

Sound production and endothermy in the horse bot-fly, *Gasterophilus intestinalis*

W. F. HUMPHREYS and S. E. REYNOLDS School of Biological Sciences, University of Bath

ABSTRACT. Adult *Gasterophilus intestinalis* (De Geer) frequently produce a 'buzzing' sound while stationary. This buzzing was always associated with heat production in the fly's thorax, although sometimes heat production occurred without audible buzzing. Thoracic temperature (T_{th}) could be elevated by as much as 12°C. As buzzing continued, the T_{th} rose, the pitch of the buzzing sound increased, the frequency being directly proportion to T_{th} . Periods of buzzing were usually, but not always, terminated by attempted flight. Often, flies showed long episodes of cycling, when periods of continuous buzzing were interspersed with periods of rest. Such cycling maintained T_{th} above ambient temperature for long periods. During sustained tethered flight, flies were able to maintain T_{th} at high, steady values for long periods. Heat loss from the thorax is restricted by a dense covering of hair, and also by active control over heat transfer between thorax and abdomen.

Introduction

Although, for the most part, insects have been considered to be ectotherms, an increasing number of species have been shown to use the relative inefficiency of muscular activity to provide a means of increasing their body temperatures (Heinrich, 1973). This endogenous heat production can be used for a variety of purposes, but the most common appears to be the use of the flight musculature to increase temperature prior to take-off, and to maintain a high temperature during flight (Kammer & Heinrich, 1978).

Here we extend the list of insects which regulate their body temperature by endothermy to include the horse bot-fly, *Gasterophilus intestinalis* (De Geer), which increases its thoracic temperature by 'shivering' prior to take-off. This pre-flight warm-up is usually associated with sound

production, which was what first drew our attention to the phenomenon.

Methods

Gasterophilus larvae were collected locally from fresh horse dung and kept at room temperature (20–24°C) while they underwent pupal and adult development. They were used for experiments within 3 days of emergence from the puparium. Bot-flies are variable in size; those used in the experiments weighed between 31 and 240 mg.

Sound produced by buzzing was recorded on a Sony TC-126 tape recorder. The characteristic frequency of the recorded sound was analysed visually on the screen of a Telequipment DM 64 storage oscilloscope.

The temperature of the thorax (T_{th}), abdomen (T_{ab}), and surrounding air (T_{am}) were measured by means of fine (0.04 mm diameter) enamelled copper/constantan thermocouple wires (Dural Plastics and

Correspondence: Dr W. F. Humphreys and Dr S. E. Reynolds, School of Biological Sciences, University of Bath, claverton Down, Bath BA2 7AY.

Engineering, Dural, N.S.W., Australia), which were encased in a flexible P.V.C. sheath (0.45 mm diameter) except at the tip. The bot-flies could take off and maintain flight while tethered to this lead if they were otherwise unrestrained. The flies survived for several days with the thermocouples implanted, a survival time similar to flies without implanted thermocouples. The flies were anaesthetized with carbon dioxide and the thermocouples inserted to a depth of about 1 mm through a hole made in the cuticle. The thermocouples were sealed in place with quick setting epoxy resin (Araldite). Thoracic thermocouples were placed slightly to one side of the mesothoracic tergum and abdominal thermocouples were placed in the tergum of segment 3. The output of the thermocouples was measured relative to that of a cold junction (0°C), and recorded on a potentiometric chart recorder (a Devices M2R recorder equipped with DC2D pre-amplifiers) or with a Comark type 1624 electronic thermometer.

Results

Buzzing and heat production

Both male and female adult bot-flies frequently produce a high-pitched buzzing

tone while stationary. They adopt a characteristic posture with the abdomen tucked ventrally at $c. 90^{\circ}$ to the thorax, and with the wings reflexed over the abdomen, with the wingtips overlaying each other. During buzzing, the wingtips can be seen to vibrate, and the wings seem likely to be the source of the sound.

Experiments with a thermocouple inserted into the thorax showed that bouts of continuous buzzing were always correlated with an increase in thoracic temperature. In a few insects, increases in T_{th} occurred which were not accompanied by audible buzzing. These insects may have been damaged during the insertion of the thermocouple wires, so that they were not able to reflex their wings properly.

Fig. 1 shows a typical record in which each of three bouts of buzzing resulted in heat production in the thorax. Very short periods of buzzing (less than 10 s) sometimes did not result in a measurable increase in T_{th} , but heat is presumably always generated during buzzing. T_{th} frequently reached as much as 12°C above ambient temperature (T_{am}) during buzzing, although the exact temperature at which buzzing (and heat production) was terminated varied considerably, even for individual flies (Fig 1).

