ESTIMATION OF THERMAL CONSTANTS: THE IMPORTANCE OF USING EQUILIBRIUM TEMPERATURE RATHER THAN AMBIENT TEMPERATURE DEMONSTRATED WITH HOVERFLIES (DIPTERA, SYRPHIDAE, GENUS *ERISTALIS*)

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Summary

Water evaporation has a marked effect on the passive rates of body temperature change of eristaline hoverflies. It results in the equilibrium temperature of these flies being significantly lower than ambient temperature. Different values for the cooling and warming constants are therefore obtained depending on whether equilibrium or ambient temperature is used as the baseline. Hence, care must be taken when estimating these constants with all animals, especially those of moderate to high permeability. It is recommended that equilibrium temperature be used in such situations. Evaporative cooling is probably also responsible for cooling constants being higher than warming constants in this and other studies.

Key words: thermal constant, temperature, cooling constant, warming constant, hoverfly, *Eristalis tenax*, *Eristalis pertinax*.

Introduction

Cooling and warming constants (k_c and k_w) reflect the speed at which an individual passively cools down or warms up in response to temperature changes in the environment (e.g. Bartholomew and Epting, 1975; Bartholomew, 1981). They can be used to help explain animal activity patterns in the field (e.g. Willmer, 1982; May, 1995).

To determine these constants, an animal is normally placed in an environment where air temperature (T_a) is different from its body temperature (T_b), and the change over time of T_b is recorded. The Newtonian equation:

$$dT_b/dt = k(T_b - T_a)$$

specifies the body temperature change per unit time (t) per unit temperature difference between an animal and its surroundings (Bartholomew, 1981), where k is the cooling or warming constant (in $°C\,min^{-1}\,°C^{-1}$, or min^{-1}). The data are conveniently analysed by plotting $\ln(T_b-T_a)$ against time, which gives a straight line if the temperature of the animal is changing at an exponential rate. The constant k corresponds to the gradient of this line.

Most previous studies (e.g. Heinrich, 1971; Bartholomew and Epting, 1975; Casey, 1976; May, 1976) have taken ambient temperature as the baseline to calculate the difference between 'external' temperature and body temperature. However, Bakken (1976) warned of the dangers of doing so. The body temperature at which an animal equilibrates is not necessarily equal to ambient temperature. It can equilibrate at a higher temperature because of metabolic heat production (endothermy) or at a lower temperature because of evaporative cooling, and different parts of the body (tagmata in insects) may equilibrate at different temperatures because of internal blood shunting. Here, we show that eristaline flies often equilibrate their thorax temperature at a temperature lower (by up to $2\,°C$) than ambient; this value is referred to as the equilibrium temperature, T_{eq}. This is likely to be a result of evaporative cooling, as these flies have been found to be quite 'leaky' (S. Bressin and P. G. Willmer, in preparation), and their rate of water loss is sufficient to account for the observed temperature depression. As Bakken (1976) showed, using ambient temperature as the baseline when it differs from equilibrium temperature gives a curved line when $\ln(T_b-T_a)$ is plotted against time. When an insect warms up or cools down, its rate of temperature change slows down with time – as it approaches its equilibrium state – in an exponential manner. Here, for a warming insect, heat exchange is slower than expected because T_a is never reached. Once the insect is at its equilibrium temperature, there is no further change: the rate of heat gain is zero, which it should not be if T_a were the end point. Thus, when $\ln(T_b-T_a)$ is plotted against time, the plot gives a curve with a decreasing slope (see Fig. 3). However, when the insect cools down, it overshoots T_a before its temperature reaches equilibrium: the rate of temperature change does not slow down as much as expected if the end point were T_a. The curve obtained has an increasing slope. These effects are very temperature-dependent because the insect's vapour pressure increases with temperature: there is more tendency for water to evaporate at higher temperatures. Thus, in this situation, it becomes very inaccurate to estimate

the value of the constant k because the slope of the plot is not constant. Also, the estimated constants are higher (cooling) or lower (warming) than expected. Bakken (1976) stated that an error of 0.1 °C in the choice of the equilibrium temperature resulted in an error of 2.2–4.1 % in the estimation of the cooling constant. He therefore recommended using the equilibrium temperature as the baseline. However, this message has been largely ignored in subsequent literature (e.g. Casey, 1980; Heinrich, 1980, 1987; Chappell and Morgan, 1987; Morgan, 1987; Morgan and Heinrich, 1987; Stavenga et al., 1993).

