in dry air, and
another for variable humidity, at $24.7 \pm 3$ °C. Yet a third set of data points can be inferred by
reason. One expects no transpiration at dewpoint, regardless of the temperature. The existance
of seperate, temperature-dependent and humidity-dependent data sets lends itself favourably to
an assumption of multiplicative seperability.

**Assumption 1** The transpiration rate is a multiplicatively, separable function of humidity
and temperature.

How reasonable is this assumption? Certainly it is consistent with, and replicates, the third,
inferred set of data points entertained above. For the ‘H’ of data which exists across the
humidity-temperature domain, one reasonably expects rates to be bounded by the inferred, wet-end and known, dry-end data, furthermore, to be close to monotonic. The multiplicatively separable result is consistent with the simplest, such surface. The perceived wisdom is, furthermore, that the domain of interest lies mainly between 16 °C and 32 °C, (although atmospheric temperatures hotter than that certainly do occur in *G. morsitans* country). This means that the detailed, 24.7 °C data are only being extrapolated over ± 8 °C, based on the known, dry-end data and consistent with the inferred, wet-end data. One would also expect any unusual, capricious behaviour, or even failure in the waterproofing, to manifest itself in dry air. The dry-air, temperature-dependent data set is, fortunately, reasonably complete and suggestive of behaviour which is simple, smooth and monotonic (pure exponential, in this case). It should also be pointed out that, even in the event that circumstances were more favourable and data in the ideal format of a grid were being interpolated, one would still ultimately be ignorant of the behaviour between grid points, with the possibility of an unpredictable value for some, unique combination of humidity and temperature. Thus, in the very likely event that water loss rates are not perfectly multiplicatively separable, multiplicative separability should not be a bad substitute.

Sensu strictu pupal-stage transpiration is not as straightforward as that pertaining to the preceding stages. Transpiration rates are determined by the temperature and humidity which prevailed during the third and fourth instars as well as at the beginning of the sensu strictu pupal stage itself. A second assumption followed by minor manipulation is required in order to resolve the apparent dependence on historical water loss.

**Assumption 2** *The transpiration rate, conditioned by a given historical water loss, is the same as the transpiration rate conditioned by an historically steady humidity, at 24.7 °C, which produced an equivalent total water loss.*

The relationships so derived in Childs (2009) are thought not to be readily improved upon, given the existing data.

## 3 The Dependence of Transpiration on Time

Time-dependent transpiration would appear to be little more than an alternation between an unprotected rate and a more protected rate associated with the sensu strictu pupal-stage. This observation is not only supported by the Bursell (1958) temporal data, it is also corroborated by the different temperature-dependent and humidity-dependent relationships which prevail prior to, then during the sensu strictu pupal stage. In both the aforementioned data sets, a clear distinction exists between data which pertain to the stages prior to the commencement of the sensu strictu pupal stage and those which pertain subsequent to its commencement.

What is less trivial is the manner in which the Bursell (1958) time-domain warps with fluctuations in temperature. The assumption that parts of the puparial duration vary with temperature, in the same way as the whole, is inevitable in the absence of any more detailed information on
the individual stages of the pupa’s development. The constituent third and fourth instars, the sensu strictu pupal stage and the pharate adult stage are all assumed to warp in unison with the entire puparial duration, predicted by the formula of Phelps and Burrows (1969) and Hargrove (2004). A uniform dependence on an overall metabolism for all parts of the puparial duration might be considered adequate justification for such an assumption.

Before the model can be extended to this, more general time frame, however, the temporal dependence on the original, constant-temperature time domain of Bursell (1958) first needs to be established.

3.1 Transpiration on Bursell’s Time Domain

Data exist for the temporal dependence of the water-loss-rate between parturition and eclosion, at 0% r.h. and 24.7 ± 3 °C (Fig. 1 of Bursell, 1958). Two, essential modes of transpiration are discernable. The rates differ by an order of magnitude in dry air at room temperature. It is the interpretation of this work that the other, intermediate rates arise largely due to transitions between these two, basic modes of transpiration. They are thought to be a consequence of protection, afforded by a thin, pupal skin and its subsequent slow, then finally-catastrophic demise. It should nonetheless be emphasized that the work is neither concerned nor dependent on any particular biological mechanism. Two different rates, with transitions between them, are simply observed to exist.

The elevated rate, that which occurs in the absence of any waterproofing, is

\[
\frac{dk}{dt}_{\text{unprotected}} (h, T) = 24 \frac{100 - h}{100} \left( e^{0.110268T - 9.92201} + 0.000354783 \right)
\]

initial-pupal-masses per day in which \( h \) denotes humidity and \( T \), the temperature (Childs, 2009). This rate prevails from parturition and persists for the duration of the third and fourth instars, only to reappear again, shortly before eclosion. The vagaries of the time-dependence during the third and fourth instars were ignored, for want of better data, and only the subsequent stages were modelled based on the Bursell (1958) Fig. 1 data. Transpiration plummets at the end of the fourth instar as a result of the new-found protection afforded by the deposition of the thin, pupal skin inside the puparium. In the subsequent, brief period before the historical conditioning is established, an interim, protected-stage rate,

\[
\frac{dk}{dt}_{\text{protected}} (h, T) = 24 \frac{100 - h}{100} e^{0.161691T - 12.9591}
\]

is adopted (Childs, 2009). This temporary, protected-stage rate assumes the same humidity-dependence as the third and fourth instars. The rate which ultimately serves as the basis to the sensu strictu pupal stage was determined to be

\[
\frac{dk}{dt}_{\text{protected}} (w, h, T) = 24 \left( c_1 + c_2 h + c_3 w + c_4 wh + c_5 h^2 + c_6 w^2 \right) e^{0.161691(T - 24.7)}
\]

initial-pupal-masses per day, in which \( w \) is the total historical water loss and the \( c_i \) are the constants of the fit (Childs, 2009). This is followed by a long, slow and slight rise in transpiration
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rates, from the initial minimum attained at the start of the sensu strictu pupal phase. One possible cause is that the puparial exuviae lose some of their competence as they age, developing minute cracks etc. as the pupa develops inside. The transpiration rate finally makes a spectacular return to its initial levels, an event which coincides with the cracking of the pupal case, shortly before eclosion.