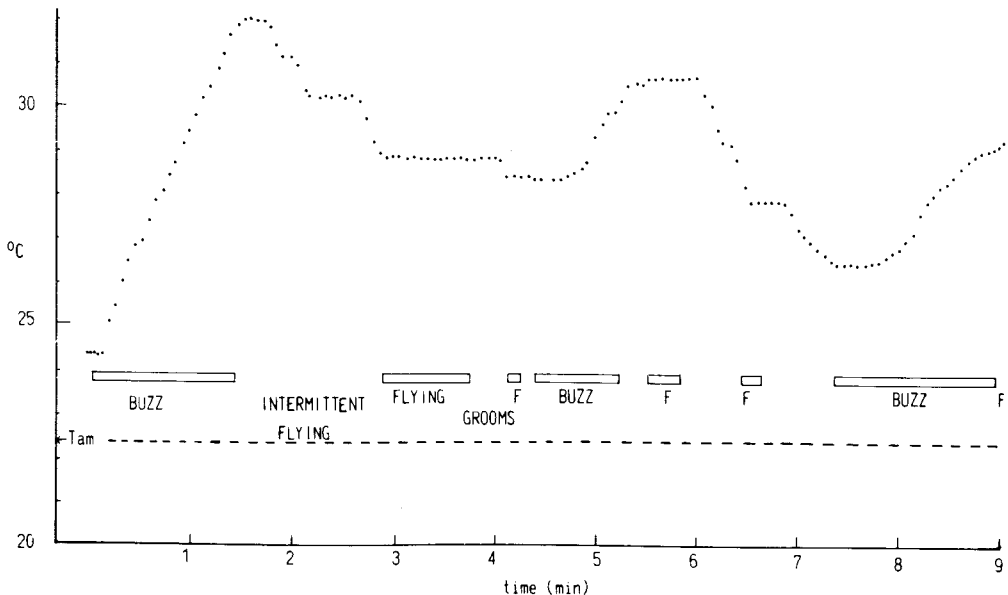


FIG. 1. Typical record of thoracic temperature ($^{\circ}\text{C}$) and behavioural state against time for *Gasterophilus*.

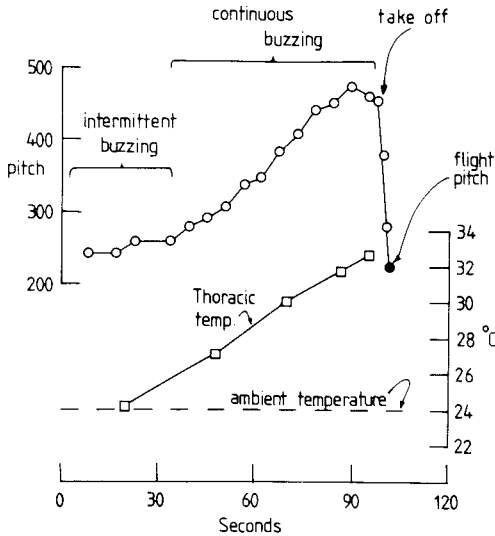


FIG. 2. The relationship between thoracic temperature and the pitch (characteristic frequency shown in Hz) of buzzing during pre-flight warm up. Note the reduction in pitch once flight began.

The sound produced by buzzing is quite distinct from that produced during flight. The pitch of the flight tone during free or tethered flight was found to be characteristically close to 200 Hz. Buzzing was quieter. The pitch increased during any one bout of buzzing from about 200 Hz to 500 Hz (Fig. 2) and was positively correlated with T_{th} (Fig. 3).

T_{th} during flight and attempted flight

The cessation of buzzing usually coincided with take-off and attempted flight. In many cases take-off also resulted in a sharp fall in T_{th} (Fig. 2). In those cases where flight

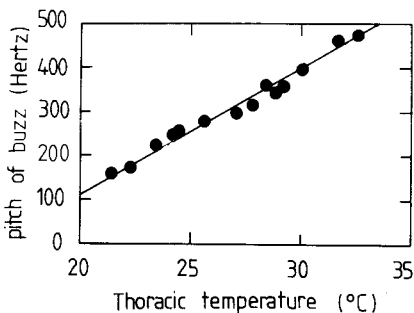


FIG. 3. Correlation between pitch and thoracic temperature during pre-flight warm up. Data plotted from Fig. 2 and an additional experiment on the same fly.

was sustained for more than a few seconds it was evident that T_{th} could be controlled during flight as the level at which constant temperatures were maintained varied even between successive bouts of flying (Fig. 1). This implies that the temperature maintained is not simply the balance between heat production due to flying and passive heat loss. That flies can maintain fairly constant temperatures during prolonged flight is shown in Fig. 4.