This work aims at reinforcing the message of Bakken (1976) by presenting a real-life example of the effect of evaporative cooling on the estimation of thermal constants in insects. It is part of an extensive study of the ecophysiology of two hoverfly species, *Eristalis tenax* and *Eristalis pertinax*. These are relatively large insects (ranging from 30 to 220 mg) of easy availability across a broad geographical range. They are often encountered in association with honeybees and bumblebees and so should be expected to share similar thermal constraints with these well-studied insects.

Both live and dead specimens of the two species were included in this study, and all were found to be affected by evaporative cooling. For clarity, we only present and analyse the results for dead *E. tenax* in detail, and give brief comparisons for dead *E. pertinax* and for live flies.

Materials and methods

Experiments were carried out in a temperature-controlled room. Humidity was not controlled; it ranged from 40 to 50 % at ambient temperatures above 18 °C, and from 50 to 80 % at ambient temperatures below 18 °C. Ambient temperature ranged from 11 to 32 °C.

The flies were caught from the field and kept in plastic boxes (in a refrigerator) at 5±1 °C and 96.5±0.4 % relative humidity with access to water. These temperature and humidity conditions mimicked those encountered by overwintering *E. tenax* and were ideal to reduce activity.

Flies were freshly killed with ethyl acetate. Thoracic temperature T_{th} was recorded using a hand-made constantan–steel thermocouple (external diameter 0.2 mm) inserted mid-dorsally in the thorax and secured in place using a minimal amount of glue (Copydex, Henkel Ltd, UK), which also prevented any haemolymph leak. The thermocouple was then inserted between two pieces of Styrofoam in a clamp held in a stand so that the fly was suspended from the thermocouple. For the warming up experiments, the fly–thermocouple assemblage was then returned to the refrigerator. When the fly had cooled down to approximately 10 °C below ambient temperature, it was transferred to a glass tank (to reduce draughts), and the thermocouple was connected to a meter (PI 8013, Portec Instruments Ltd, UK) and a chart recorder (L6512, Linseis GmbH, Germany, or BS-272, Gould instruments, UK). Body temperature and ambient temperature were continuously recorded until the fly reached its equilibrium temperature.

For the passive cooling experiment, the flies were warmed with a 60 W bench lamp to approximately 10 °C above ambient temperature before being transferred to the tank. Each experiment with each fly was repeated twice.

Recording errors due to heat loss from the thermocouple wires have been shown to be insignificant (Stone and Willmer, 1989).

The flies were weighed at the beginning and end of the experiment (electronic balance, Sartorius Handy H260, Sartorius Ltd, UK; accuracy 1 mg), and the mean of these values was used.

Statistical methods

Time and the difference between body and baseline (ambient or equilibrium) temperature were measured directly from the curves traced by the chart recorder. From plots of $\ln(T_{th}-T_{ref})$ against time, where T_{ref} is either equilibrium or ambient temperature, the gradient of the best fitted line (i.e. k) was obtained. When T_a was used as a baseline, the plots obtained being curved, measurement consistency was achieved by using the gradient of the best fitted line for body temperatures 5–2 °C above or below T_a.

All tests were carried out using 'Minitab' version 8.2. All data sets were tested for normality and, if necessary, transformed before using any parametric test. Values are given as means and standard errors (S.E.M.). The differences between the means of two populations or of matched samples were assessed using a t-test. Regression was used to analyse the effect of one or more continuous variables on another.

The general linear model was employed (because of unequal sample sizes) to investigate the effect of several variables (non-continuous and continuous, in which case a covariance analysis was used) on one other variable. Various models were fitted, with a number of interactions between the variables. To reduce the degrees of freedom used, those interactions and predictors (such as season and sex) that were not significant and not essential for the analysis were omitted. Each individual fly contributed one data point, which is a mean of two values.