A weighted average was used to model the mix of the two rates. The mix of the two rates for the days following the fourth instar was determined to be as depicted in Fig. 1, by taking the unprotected rate, as a fraction of initial pupal mass in dry air at room temperature, to be $0.001102571 \ h^{-1}$ and the pupal rate to initially be $0.000135555 \ h^{-1}$.

![Figure 1: The degree to which the organism is unprotected by the thin pupal skin inside the puparium is the proportion Eq. (1) contributes to the transpiration rate.](image)

The functions in Fig. 1 were consecutively used to model the relative mix of the two rates with the following results, in which $\bar{t}$ denotes time in the constant temperature time frame.

**The Period $\bar{t} = (4, 8)$**

During this stage there is an adjustment from the unprotected rate down to the protected rate dictated by exponential decay, so that the transpiration rate was deemed to be

$$
\frac{dk}{dt} = \left[ 1 - \left( 14.0727 e^{-0.630097 \bar{t}} - 0.0952632 \right) \right] \frac{dk}{dt} \text{ protected } + \left( 14.0727 e^{-0.630097 \bar{t}} - 0.0952632 \right) \frac{dk}{dt} \text{ unprotected } .
$$

(2)
The Period $\bar{t} = (8, 21)$

Transpiration during this phase is predominantly at the pupal-stage rate. That is,
\[
\frac{dk}{dt} = [1 - (0.00262629 \bar{t} - 0.0236366)] \frac{dk}{dt} \text{protected} \\
+ (0.00262629 \bar{t} - 0.0236366) \frac{dk}{dt} \text{unprotected}.
\] (3)

The Period $\bar{t} = (21, 29)$

Transpiration then begins its return to the unprotected rate during the pharate adult phase. That is,
\[
\frac{dk}{dt} = \left[1 - \left(6.08165 \times 10^{-7} e^{0.454697\bar{t}} + 0.0443087\right)\right] \frac{dk}{dt} \text{protected} \\
+ \left(6.08165 \times 10^{-7} e^{0.454697\bar{t}} + 0.0443087\right) \frac{dk}{dt} \text{unprotected}.
\] (4)

All these rate formulae, together with Eq. 1, constitute a sequence of first order, ordinary differential equations which one expects to be Lipshitz continuous over each of the stages identified (likely, even a contraction over the greater portion of the time domain).

3.2 Mapping Bursell’s Time Domain into Real Time

The circumstances one is confronted with in reality are usually not those for which the temperature is constant, let alone a constant 24.7°C. What if the temperature varies? The problem is that as the temperature varies, so the metabolic process of the organism is either accelerated, or deaccelerated. As the temperature varies, so the duration of the various developmental stages, established on the constant-temperature time interval, warp; stretching at low temperature and shrinking at high temperature.

What the Bursell (1958) temporal data have so far been used to establish is the metabolic timetable for transpiration as a function of the constant-temperature time-frame or, more succinctly, a function $\frac{dk}{dt}(\bar{t}, w, h, T)$ dependent on $\bar{t} \in [0, 30]$. This time-frame is rooted in a particular metabolic rate, namely that associated with a constant temperature of 24.7°C, whereas what is of interest is the total water loss associated with more general temperature conditions. If time-dependent transpiration rates really can be simplistically regarded as being dependent on the stage of pupal development and the historical conditioning of the puparium alone (ignoring the ultimate, underlying dependence of humidity and temperature on time for the present), then the solution to the problem lies in a mere change in the variable of integration; should this be possible. That is,
\[
\int_{0}^{\tau_0} \frac{dk}{dt}(t, w, h, T)\ dt = \int_{0}^{30} \frac{dk}{dt}(t(\bar{t}), w, h, T) \frac{dt}{d\bar{t}}\ d\bar{t},
\]
in which \( \tau_0 \) is the puparial duration for the temperature history in question. Only the lack of a knowledge of one function prevents the integral on the right from being evaluated. Only the lack of knowledge of \( t(\bar{t}) \) prevents the performance of the integration in the \( \bar{t} \) time-frame. Since its derivative is obviously required and since one’s only knowledge of temperature and humidity data can be expected to be in terms of the actual time, \( t \), a one-to-one, invertible mapping from the Bursell (1958), time domain, into the pertinent time-frame of some, more general case at hand, must be formulated. By knowing only the function \( t(\bar{t}) \), or its derivative, \( \frac{dt}{d\bar{t}} \), either one can be deduced from the other and the correct \( T(t(\bar{t})) \) and \( h(t(\bar{t})) \) data be retrieved.

The formula for the puparial duration is the only, potentially exploitable information in this regard and could be the key to what is sought. The puparial duration in days, \( \tau \), has been found to vary according to the formula

\[
\tau = \frac{1 + e^{a+bT}}{\kappa},
\]

in which \( T \) is temperature (Phelps and Burrows, 1969). For females, \( \kappa = 0.057 \pm 0.001 \), \( a = 5.5 \pm 0.2 \) and \( b = -0.25 \pm 0.01 \) (Hargrove, 2004). For males, \( \kappa = 0.053 \pm 0.001 \), \( a = 5.3 \pm 0.2 \) and \( b = -0.24 \pm 0.01 \) (Hargrove, 2004). The puparial durations of all species, with the exception of \( G. \) brevipalpis, are thought to lie within 10% of the value predicted by this formula (Parker, 2008). \( G. \) brevipalpis takes a little longer. To expect parts of the pupa’s development to be effected by temperature in the same way as the whole, that there are no ‘bottle-necks’ in the pupa’s development, seems a reasonable assumption in the absence of any information to the contrary.