Although take-off was generally preceded by a bout of buzzing, and thus occurred when T_{th} had been raised, the flies could sometimes be induced to fly from cold by eliciting the 'tarsal reflex' (Fig. 4).

In many flies, buzzing followed a cyclic pattern of activity (Fig. 5) in which T_{th} increased for a period (variable but generally in the order of 20 s), decreased when buzzing ceased and then rose again when buzzing recommenced. This pattern of activity could lead to either an increasing or decreasing trend in the thoracic temperature. Cyclical increase in the temperature trend was usually terminated by flight.

Since this pattern of activity was observed on many occasions, not only in flies which had implanted thermocouples, but also those living free in a cage in the laboratory, it would seem to be a natural behaviour rather than an experimental artefact.

Regulation of heat loss from the thorax

The ability of the flies to maintain different constant temperatures during successive bouts of flying suggests that both heat loss and heat production can be regulated. Adult *Gasterophilus* show a number of morphological adaptations which may restrict the rate of heat loss. One such is the hairiness of the thorax. We investigated the insulative properties of this by determining the rates of cooling in the thorax of freshly killed flies immediately before and after as much as the hair as possible had been shaved from the dorsum of the thorax with a scalpel blade (Fig. 6). The rate of cooling of the thorax was increased by 14% after this operation. (ANOVA to test equality of slopes: $F_{1,26} = 4.68$, $P < 0.05$). Similar results were obtained in four experiments on three individuals.

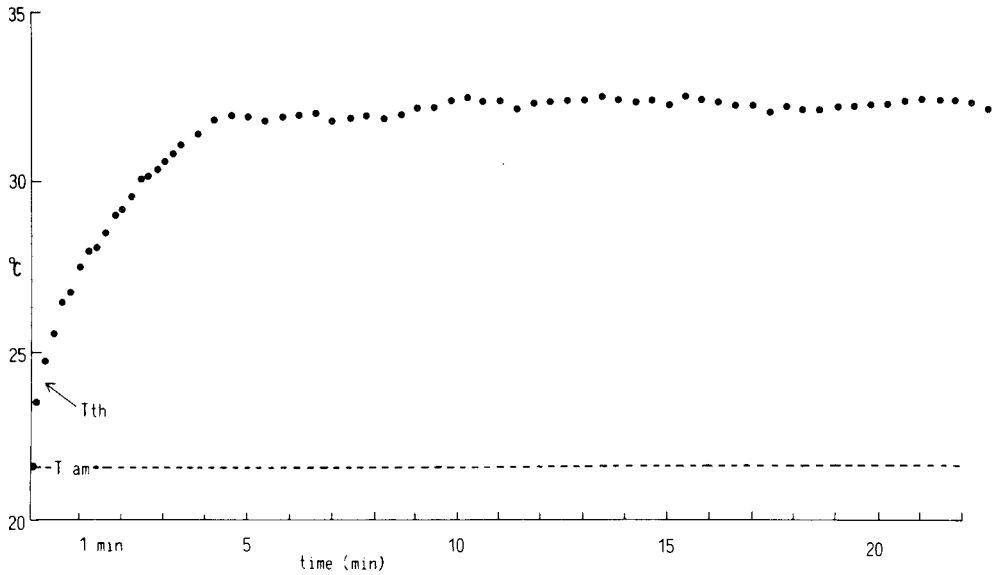


FIG. 4. Maintenance of steady thoracic temperature during >20 min continuous flight following take-off from cold. Flight was induced by the 'tarsal reflex', when the ambient temperature was 21.6°C.