To represent graphically, in multiple regression analyses, the effect of a second variable (x_2) on another (y) having controlled for a first variable (x_1), we plot the residuals obtained from the simple regression of y on x_1 as a function of x_2.

Results

Difference between ambient and equilibrium temperature

The difference between ambient and equilibrium temperature (T_{diff}) shows a positive relationship with ambient temperature, but no relationship with mass m (multiple regression for warm-up data: $T_{diff}=-0.297-0.0022m+0.056T_a$, $r^2=0.55$, $N=34$, T_a $P<0.001$, mass $P=0.136$; multiple regression for cooling down data: $T_{diff}=-0.295-0.0016m+0.050T_a$, $r^2=0.59$, $N=36$, T_a $P<0.001$, mass $P=0.186$).

As ambient temperature increases, the equilibrium temperature is increasingly below ambient temperature (by up

to 2 °C), in accordance with the evaporative cooling effect increasing.

Cooling and warming constants

The mean values for cooling and warming constants for *E. tenax* and *E. pertinax* are shown in Table 1. There was no significant difference between the T_{eq} k_w for dead and live *E. tenax* (paired *t*-test: $N=35$, $t=-0.19$, $P=0.85$), suggesting that only passive physical processes are involved in warming under these conditions. However, the T_{eq} k_c was larger for dead flies than for live ones (paired *t*-test: $N=36$, $t=-4.01$, $P=0.0003$), suggesting that live flies have some way of regulating their rate of heat loss.

Equilibrium temperature as the baseline

Fig. 1A illustrates the negative relationship between mass and the logarithm of the warming constant. The residuals from this simple regression are then plotted against T_{eq} in Fig. 1B, which shows that there is no significant relationship between equilibrium temperature and the logarithm of the warming constant (results of the multiple regression of $\log k_w$ on mass and T_{eq}: $N=53$, $r^2=0.55$, mass $P<0.001$, T_{eq} $P=0.171$).

As shown in Fig. 1C,D, there is a negative relationship between the logarithm of the cooling constant and mass, and a positive relationship between the logarithm of the cooling constant and equilibrium temperature ($N=50$, $r^2=0.57$, mass $P<0.001$, T_{eq} $P<0.001$).