**Assumption 3** *The duration of any fraction of the puparial duration varies with temperature as the whole.*

That the duration of all the stages in the pupa’s development are determined by the same set of endothermic reactions, known collectively as the metabolism, could be considered justification for such an assumption. At constant temperature, a claim that the increments are related according to the formula

\[
\frac{d\bar{t}}{dt} = \frac{30}{\tau(T)} dt
\]

would then certainly be correct. What about real-life scenarios in which the temperature varies? The relationship between the increments is readily extended to variable-temperature scenarios to yield

\[
\frac{dt}{d\bar{t}} = \frac{\tau(T(t))}{30}.
\]

Once \( \frac{dt}{d\bar{t}} \) has been formulated, the actual time, \( t \), corresponding to a point, \( \bar{t} \), within the interval of integration can be recovered from

\[
\bar{t}(t) = \int_0^t \frac{d\bar{t}}{dt}(t')dt' = \int_0^t \frac{1}{\frac{dt}{d\bar{t}}(t')}dt',
\]

(7)
in which \( t' \) is a dummy variable of integration. It is in this way that a mapping between the respective time domains is formulated to facilitate integration on the constant-temperature time domain. It is in this way that pertinent temperature and humidity input-data can be retrieved to facilitate integration on the constant-temperature time domain. Should there be no interpolation between discrete, daily temperature data, the integral in Eq. 7 naturally becomes a summation (usually terminating with a fraction of a day’s contribution). An exposition of this is provided in Section 5.2.

4 Adapting the *G. morsitans* Model to Other Species

Transpiration-related rates for a number of species were measured at both the unprotected and protected stages, by Buxton and Lewis (1934) and Bursell (1958). The rates are quoted in units of mg h\(^{-1}\)cm\(^{-2}\)(mm Hg)\(^{-1}\). Surface-area data for pupae in the wild are likewise available and all are tabulated in Table 6 of Bursell (1958).

In terms of fluxes, if \( \rho \) denotes the density of a fluid, \( \mathbf{v} \) denotes the nett velocity of that fluid through the surface of the organism, \( \mathbf{n} \) denotes the normal to that surface, \( P \) is a pressure and \( ds \) is an element of area, then the quantities supplied in Table 6 of Bursell (1958) are

\[
p = \frac{\rho \oint \mathbf{v} \cdot \mathbf{n} ds}{P \oint ds} \quad \text{and} \quad s_{\text{species}} = \oint ds,
\]

whereas

\[
\frac{dk}{dt} = \rho \oint \mathbf{v} \cdot \mathbf{n} ds = P \times s_{\text{species}} \times P,
\]

is the transpiration rate sought. One can alternatively regard transpiration through the integuments of each species to be governed simplistically by something like Darcy’s law, since permeability is pressure dependent. The transpiration rate per fly is again the relevant rate multiplied by the surface area and a pressure.

Using either argument, a species conversion factor for unprotected-stage rates can be defined as

\[
\delta_{\text{unprotected}} \equiv \frac{P_{\text{unprotected}}}{P_{\text{morsitans unprotected}}} \times \frac{s_{\text{species}}}{s_{\text{morsitans}}},
\]

in which \( P_{\text{morsitans unprotected}} \) and \( P_{\text{unprotected}} \) are the unprotected-stage rates for *G. morsitans* and the species in question, respectively, and \( s_{\text{morsitans}} \) and \( s_{\text{species}} \) are the surface areas of *G. morsitans* and the species in question, respectively.
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| Group  | Species         | $\delta_{\text{unprotected}}$ | $\delta_{\text{protected (for minima)}}$ | $\delta_{\text{protected (for maxima)}}$ |
|--------|-----------------|-------------------------------|------------------------------------------|------------------------------------------|
| morsitans | austeni         | 1.60                          | 0.712                                    | 0.722                                    |
| morsitans |                  | 1                             | 1                                        | 1                                        |
| pallidipes |                | 1.50                          | 1.24                                     | 1.31                                     |
| submorsitans |             | 1.59                          | 0.949                                    | -                                        |
| swynnertoni |            | 0.830                         | 0.869                                    | 0.892                                    |
| palpalis | palpalis        | 2.54                          | 1.41                                     | 1.36                                     |
| tachinoides |             | 0.818                         | 0.743                                    | -                                        |
| fusca    | brevipalpis     | 10.3                          | 4.57                                     | 3.06                                     |
| fuscipleuris |           | 8.84                          | 4.45                                     | 3.16                                     |
| longipennis |            | 3.62                          | 2.45                                     | 2.30                                     |

Table 1: Species conversion factors for the model calculated from data, ultimately sourced from Buxton and Lewis (1934), presented in Bursell (1958).

A dimensionless, species conversion factor for sensu strictu pupal-stage rates can be defined, similarly, as

$$\delta_{\text{protected}} = \frac{P_{\text{protected}}}{P_{\text{morsitans protected}}} \times \frac{S_{\text{species}}}{S_{\text{morsitans}}}$$

in which $P_{\text{morsitans protected}}$ and $P_{\text{protected}}$ are protected-stage transpiration rates for *G. morsitans* and the species in question, respectively. The same surface area is used for both the puparium and the third instar larva, the justification being that the puparial exuviae render the puparium marginally bigger while the larval surface is not as regular. Actual values of $\delta_{\text{unprotected}}$ and $\delta_{\text{protected}}$, for ten different species, are tabulated in Table 1.

Notice that the ratio of the unprotected to protected conversion factors in Table 1 is curiously close to two for hygrophilic species, whereas it is around unity for both the mesophilic and xerophilic categories. (It could suggest these categories have a double layer of some, or other, protection). Both the exceptions to this rule, *G. submorsitans* and *G. longipennis*, occur in Sudan as well as Ethiopia and the aforementioned ratio is around 1.5 in both. Notice, also, that conversion factors alternatively deduced from the transpirational maxima, then minima, during the sensu strictu pupal stage, are remarkably similar for a given species (for all except *G. brevipalpis* and *Glossina fuscipleuris*). This is very encouraging and immediately suggestive of a similar slope in the time-dependence of the transpiration rate across all species. The suggestion is that the ratios in Table 1 are fixed over time, *G. brevipalpis* and *G. fuscipleuris* being the possible exceptions (a lower, maxima-based value in the case of *G. brevipalpis* might be an error which can be attributed to the slightly longer puparial duration). The conversion factors alternatively deduced from the transpirational maxima, then minima suggest no behavioural differences. With this observation comes the dawning realization that the strategies,
the metabolic time-table, adopted by the different species could all be very similar and that these conversion factors might be all that is necessary to enable both the unprotected and protected transpiration rates for another species to be calculated from \textit{G. morsitans} values. Such speculation is further reinforced by the known, third-instar transpiration rates for both \textit{Glossina brevipalpis} and \textit{Glossina palpalis}, although very little data is available for other species. Conversion of third-instar, \textit{G. morsitans}-model, transpiration rates to \textit{G. brevipalpis} and \textit{G. palpalis} values, on this basis yields errors of 6\% and 10\% respectively (Childs, 2009). Such observations present a strong argument for asserting that the only difference between the species, so far as water loss is concerned, are permeability, surface area and a fourth-instar excretion.