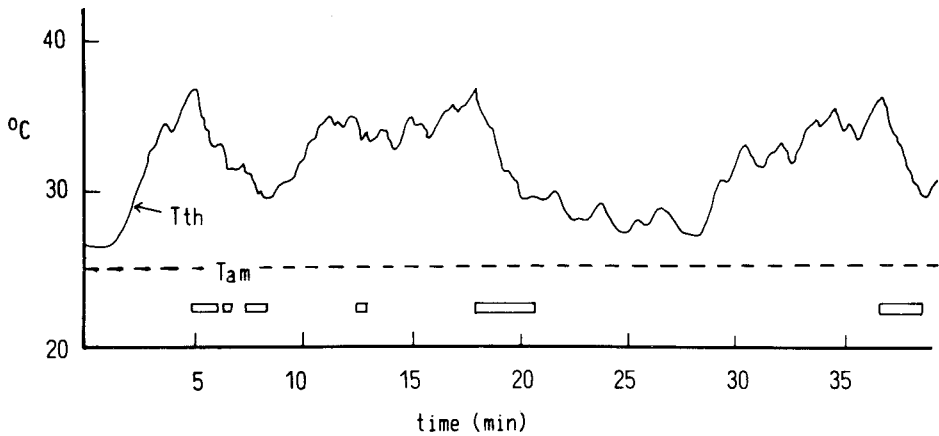


FIG. 5. Record of a long period of elevated thoracic temperature in a female (125 mg) *Gasterophilus* showing the fluctuation of the temperature associated with alternate buzzing and flight. Bars indicate periods of continuous flight. Rising temperature was always associated with buzzing (typically in c. 20-s bouts), and falling temperature with cessation of buzzing or the start of flight.

Fig. 7 shows the result of an experiment in which a male fly was equipped with both thoracic and abdominal thermocouples. As can be seen, in this insect, stationary heat production (this particular fly did not buzz audibly) resulted in a rise in T_{th} (of 3.0°C) which was accompanied by only a small change in T_{ab} (of 0.8°C). When the fly was

induced to fly using the tarsal reflex (Fig. 7B) the initial effect was to increase T_{th} sharply, while T_{ab} actually fell below T_{am} by 0.3°C, perhaps due to evaporative heat loss from the abdomen in the moving air stream from the wings. A brief pause in flight activity was accompanied by a fall in T_{th} and a corresponding rise in T_{ab} (see below). Con-

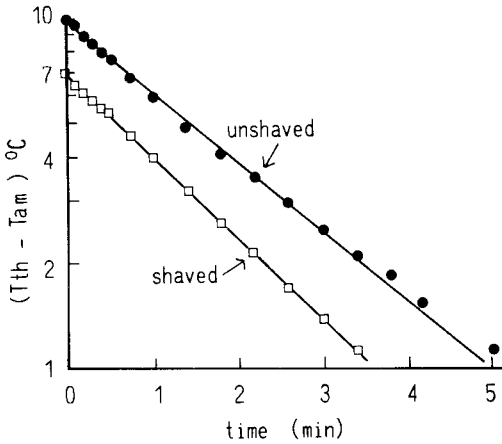


FIG. 6. Example of thoracic cooling curves in a freshly killed female *Gasterophilus* (174 mg) before and after incomplete shaving of the dense dorsal coat of thoracic hair.

tinued flight did not raise T_{ab} back above T_{am} until T_{th} was more than 6°C above T_{am} . Finally, T_{th} stabilized at about $31\text{--}32^{\circ}\text{C}$ ($7\text{--}8^{\circ}\text{C}$ above T_{am}) with T_{ab} about 0.8°C above T_{am} .

Fig. 7C shows the effect of heating the fly, during its continued tethered flight, with a high intensity microscope lamp. The shapes of the two curves are not important because no attempt was made to control the relative amounts of radiation incident on the abdomen and thorax. However, once the exogenous heat source had been removed, and the thorax and abdomen began to cool, the two curves indicate the relative rates of heat loss from the two structures. These curves obtained from the living fly were compared with those determined later in the same fly after death (Fig. 8).

It can be seen that whereas loss of heat from the thorax is more rapid in the living fly than the dead one, the reverse is true for heat loss from the abdomen. The simplest explanation for this is that under the heat stress imposed by the experiment the living fly is able to transfer heat from the thorax to the abdomen, presumably by haemolymph circulation, whereas the dead fly cannot.

However, this route for heat loss is evidently restricted at times when the insect is not heat stressed; Fig. 7D shows that on resumption of