Therefore, large flies warm up and cool down more slowly than small flies, reflecting the well-documented (e.g. Bartholomew, 1981; Willmer and Unwin, 1981; Casey, 1988) physical effect of size on passive heat exchange. Moreover, with equilibrium temperature as the baseline, the cooling
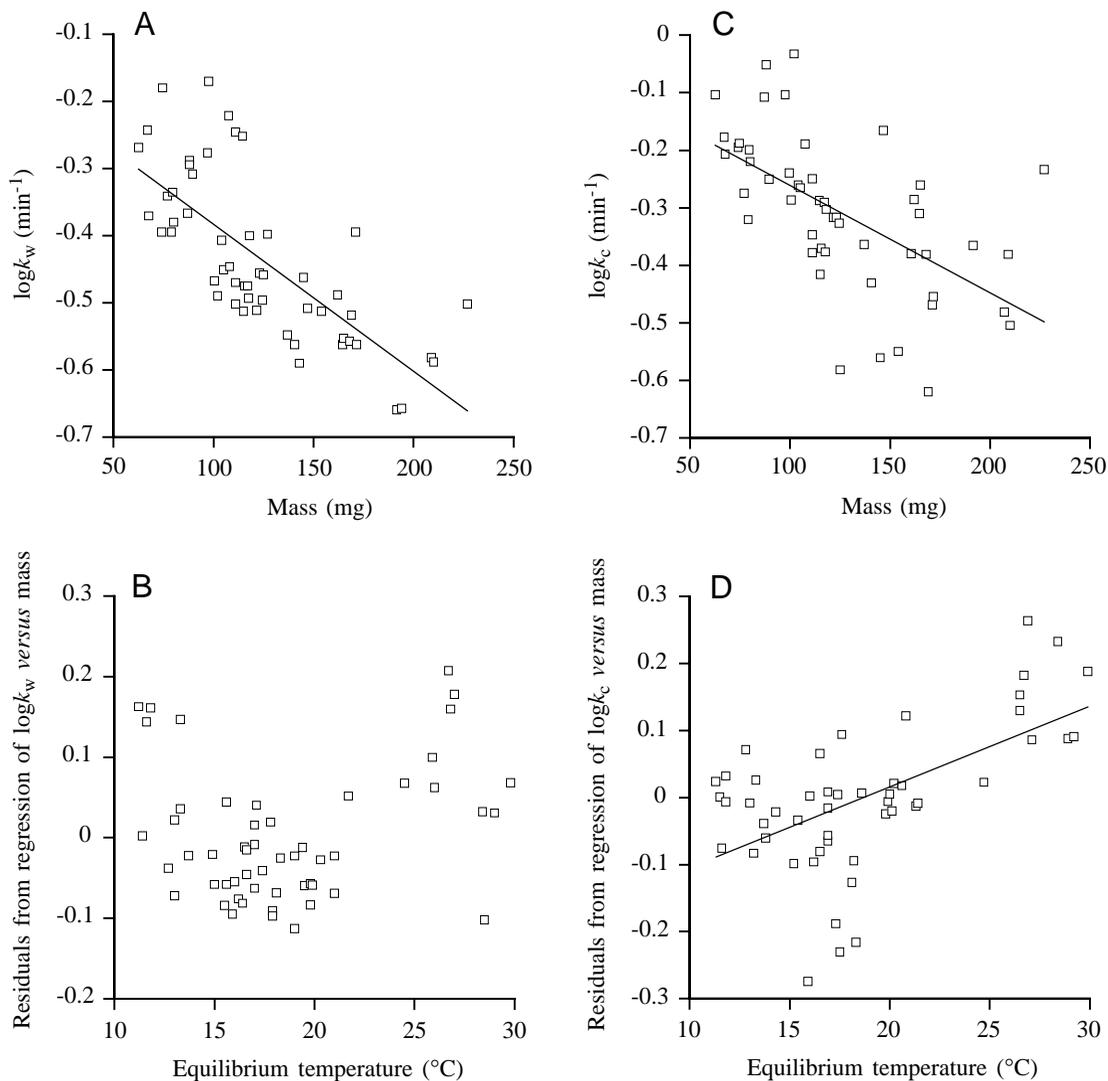

Fig. 1. Passive warm-up and cooling in dead *Eristalis tenax* taking equilibrium temperature (T_{eq}) as the baseline: (A) $\log k_w$ *versus* mass, $y=-0.002x-0.163$ ($r^2=0.533$, $P<0.001$); (B) residuals of the regression of $\log k_w$ *versus* mass against T_{eq}; (C) $\log k_c$ *versus* mass, $y=-0.002x-0.074$ ($r^2=0.338$, $P<0.001$); (D) residuals of the regression of $\log k_c$ *versus* mass against T_{eq}, $y=0.012x-0.224$ ($r^2=0.334$, $P<0.001$). k_c, cooling constant; k_w, warming constant.

constant increases with temperature: at high temperature, flies cool down faster than at low temperature. However, the warming constant is independent of temperature.

Ambient temperature as the baseline

When ambient temperature is used as the baseline, the negative relationship between mass and the warming constant is still present, but in addition there is a negative relationship between ambient temperature and the logarithm of the warming constant as shown in Fig. 2A,B ($N=41$, $r^2=0.50$, mass $P<0.001$, T_a $P=0.003$). Thus, at high ambient temperature, the warming constant is lower than at low ambient temperature.

Fig. 2C,D shows that, for the cooling constant, the relationships with mass and temperature are similar to those when equilibrium temperature is the baseline, i.e. a negative

relationship between mass and the cooling constant and a positive relationship between temperature and the cooling constant ($N=38$, $r^2=0.58$, mass $P<0.001$, T_a $P=0.001$).

Comparison of cooling and warming constants

A subsample was selected to have matched pairs (from the same flies) of cooling and warming constants (each with both ambient and equilibrium temperature as baselines). The cooling constant is larger than the warming constant when either equilibrium or ambient temperature is used as the baseline (equilibrium temperature: $N=31$, $t=6.31$, $P<0.0001$; ambient temperature: $N=32$, $t=8.59$, $P<0.0001$).