\textbf{Assumption 4} \textit{The water management strategies of the majority of tsetse fly species differ only with respect to relative pupal surface area, relative unprotected and protected transpiration rates and the different amounts excreted during the fourth instar.}

On the face of it, Assumption 4 is certainly the most tenuous. How valid is it? Does such a simplistic approach work? No dependence with pronounced variation in temperature and humidity is indicated, since the pressures cancel when using the original data on which Table 1 is based. It is of considerable comfort that the conversion of the \textit{G. morsitans} model to other species involves relative rates. Assuming continuity, one should at least be able to have confidence in results that are within a few degrees of room temperature. The perceived wisdom is that the region of interest lies mainly between 16 \textdegree C and 32 \textdegree C.

\section{The Resulting Model for Pupal Water Loss}

Collecting together all prior derivation and assumptions gives rise to a series of governing equations. Their application to any, specific temperature and humidity data set is then facilitated by a mapping of the time domain and its derivative alone.

\subsection{The Governing Equations}

The total water loss of the organism is formulated as a series of five integrals, based on a decomposition of the temporal domain and an excretion. Note that water losses are in units of \textit{G. morsitans}, initial-pupal-masses (31 mg).

\textbf{The Period} \( \bar{t} = [0, 4] \)

The water loss rate for the greater part of the third and fourth instars is at the unprotected rate. Generalising Eq. 1 to all species and integrating leads to

\[
k |^4_0 = \int_0^4 24 \left( e^{0.110268T - 9.92201} + 0.000354783 \right) \frac{100 - h}{100} \frac{p_{\text{unprotected}}}{p_{\text{morsitans unprotected}}} \frac{s_{\text{species}}}{s_{\text{morsitans}}} dt d\bar{t}.
\]
**The Period** $\bar{t} = (4, 8)$

During this period there is an adjustment from the unprotected rate down to the protected rate dictated by the exponential decay in Eq. 2. Generalising to all species and integrating leads to the expression

$$
\int_{4}^{8} 24 \left[ 1 - \left( 14.0727 e^{-0.630097\bar{t}} - 0.0952632 \right) \right] e^{0.161691(T-12.9591) / p_{morsitans\ protected}} \\
+ \left( 14.0727 e^{-0.630097\bar{t}} - 0.0952632 \right) \left( e^{0.110268T-9.92201 + 0.000354783} \right)
$$

the overall dependence on humidity being the one which exists prior to the dependence on historical water loss.

**Excretion**

If water loss is sufficiently low during the first $\bar{t} = 8$ days of the puparial duration, cognizance must be taken of excretion. In such scenarios the formula

$$
k |_{4}^{8} = 0.0750 + \frac{h_{3rd\ instar}}{100} (0.0885 - 0.0750)
$$

was implemented for members of the *morsitans* group. Values of 0.0585 and 0.06 of the pupal mass were used for *G. brevipalpis* and *G. palpalis*, respectively, for want of any greater wisdom. The only fourth instar excretion data are those for *G. morsitans*, *G. palpalis* and *G. brevipalpis*. The *G. morsitans* rate was proportionally adjusted for other members of the *morsitans* group.

**The Period** $\bar{t} = (8, 21)$

Transpiration during this phase is predominantly at the protected rate. There is also deemed to be a small component of loss at unprotected rates, which increases linearly over time, according to Eq. 3. Generalising to all species and integrating leads to

$$
k |_{8}^{21} = \int_{8}^{21} 24 \left[ 1 - (0.00262629 \bar{t} - 0.0236366) \right] e^{0.161691(T-24.7) / p_{morsitans\ protected}} \\
+ (0.00262629 \bar{t} - 0.0236366) \left( e^{0.110268T-9.92201 + 0.000354783} \right)
$$

The overall dependence on humidity being the one which exists prior to the dependence on historical water loss.
The Period $\bar{t} = (21, 29)$

Transpiration begins its return to unprotected rates during the pharate adult phase. Generalising the exponential growth in Eq. 4 to all species and integrating leads to

$$k \bigg|_{21}^{29} = \int_{21}^{29} 24 \left[ 1 - \left( 6.08165 \times 10^{-7} e^{0.454697\bar{t}} + 0.0443087 \right) \right] e^{0.161691(T-24.7)}$$

$$\left( c_1 + c_2 h + c_3 w + c_4 w h + c_5 h^2 + c_6 w^2 \right) \frac{p_{\text{protected}}}{p_{\text{morsitans protected}}}$$

$$+ \left( 6.08165 \times 10^{-7} e^{0.454697\bar{t}} + 0.0443087 \right) \left( e^{0.110268T-9.92201} + 0.000354783 \right)$$

$$\frac{100 - h}{100} \frac{p_{\text{unprotected}}}{p_{\text{morsitans unprotected}}} \right] \frac{s_{\text{species}}}{s_{\text{morsitans}}} \frac{dt}{d\bar{t}}.$$  

The Period $\bar{t} = (29, 30)$

There is a return to unprotected rates shortly before eclosion and Eq. 1 once again becomes the applicable integrand, that is

$$k \bigg|_{29}^{30} = \int_{29}^{30} 24 \left( e^{0.110268T-9.92201} + 0.000354783 \right) \frac{100 - h}{100} \frac{p_{\text{unprotected}}}{p_{\text{morsitans unprotected}}} \frac{s_{\text{species}}}{s_{\text{morsitans}}} \frac{dt}{d\bar{t}}.$$  

5.2 A Discrete Function of Time and its Derivative

If discrete values are used in Eq. 7, instead of interpolating between the $\tau$ predicted by daily temperature, the integral becomes a sum whose final term is usually some fraction of a day’s contribution,

$$\sum_{i=1}^{\text{floor}(t(\bar{t}))} \frac{1}{d\bar{t}(i)} + \frac{t - \text{floor}(t)}{d\bar{t} (\text{floor}(t) + 1)} = \bar{t}.$$  

The slightly-involved-in-appearance expression is suggestive of the method of solution. A progressively increasing number of days’ contributions are summed, until the contributing interval, itself, overtakes the sum. Newton’s method is then implemented to determine what fraction of the last day is necessary.