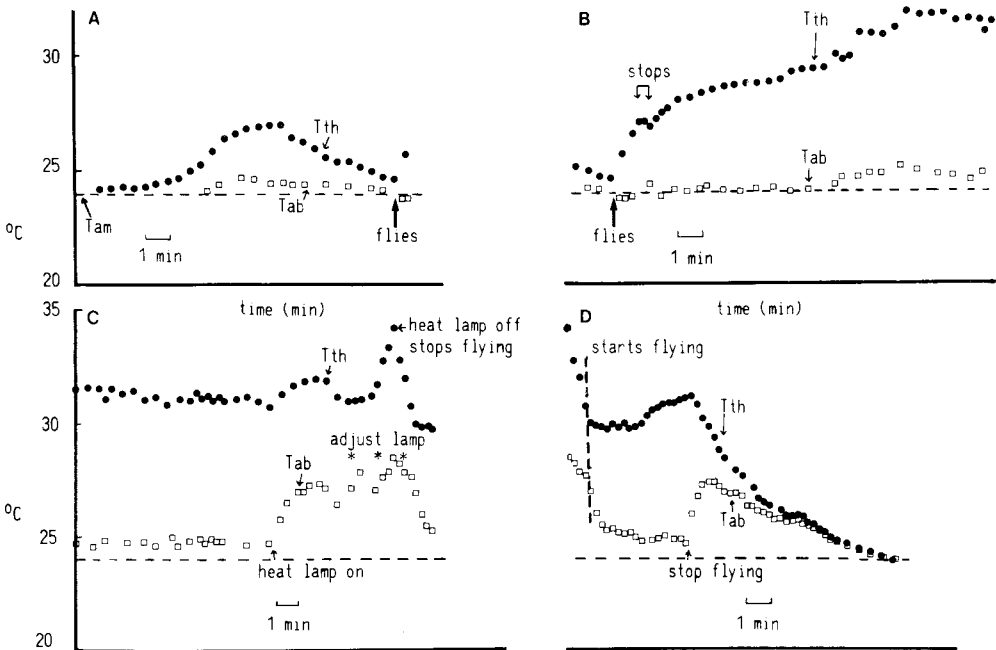


FIG. 7. Record of changes in thoracic and abdominal temperature in a single *Gasterophilus* (male, 85 mg). The records shown in A–D are continuous and have been divided (with some overlap) for ease of discussion in the text. In C a lamp to heat the fly was switched on and adjusted as indicated (asterisks).

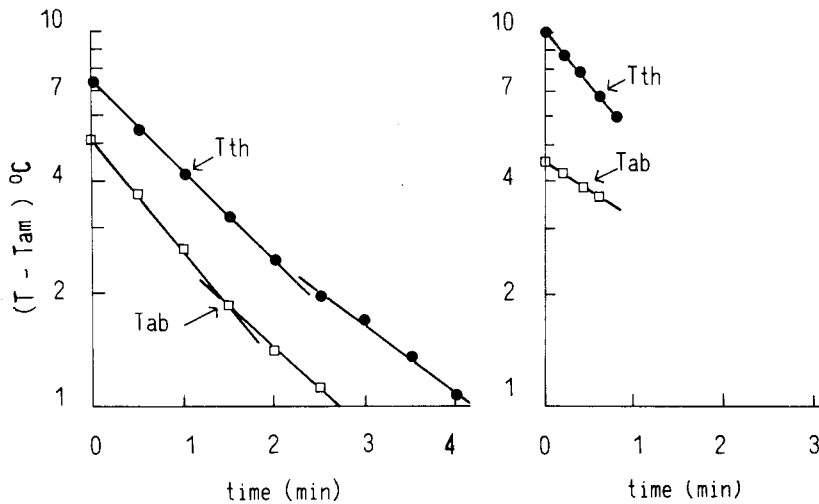


FIG. 8. Cooling curves for the fly from Fig. 7. Abdominal and thoracic cooling curves were determined following external heating with a microscope lamp, (A) after death, and (B) in the living fly (data of Fig. 7C). The slight bend in the cooling curves is common and probably due to unilateral heating of the fly. In (A) the time for 50% heat loss in the upper and lower halves of the cooling curves are, respectively, for the thorax 75 s and 99 s, and for the abdomen 60 s and 81 s. Abdominal cooling in each case is about 1.25 times faster than that of the thorax. In (B) the time for 50% heat loss is 66 s for the thorax and 117 s for the abdomen, with the abdomen cooling at about half the rate of the thorax.

flight after the lamp has been switched off there occurred simultaneously the stabilization of T_{th} and an abrupt decrease in T_{ab} . Furthermore, when flight ceased T_{th} fell abruptly and T_{ab} increased considerably. The simplest explanation is that the resumption of flight leads to greater heat production in the thorax, and closing off the circulation to the abdomen results in lowered heat loss by the thorax plus lowered heat gain by the abdomen. When flight ceases, the haemolymph circulation is restored and heat is transferred from the thorax to the abdomen.