A multiple regression of $\log(k_c-\log k_w)$ on temperature (equilibrium or ambient) and mass shows a positive relationship but with no influence of mass (equilibrium temperature, $r^2=0.24$, $N=31$, T_{eq} $P=0.007$, mass $P=0.644$;
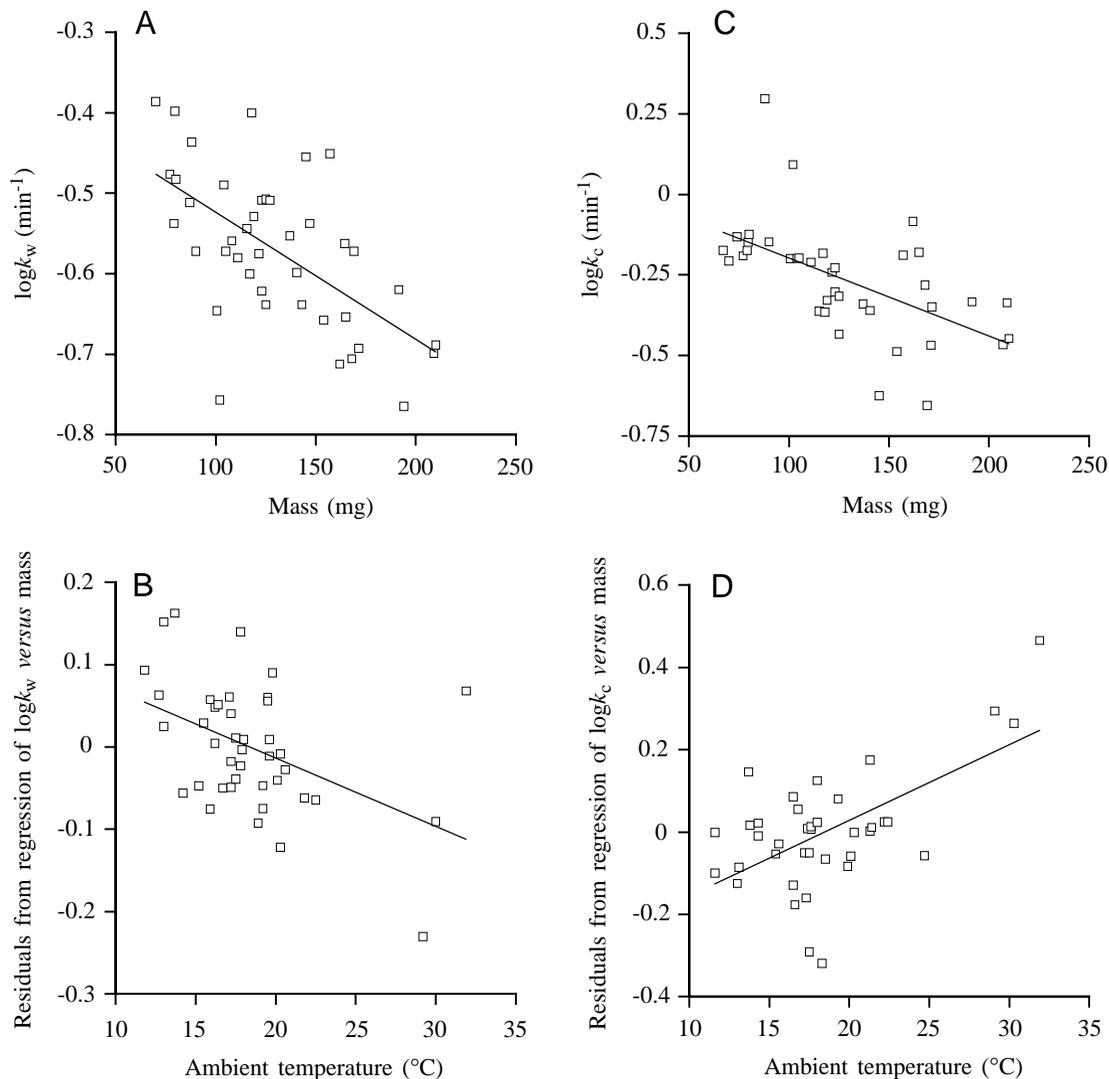