5.3 Numerical Integration

Issues of non-differentiabilty and discontinuity dictate that a low order integration rule be used. The preferred choice in Childs (2009) was Euler’s method. Euler’s method is, however, no longer appropriate as the time-dependence is now exponential (‘stiff’) over parts of the domain.
The midpoint rule is as distasteful from the point of view of its error. The local error per step, of length $\Delta t$, is $O(\Delta t^3)$. Since the required number of steps is proportional to $\frac{1}{\Delta t}$, the global error is $O(\Delta t^2)$. This is indeed primitive. The real strength of the midpoint rule and other low order methods lies in their robustness at discontinuities and points of non-differentiability. The maximum, additional error introduced at such points is of a similar order as the method’s global error. The same cannot be said for the higher order methods. Using the midpoint rule, one has one problem to solve, whereas using one of the higher order methods entails solving five, separate problems; each confined to its own respective domain of Lipschitz continuity etc. The handicap of a poor error is easily overcome computationally by using a small step length. When considering the original pupal material used, the known error in the data, that an engineering-type accuracy is anticipated from the model and a host of other factors, two significant figures are more than what are sought. Since the problem is not intractably large, expedience takes precedence over taste and the more pedestrian midpoint rule is considered the appropriate choice.

### 6 Survival to Eclosion

How does one translate cumulative water loss into survival? Buxton and Lewis (1934) and Bursell (1958) collected pupal emergence data for a variety of species, over a range of humidities at 24 °C (all reported in Bursell, 1958). Two challenges arise in using this pupal emergence data. The first is to establish a credible relationship between the proportion which eclode and the humidity of the substrate, while the second is how to relate this survival to total water loss when it is only known as a function of humidity at 24 °C. Of course, the former problem reduces to an exercise in curve-fitting, once a suitable function has been determined.

What is the relationship between pupal emergence and humidity in Fig. 12 of Bursell (1958)? Assuming the usual intra-specific variation, the simplest point of departure is that some pupae will be slightly bigger, have slightly bigger reserves and more competent integuments. Yet others will be slightly smaller, have slightly smaller reserves and less competent integuments. This justifies the following interpretation of the Bursell (1958) Fig. 12 data.

**Assumption 5** The relationship between pupal emergence and humidity is a Gaussian curve, or a part thereof.

The parameters in

$$E(h_{24}) = a \exp \left[ -\frac{(h_{24} - b)^2}{2c^2} \right],$$

for each species, are provided in Table 2. Convincing fits to the data are obtained in this way, regardless of whether or not the underlying logic is correct (Fig. 2 and Table 2).
Figure 2: Percentage emergence data modelled as a Gaussian curve (Childs, 2009) for a variety of species. All are at 24 °C, except *G. tachinoides* (30 °C). A straight line had to be fitted to the only two data points for the single exception, *G. longipennis*

A similar challenge to that encountered for historically-conditioned transpiration, compounds matters when it comes to the survival to eclosion for each species. Humidities are steady, furthermore, the data were obtained at a constant 24 °C.

**ASSUMPTION 6** *The pupal survival to eclosion, for a given water loss, is the same as that for the steady humidity, at 24 °C, that produced an equivalent total water loss.*

In other words, it is assumed that survival to eclosion can be re-expressed in terms of total water loss. Do different histories in temperature and humidity, which produce the same water loss, imply the same pupal emergence, or is the level of the organism’s water reserve at some particular stage more relevant to the pupa’s survival to full term? Such dependence is beyond the scope of this work, as well as that of Bursell (1958). In practice, it is far easier to convert a total computed water loss to a corresponding steady-humidity-at-24 °C, instead of the other way around. The problem is that the water loss algorithm is relatively involved and voluminous. The results of the water loss algorithm at 24 °C constitute a monotonic decline with steady humidity, barring excretion. These are ideal circumstances for the implementation of an half interval search. (The rate of convergence is not bad in this instance.)
Improved Temporal Formulation of Pupal Transpiration in *Glossina*

| group      | species       | parameter a | parameter b | parameter c |
|------------|--------------|-------------|-------------|-------------|
| *morsitans*| austeni      | 101.663     | 73.1591     | 30.6468     |
| *morsitans*|              | 94.4792     | 70.6391     | 77.3495     |
| *pallidipes*|             | 86.6257     | 71.5636     | 54.9713     |
| *submorsitans*|          | 94.5092     | 81.1895     | 75.4474     |
| *swynnertoni*|            | 94.0194     | 62.4064     | 75.2339     |
| *palpalis*  | palpalis     | 95.8732     | 78.8419     | 23.4835     |
| *tachinoides*|            | 98.8383     | 79.6877     | 40.8616     |
| *fusca*     | brevipalpis  | 94.0057     | 84.0199     | 13.6433     |

Table 2: Parameters for the fit of a Gaussian curve to the Bursell (1959) and Buxton and Lewis (1934) pupal emergence data for a variety of species (Childs, 2009). All are at 24 °C, except *G. tachinoides* (30 °C).