Discussion

Our measurements of thoracic temperatures have shown that the characteristic buzzing sound made by stationary horse bot-flies is associated with endothermic heat production in the thoracic musculature.

The finding that the characteristic frequency of the buzzing sound is directly related to the temperature of the thorax is not surprising. The sound is produced by vibrations of thoracic structures (probably the

wings) which are moved in turn by asynchronous (fibrillar) muscles in the thorax. The frequency of oscillation of asynchronous muscles is determined only by the loading characteristics of the mechanical system in which they operate, and by the temperature. A number of authors (see Chadwick, 1953) have noted that the wing beat frequency of *Drosophila* and other small insects (presumably, non-thermoregulators) bears a similar, simple relationship to T_{am} .

Periods of buzzing are usually, but not always, terminated by attempted flight. Buzzing may thus be described as pre-flight warm-up. As is the case in other insects which show this kind of behaviour, there does not seem to be an absolute temperature at which flight is initiated. Rather, the probability of take-off is greatly increased at higher body temperatures. Spontaneous attempts to fly were most common in *Gasterophilus* after buzzing activity had raised T_{th} to values close to 31–32°C (the preferred temperature range for spontaneous flight was $T_{am} = 20–24^{\circ}\text{C}$). We never observed spontaneous take-off at values of T_{th} less than 28°C.

The significance of pre-flight endothermy in bot-flies may be in increasing their readiness for take-off at short notice, when a suitable host appears. The cycling behaviour which we saw and which has also been observed in beetles (Bartholomew & Casey, 1976), certainly serves to maintain T_{th} above T_{am} for long periods during which flight attempts are only intermittent. In this respect, it is interesting that long-term records from flies implanted with thoracic thermocouples only showed cycling of T_{th} during the day, remaining quiescent when the lights were switched off. On the other hand, warm-up can be rapid. One fly raised T_{th} from ambient (21.8°C) to 30.5°C in 39 s.

Confined in a cage in the laboratory, bot-flies only rarely fly spontaneously for more than 1–2 s and so scarcely need the kind of temperature regulatory abilities we have postulated in this paper. However, in the field, female bot-flies have been observed to hover over their egg-laying sites on the host's legs (Dinulescu, 1932). Perhaps regulation of thoracic temperature may be important in allowing this.

The flies seem to have considerable control over heat exchange between the thorax and abdomen (Fig. 8). A similar control of heat loss from the thorax has been shown in bumble-bees (Heinrich, 1976). During flight, *Gasterophilus* appears to restrict the circulation of haemolymph between thorax and abdomen, and thus limit heat losses from the thorax. The thick layer of thoracic hair also serves to conserve heat (Fig. 6). Even incomplete shaving of this coat increased the rate of heat loss substantially. In a forced draught, such as would be experienced during flight, insulation by the hairs would conserve relatively more heat.

Despite such measures to conserve thoracic heat, the metabolic cost to the fly to keeping warm must be considerable. A rough calculation illustrates this: the fly from which the data of Figs. 7 and 8 were obtained weighed 85.1 mg (an average size for a male) of which 53.1 mg was the thorax. Assuming a specific heat of $3.35 \text{ j g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ (from Heinrich & Pantle, 1975) and a rate of cooling of $6.6^{\circ}\text{C min}^{-1}$ at $T_{am} = 24^{\circ}\text{C}$ and $T_{th} = 31^{\circ}\text{C}$ (taken from Fig. 8), then the fly would need to expend at least 1.17 j min^{-1} ($22.1 \text{ j min}^{-1} \text{ g}^{-1}$)

in order to maintain T_{th} constant at the preferred flight temperature.

Most attempts to explain the adaptive significance of endothermy in insects have concentrated on the advantages which accrue to foraging insects when they extend the time available for gathering food (Heinrich, 1972). *Gasterophilus* has no mouthparts in the adult stage and cannot feed, living only a few days. Thus the adoption of even partial endothermy, particularly when a sustained elevation of T_{th} was not always associated with attempted flight, is surprising. The caloric content of an 85-mg fly would be approximately 640 joules (assuming 70% water and a caloric value of 25 j mg^{-1} dry weight; from Cummins & Wuycheck, 1971). Stabilization of T_{th} by endothermy at 7°C above T_{am} (calculated above to require about 70 j h^{-1}) would thus completely consume the fly in only 9 h. The length of time which can be spent in thermo-regulating must therefore be much less than this.

This apparent squandering of energy doubtless has selective advantage; improved performance associated with high body temperature presumably aids such behaviour as mate-finding, host-finding or egg-laying.

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