Fig. 2. Passive warm-up and cooling in dead *Eristalis tenax* taking ambient temperature (T_a) as the baseline: (A) $\log k_w$ *versus* mass, $y=-0.002x-0.366$ ($r^2=0.365$, $P<0.001$); (B) residuals of the regression of $\log k_w$ *versus* mass against T_a, $y=-0.008x+0.152$ ($r^2=0.207$, $P<0.001$); (C) $\log k_c$ *versus* mass, $y=-0.002x+0.04$ ($r^2=0.316$, $P=0.003$); (D) residuals of the regression of $\log k_c$ *versus* mass against T_a, $y=0.018x-0.340$ ($r^2=0.350$, $P<0.001$). k_c, cooling constant; k_w, warming constant.

Table 1. *Mean values for* k_c *and* k_w *for* Eristalis tenax *and* E. pertinax

	Cooling constant, k_c (min^{-1})	Warming constant, k_w (min^{-1})
E. tenax dead		
Baseline T_{eq}	0.545±0.025 (36)	0.394±0.021 (34)
Baseline T_a	0.592±0.048 (38)	0.275±0.010 (41)
E. tenax live		
Baseline T_{eq}	0.495±0.024 (36)	0.399±0.019 (34)
Baseline T_a	0.571±0.043 (26)	0.320±0.018 (35)
E. pertinax dead		
Baseline T_{eq}	0.646±0.041 (21)	0.608±0.050 (21)
Baseline T_a	0.797±0.061 (21)	0.498±0.050 (21)
E. pertinax live		
Baseline T_{eq}	0.577±0.036 (21)	0.528±0.045 (22)
Baseline T_a	0.619±0.040 (21)	0.432±0.052 (22)

T_a, ambient temperature; T_{eq}, equilibrium temperature.
Values are means ± S.E.M., N values are in parentheses.

ambient temperature, $r^2=0.51$, $N=32$, T_a $P<0.001$, mass $P=0.457$).

Therefore, dead *E. tenax* cool down faster than they warm up, and the difference in rates increases with temperature. However, small flies are not more affected than large ones. Using either reference temperature as the baseline does not affect these results.

Comparison of constants calculated with equilibrium and ambient temperature as baselines

A paired *t*-test reveals that the warming constant calculated with equilibrium temperature as the baseline ($k_w=0.394±0.021\,\text{min}^{-1}$) is larger than if calculated with ambient temperature as the baseline ($k_w=0.275±0.010\,\text{min}^{-1}$) ($N=31$, $t=-4.98$, $P<0.0001$). Similarly, the T_{eq} cooling constant ($k_c=0.545±0.025\,\text{min}^{-1}$) is smaller than the T_a cooling constant ($k_c=0.592±0.048\,\text{min}^{-1}$) ($N=30$, $t=-4.55$, $P=0.0001$).

Discussion

Cooling curves have been widely used in insect thermal biology to estimate thermal constants and conductance. Because of the controlled conditions in laboratory experiments, they cannot reflect the complex avenues of heat exchange experienced in the field by insects, but they do represent a simple means of comparing insulation and the effects of temperature changes in various insects or in different tagmata of one insect. In addition, the influence of wind or flight speed on thermoregulation can be quantified by the use of cooling curves.

For such studies, dead and/or live animals can be used, both having their advantages and drawbacks. In live animals, the rates of heat exchange are not necessarily passive but can be regulated (endothermy, haemolymph shunting, etc.). In dead specimens, the rates are passive but might be different from those experienced by live subjects: an insect might die with its spiracles open, increasing water evaporation and cooling, or the means of killing might itself alter the rates (ethyl acetate, for example, could in theory damage the waxy components of the cuticle, increasing water loss). For these reasons, both live and dead animals were used in this study; where there were obvious signs of endothermy and/or thermoregulation (shivering, abdominal pumping, etc.), the data were excluded. The fact that both live and dead (warming up and cooling down) flies did equilibrate their body below ambient temperature confirms that the phenomenon under consideration is not an artefact. The error in measured rates of temperature change (10–30 %; see Table 1) is relevant for this kind of study; it is of the same order as the increase in conductance (26–28 %) when the insulating elytra and wings are removed from dung beetles (Bartholomew and Heinrich, 1978) or as the difference in conductance (20–40 %) between the thorax and the abdomen of the sphinx moth *Hyles lineata* (estimated from Casey, 1976).