7 Results

Validating the model presents something of a challenge. The deficiency in data, synonymous with the need for a model, is extreme in this case. Almost all available data has been incorporated into the model. The veracity of the results is, to a certain extent, suggested by consistancy with the model itself. For example, transecting the Figs. 3–8 surfaces of emergence at 24 °C should replicate the Gaussian curves in Fig. 2 and they do. Predicted pupal mortalities due to water loss should also, logically, never exceed any pupal mortalities observed in the field for similar humidity and temperature conditions. It is also relevant to Figs. 4 and 6 that Onderstepoort Veterinary Institute (O.V.I.) keep their *G. austeni* and *G. brevipalpis* colonies at 75% r.h. (De Beer, 2013).

One set of data on which the model is not based is the measured initial water reserves for the various species (Table 3). In a world in which normal distributions are assumed in the absence of any other information, the measured, critical water loss contour should correspond to that of the median, or 50%, emergence contour. The sensitivity of *G. brevipalpis* and *G. palpalis* pupae to dehydration makes these species arguably the most challenging tests, as well as of particular interest to this work. *G. brevipalpis* is, furthermore, a topic of intense interest in South Africa at the moment and *G. palpalis* is known to be a major culprit in the spread of human trypanosomiasis. Information on the fourth instar excretions is available for both species, as well as *G. morsitans*. The critical water reserve is also known for three other species for which, it is hoped, *G. morsitans*-proportionate, fourth instar excretions will suffice.
Figure 3: *G. morsitans* pupal emergence (top left) and water loss (top right); *G. morsitans* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
Improved Temporal Formulation of Pupal Transpiration in *Glossina*

Figure 4: *G. austeni* pupal emergence (top left) and water loss (top right); *G. austeni* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
Figure 5: *G. palpalis* pupal emergence (top left) and water loss (top right); *G. palpalis* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
Improved Temporal Formulation of Pupal Transpiration in *Glossina*

Figure 6: *G. brevipalpis* pupal emergence (top left) and water loss (top right); *G. brevipalpis* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
Figure 7: *G. pallidipes* pupal emergence (top left) and water loss (top right); *G. pallidipes* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
Figure 8: *G. swynnertoni* pupal emergence (top left) and water loss (top right); *G. swynnertoni* pupal emergence for a 35 °C and 25% r.h. heat wave on the first two days after larviposition (bottom left) and on day fifteen and sixteen (bottom right).
The three species in question are *G. austeni*, *G. pallidipes* and *G. swynnertoni*.

|        |        |        |        |        |        |
|--------|--------|--------|--------|--------|--------|
| *G. austeni* | *G. brevipalpis* | *G. morsitans* | *G. pallidipes* | *G. palpalis* | *G. swynnertoni* |
| 5.3 mg | 18.7 mg | 8.8 mg | 10.5 mg | 7.7 mg | 8.5 mg |

Table 3: Initial water reserves after Bursell (1958).

It was also decided to simulate an heat wave, more especially, experiment with the timing thereof, for reasons expounded in the conclusion and which have their origins in Section 4. A hypothetical heat wave of 35 °C and 25% r.h., lasting two days, was based on the kind of atmospheric temperatures and humidities one might expect in *G. morsitans* habitat. The heat wave was modelled to alternatively coincide with the first two days after parturition and day fifteen and sixteen of the puparial duration, these times usually occurring within the unprotected and sensu strictu pupal stages, respectively. In what way atmospheric conditions relate to those within the substrate of larviposition is not really known, however, the mesophilic and xerophilic results may shed some light on the matter.

The same axes for the presentation of the results are used throughout, to facilitate easy comparison. The reason that results above 35 °C are blanked out in relevant plots, is that heat wave experiments above 35 °C are obviously meaningless insofar as the “heat wave” still being an heat wave is concerned. Relevant results below 15 °C are similarly blanked out, since day fifteen and sixteen no longer occur within the sensu strictu pupal stage once the temperature falls to a little above 15 °C.

8 Conclusions

This work gives rise to a series of integrals and an algorithm which predict pupal water loss and consequent survival, given the prevailing soil temperatures and humidities. High transpiration rates are generally a consequence of high temperatures and low humidities. They lead to a dehydration of the tsetse pupa which can be fatal. Although the diametrical opposite is true of transpiration rates at low temperatures, metabolic processes are slowed, the puparial duration becomes too long and the cumulative effect of transpiration can be just as fatal. The low transpiration rates, associated with low temperatures, are more than compensated for by the lengthening of the duration over which they prevail. High water losses, in the days immediately following larviposition, also trigger a more conservative mode of transpiration during the sensu strictu pupal stage.

The correspondence between actual, measured critical water losses and those predicted by the model is such that very little error is discernable (comparing the position of the critical water loss contour with the median, or 50%, emergence contour in Figs. 3–8). In fact, the results could be said to be remarkable in this regard. It would therefore appear that the behavioural
similarities between different species of pupae far exceed initial expectations. The agreement between measured and calculated critical water losses reinforces the assumption that the water-management strategies and the metabolic time-tables for development differ little from species to species. This is since the premise on which the *G. morsitans* model is extrapolated to other species, is that the only differences between species are their relative surface areas, the relative permeability of their unprotected-stage and protected-stage integuments and their different fourth instar excretions. One might therefore suspect that all tsetse species share a common strategy to actively minimise water loss for the majority of modern habitats and have hydrational mechanisms preventative of dehydration. Despite the good correspondence in measured and calculated critical water losses, some caution may still be necessary at temperatures remote from 24.7 °C. The relative permeability of the species’ membranes could change at such extremes.

The *G. morsitans*-based model seems to provide a reasonably reliable and concise definition of hygrophilic species, this by way of the factors which facilitate its extrapolation to other species (in Table 1). The ratio of the unprotected to protected conversion factors is curiously close to two for all hygrophilic species, whereas it is around unity for the mesophilic and xerophilic species (it could suggest the latter categories have a double layer of some or other protection during the instars). Both exceptions to this rule, *G. submorsitans* and *G. longipennis*, occur in Sudan as well as Ethiopia and the aforementioned ratio is remarkably similar, having a value of around 1.5. A third denizen of these climes, *G. tachinoides*, has an extraordinarily mesophilic pupal stage for a *palpalis* group fly and the 30 °C exception in the Fig. 2 data can be misleading. Of course, both *G. tachinoides* and *G. submorsitans* also have habitat in Chad (Ford and Katondo, 1977) and it is such habitats that elicit interest in a hot, dry spell.

There would therefore appear to be a certain merit in advancing the course of this enquiry by way of experimenting with a simulated, two-day heat wave, the intention being to further elucidate the classification of species as hygrophilic, mesophilic or xerophilic and thereby contribute novel biological insight. The xerophilic and mesophilic categories are found not to be as distinct from one another in such a context as the hygrophilic category is. The classification of tsetse into separate mesophilic and xerophilic entities could, possibly, even be considered artificial, in the sense of the one merely being a more extreme adaption. The responses in Figs. 3, 7 and 8 are all qualitatively similar. One clear distinction to emerge between them and the hygrophilic species is that the latter are far more vulnerable to dehydration during the early stages not protected by the thin, pupal skin inside the puparium. If the onset of the heat wave is timed to coincide with the days immediately following larviposition, instead of during the sensu strictu pupal phase, the effect on hygrophilic species is profoundly detrimental. In the case of *G. brevipalpis* it is nothing short of catastrophic (Fig. 6). In contrast, the suggestion for mesophilic and xerophilic species is that such an heat wave is only mildly detrimental and, even then, mostly effects the higher levels of survival. More than one mechanism is thought to be responsible for the mesophilic and xerophilic response. One is the historical conditioning of the transpiration rate (already referred to), a mechanism by which hot, dry weather galvanizes the puparium against later losses. Although sensu strictu pupal transpiration rates are an order of magnitude lower than the earlier transpiration rates which condition them, any difference in them prevails for a much longer duration and so the cumulative effect is potentially as, or more,
damaging. Higher transpiration rates associated with a hot and dry spell are also compensated for, to a certain extent, by a metabolic quickening of the relevant part of the third and fourth instars over which they prevail. For hygrophilic species, unprotected transpiration rates are, however, simply too high for either mechanism to make a difference. An hot, dry spell in the days immediately following larviposition is profoundly detrimental to hygrophilic species.

A lack of any early conditioning of the puparium gives rise to one further, surprising feature of mesophilic and xerophilic species: An hot, dry spell is more detrimental when it coincides with the sensu strictu pupal stage, than when it occurs immediately after larviposition (e.g. Fig. 3). This certainly is counter-intuitive when the transpiration rates in Bursell (1958) Fig. 1 are contemplated in isolation, as well as being in stark contrast to the hygrophilic responses (Figs. 4–6). Yet, however compelling it may be to propose an artefact, it would be difficult to refute the existence of the phenomenon in the case of the *G. morsitans* calculation for dry air at room temperature. Such adaption begs the question of whether an heat wave could be expected to manifest itself in a different way in the pupal environment, at different stages of pupal development. Just how the atmospheric conditions associated with an heat wave manifest themselves in soil, rot holes, compost etc. is unknown, however, one would certainly expect atmospheric conditions to prevail at the start of the third instar. Thereafter, one might wonder about a departure from atmospheric conditions, the pupal environment having become more insulated. This might explain the phenomenon if, indeed, it is an adaption. Of course, a more prosaic explanation might be that the mesophilic and xerophilic species have the capability to survive adversely hot and dry conditions. They respond by taking countermeasures against any further, unnecessary losses and by preparing themselves for the worst, something which may not then materialise. For the hygrophilic species, however, the opposite is true. For the hygrophilic species, things are as expected: An hot, dry spell which occurs immediately after larviposition is more detrimental than one coinciding with the sensu strictu pupal stage.

On these grounds one might argue an apparent qualitatively-different response for hygrophilic species. For the hygrophilic species it can certainly be said that third and fourth instar water losses are extremely high. Not only can they be as much as ten times those of *G. morsitans*, perhaps more important is the fact that the ratio of the unprotected to protected conversion factors (in Table 1) is double the same ratio for the mesophilic and xerophilic species. At what point these early losses render the long duration of the sensu strictu pupal stage irrelevant is not clear. Whether a milder heat wave would reproduce the mesophilic and xerophilic response in hygrophilic species, is not known. Whether a milder heat wave would induce the same preference for an early, rather than later, exposure to hot and dry conditions is a question not answered in this research. One wouldn’t expect atmospheric conditions anywhere near as dry as those used for the hypothetical heat wave in *G. austeni* habitat, although they certainly do occur in some *G. brevipalpis* country. Despite this, the soil humidity and soil temperature within the levees and river terraces of large drainage lines, which are so often the habitat of *G. brevipalpis*, could differ altogether from those which characterise the atmosphere. For that matter, it is not known how atmospheric conditions manifest themselves in the pupal environments of any of the other species, either. In many tsetse habitats the mean daily temperatures seldom change by more than two degrees at a time, and a change of four degrees from one day to the next may be considered extreme. The transition to and from the heat wave condition is therefore completely
unrealistic for large parts of the domain investigated. Experimenting in such a manner does, however, allow the conclusions to be generally stated and so makes for an interesting study nonetheless. One might otherwise have been tempted to conclude that there is a continuous progression from G. pallidipes to G. austeni, that G. austeni is simply a more extreme adaption. It is not, if the experiments with the hypothetical heat wave can be regarded as relevant.

An explanation for the ‘squiggle’ associated with low temperature in the lower, right plots of Figs. 3–8 might be in order. At very low temperatures the puparial duration becomes so long that an heat wave, generally timed to occur during the sensu strictu pupal phase, falls within the fourth instar, instead. As the temperature drops and the metabolism slows, the puparial duration lengthens. The steep transition zone between the unprotected and protected transpiration rates (evident in Fig. 1) begins to enter the fifteenth day, the day scheduled for the commencement of the intended, sensu strictu pupal-stage heat wave. The pupa then begins to incur the massive water losses that a lack of waterproofing implies. At some point this rapid transition in rates enters the heat wave just enough for the optimum water loss to be incurred. At some point the water loss is just sufficient to produce a maximum conditioning for a minimum tax on reserves, thereafter it becomes more damaging; hence, the ‘squiggle’ in the results which occurs between 15 °C and 20 °C. At a little above 15 °C, the coincidence of the fourth instar and the heat wave becomes complete. The heat wave no longer coincides with any part of the sensu strictu pupal stage, as intended, hence the omission of the results at temperatures any lower than 15°C.