Why are ambient temperatures problematic as a baseline in thermal studies? In essence, difficulties in calculating cooling and warming constants are encountered because the temperature at which an animal equilibrates is often lower than ambient temperature. It is likely that, in the flies studied here, this phenomenon stems from the cooling effect of water evaporation because it has been shown that the difference between equilibrium and ambient temperature increases with ambient temperature. A similar explanation may apply to many other animals, and is to be expected, because the rate of water loss (and thus evaporative heat loss) is independent of the difference between body and ambient temperature, but depends on body temperature (May, 1985). In addition, the fact that air humidity is in general higher at lower temperatures will also influence evaporative cooling. Ideally, humidity should be controlled, but this does not affect the conclusions drawn here. The role of cutaneous water loss was confirmed by a series of experiments using desiccated specimens: in contrast to fresh material, these did not show any temperature deficit (i.e. $T_{eq}=T_a$). This experiment also eliminates the possibility that the evaporation of any water that had condensed on the flies' body surface (when the animals were brought from a cold to a warm environment) could have caused the temperature deficit, because the same effect would have occurred with desiccated specimens. This possibility is also refuted by the fact that cooling flies (which had not been exposed to the cold) equilibrated at a body temperature below ambient.

It has been shown elsewhere (S. Bressin and P. G. Willmer, in preparation) that *E. tenax* and *E. pertinax* are quite 'leaky' flies, i.e. that their rate of water loss (ranging from 18 to 25 mg g^{-1} h^{-1} at 20 °C, 46 % relative humidity) is in the upper part of the range for other mesic insects (cf. Table 5.3 in Willmer et al., 2000) and, in particular, for flies (e.g. Bursell, 1957, 1958). Thus, these eristalines lose water (through the spiracles and/or the cuticle) at a high rate. This probably reflects the easy availability of water in their food (nectar), and
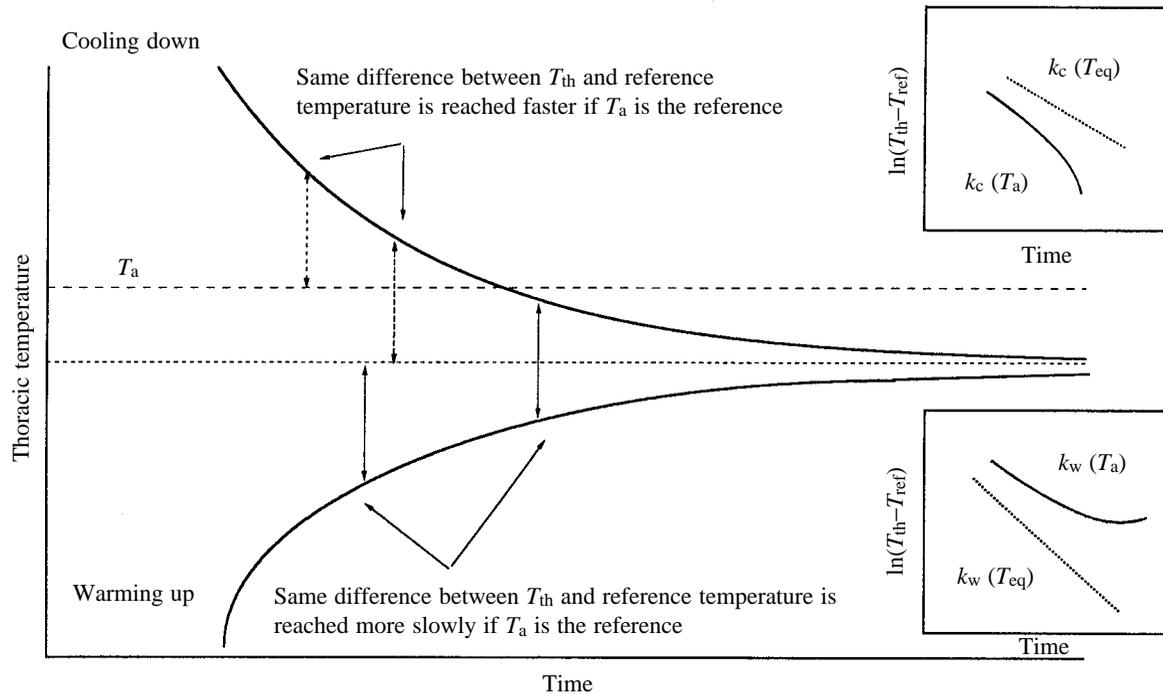
Fig. 3. Effect of water evaporation on the calculation of thermal constants. k_c, cooling constant; k_w, warming constant; T_a, ambient temperature; T_{eq}, equilibrium temperature; T_{ref}, reference temperature (i.e. T_a or T_{eq}); T_{th}, thoracic temperature.

from drinking, and the selection of very humid sites by overwintering flies (Bressin, 1999). We calculate that a fly losing water at a rate between 18 and $25\,mg\,g^{-1}\,h^{-1}$ would maintain a temperature deficit ranging from 0.54 to $0.74\,°C$; this assumes that the tissue specific heat capacity is $3.4\,J\,g^{-1}\,°C^{-1}$, the heat of evaporation of water is $2400\,J\,g^{-1}$ (Schmidt-Nielsen, 1990) and the thermal constant is $0.4\,min^{-1}$. This value is well in accord with the temperature deficits observed in the present study.

Cooling and warming constants correspond to the gradient of the line of the plot of $\ln(T_{th}-T_{ref})$ against time. When ambient temperature is taken as the baseline, the thoracic temperature of the warming flies does not reach this baseline (see Fig. 3). Thus, when $\ln(T_{th}-T_a)$ is plotted against time, a curve is obtained (lower inset, Fig. 3) because the time taken to move a unit of temperature closer to ambient temperature increases as the fly warms up, but not exponentially: this duration tends towards infinity because the fly never reaches ambient temperature. The same plot would give a straight line if the fly reached the baseline temperature (assuming that the only cause of warming is the temperature difference between the fly and its environment) because the time taken to move a unit of temperature closer to ambient temperature then does increase exponentially. Therefore, the estimates of warming constants using ambient temperature are not accurate and vary depending on which part of the curve is used for the estimation.

The problem is different for the cooling constant because thoracic temperature then overshoots the baseline (Fig. 3). Plots of $\ln(T_{th}-T_a)$ *versus* time also result in curves (upper inset, Fig. 3). However, because the body temperature used

here varied from 7 to $1\,°C$ above ambient temperature, the plot obtained is in the straighter part of the curve. The curve will bend more strongly when body temperatures below ambient temperature are included (see also Bakken, 1976). Thus, less difficulty is encountered for the estimation of the cooling constant; but this does not imply that the cooling constants thus calculated are the 'correct' ones.

Calculating the warming and cooling constants with the equilibrium temperature as reference solves these problems. Straight lines are obtained, and the negative relationship between warming constant and temperature disappears. For cooling, the effects of the temperature depression below ambient are removed. However, an additional effect of evaporative cooling on the T_{eq} cooling constant persists (because T_b is well above 'external temperature') and still gives a positive relationship with 'external' temperature. These flies still cool down faster at high temperature because of the inevitable increase in water evaporation that is occurring.

The effect of using either ambient or equilibrium temperature for estimating thermal constants is well demonstrated here. Warming constants based on T_a are smaller than warming constants based on T_{eq}. In contrast, estimates for the T_a cooling constant are larger than estimates for the T_{eq} cooling constant. This is to be expected, because a warming fly takes more time to reach the same $T_{th}-T_{ref}$ if ambient rather than equilibrium temperature is the reference temperature: a smaller warming constant is obtained (Fig. 3). Conversely, a cooling fly reaches the same $T_{th}-T_{ref}$ faster if ambient rather than equilibrium temperature is the baseline: the cooling constant is larger.

Thus, thermal constants are artificially decreased or increased if ambient temperature is used as the baseline. Using equilibrium temperature as the baseline gives a more accurate estimate of these constants. This is in accordance with Bakken's (1976) findings, and his recommendations for the use of T_{eq} should be much more widely adopted in studies of all but very large and/or very low permeability animals.

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