The model formulated is obviously intended for more ambitious purposes than the mere interpretation and a visualization of data. It, nonetheless, proves to be an invaluable tool in the interpretation and visualization of the Bursell (1958) endeavour. A substantial body of literature is of the opinion that pupal mortality due to dehydration is either irrelevant, can be assumed constant, is linearly dependent on temperature, or dependent on temperature alone. Dehydration does tend to be more temperature-dependent in the mesophilic and xerophilic species (e.g. Fig. 3), although, even then, that dependence is certainly not linear. Notice that even in the morsitans group, daily pupal mortality is neither linear, nor a function of temperature alone. Even for a hardy fly such as G. morsitans, its prospects deteriorate rapidly once out of favourable habitat. When it comes to a species such as G. brevipalpis, however, there would be more merit in assuming dehydration-related survival to be entirely humidity-dependent (Fig. 6). When it comes to hygrophilic species, that pupal mortality due to dehydration is both relevant and palpable is beyond contention. The effect of soil humidity is profound. Soil humidity defines habitat. This is despite the fact that water loss and any consequent pupal mortality are also very different things (water loss may culminate in and ultimately take its toll on the teneral). If it could be said that there was a single, overriding fact that the Bursell (1958) investigation was able to reveal, it is that the water reserve is a limiting factor in tsetse pupae. Dehydration is a challenge to pupae, if not a major threat and it is generally accepted that most of the Glossina genus is not well adapted to arid environments (Glasgow, 1963). The Glossina genus may well derive from a common, tropical, rain-forest dwelling ancestor, adjusted to moist, warm climates (Glasgow, 1963).

Notice that this is not to claim that pupal mortality due to dehydration is always decidedly higher than any other causes of mortality, only that other causes of mortality are otherwise geographically more uniform, random, or cyclical. It should also be borne in mind that there
may be additional mortality, only indirectly attributable to dehydration. A dearth of humid substrates might lead to more localised, therefore higher, pupal concentrations and, consequently, to increased predation and parasitism. The results in Figs. 3–8 point to the fact that pupal sites for some species are very much confined in the dry season, particularly in the case of South Africa’s two extant species, *G. austeni* and *G. brevipalpis*. These would be obvious places in which to concentrate control measures and one immediate application of this work. Barriers of the type modelled in Childs (2010) might be far more efficacious if deployed in the immediate vicinity of pupal sites, rather than for the purposes of containment. While early stage mortality is considered to be the most significant, by far, in any model of tsetse population dynamics it is of even greater relevance when in the context of control measures. This is since the pupal stage, alone, is neither susceptible to targets nor aerial spraying. In this regard, it is noteworthy that the eclosion rates in Childs (2011) should probably be closer to those in Childs (2013), if not higher, for a steady-state equilibrium and the outcomes can be adjusted by a very similar factor. The humidities and temperatures referred to in this research have generally been attributed to the pupal substrate, however, one might still wonder whether atmospheric conditions can be wholly ignored. Hygrophilic species’ apparent vulnerability during the third instar, coupled with the discovery that atmospheric conditions may sometimes be profoundly different from those which characterise the pupal substrate (Childs, 2014) are a cause for such concern, while the reality of some *G. brevipalpis* climates suggest the opposite. The habitats of certain tsetse species might otherwise be used in conjunction with Figs. 3–8 as an indicator of soil moisture content. Some caution still needs to be exercised, however, as Childs (2014) reveals why the highly eccentric strategy of *G. brevipalpis* (Fig. 6) succeeds, as well as how the equally contradictory pupal and adult strategies of *G. longipennis* perfectly compliment those of *G. brevipalpis*.

The results in Figs. 3–8 must ultimately be described as projections, nonetheless. They are no substitute for real data, were such data to exist. The historical conditioning of the puparium, in all species, has been based proportionately on that of *G. morsitans*, as has a *G. morsitans*-proportionate excretion been used for the other species belonging to the *morsitans* group. Conversely, both phenomena appear to be somewhat irrelevant in the case of hygrophilic species. Consider then, for instance, that if Bursell (1958) is mistaken insofar as it attributes waterproofing to being the cause of the precipitous drop in transpiration rates concomitant with the end of the fourth instar, if that drop was, instead, actually the result of a puparium, of finite water content, drying and the timing thereof coincidental, such a mistake might introduce large errors to the calculation. Such pessimism should be tempered by the observation that the model has already been demonstrated sufficiently sound, to the extent that any contrary data can be used to effect improvements. For example, any data which would demonstrate Assumption 3 to be problematic could be used to replace $\frac{dt}{d\bar{t}}(T)$ with a new function, $\frac{dt}{d\bar{t}}(\bar{t}, T)$, predicting how the different parts of the puparial duration change with temperature. The same data which would demonstrate Assumption 4 to be problematic at the extrema of the temperature domain, could similarly be used to replace $\delta$ with a new relative-to-*G. morsitans*-rate, $\delta(T)$, one dependent on temperature. Lastly, Phelps (1973) substantiated the Bursell (1958) claim that the laboratory pupae in question were slightly inferior, showing that they only correspond to pupae in the wild during the most unfavourable season. Consider, however, that if a model is able to be adapted and successfully make predictions with respect to other species, how much more suitable must
it be for adaption to the phenotypic plasticity within the same species. Since pupal dehydration would appear to be the most challenging aspect of modelling early stage mortality, the prognosis for this model would be one of greater significance than any problems arising from issues such as inferior quality pupae and differing puparial durations and the shortage of statistically significant data can be corrected at some stage. It is with the remainder of the pupal reserves that the newly-eclosed teneral fly either hops onto a hock, for example, at sunset (Vale et. al. 1976) or more likely, waits through the night until dawn to feed; the topic of Childs (2014).

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