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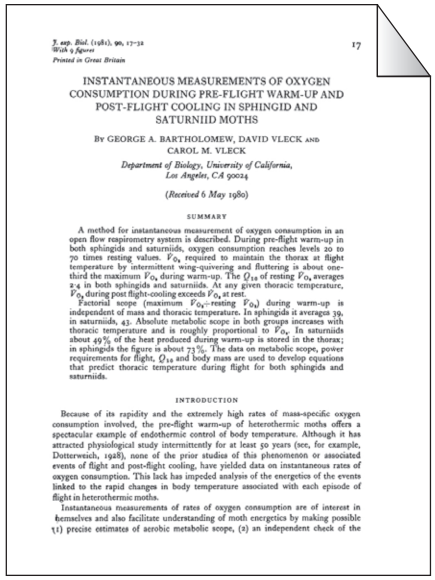
'INSTANTANEOUS' METABOLIC MEASUREMENT



J. exp. Biol. (1981), **90**, 17–32
With 9 figures
Printed in Great Britain

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INSTANTANEOUS MEASUREMENTS OF OXYGEN CONSUMPTION DURING PRE-FLIGHT WARM-UP AND POST-FLIGHT COOLING IN SPHINGID AND SATURNIID MOTHS

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(*Received 6 May 1980*)

SUMMARY

A method for instantaneous measurement of oxygen consumption in an open flow respirometry system is described. During pre-flight warm-up in both sphingids and saturniids, oxygen consumption reaches levels 20 to 70 times resting values. $\dot{V}_{O_2}$ required to maintain the thorax at flight temperature by intermittent wing-quivering and fluttering is about one-third the maximum $\dot{V}_{O_2}$ during warm-up. The Q_{10} of resting $\dot{V}_{O_2}$ averages 2·4 in both sphingids and saturniids. At any given thoracic temperature, $\dot{V}_{O_2}$ during post flight-cooling exceeds $\dot{V}_{O_2}$ at rest.

Factorial scope (maximum $\dot{V}_{O_2}$ ÷ resting $\dot{V}_{O_2}$) during warm-up is independent of mass and thoracic temperature. In sphingids it averages 39, in saturniids, 43. Absolute metabolic scope in both groups increases with thoracic temperature and is roughly proportional to $\dot{V}_{O_2}$. In saturniids about 49% of the heat produced during warm-up is stored in the thorax; in sphingids the figure is about 73%. The data on metabolic scope, power requirements for flight, Q_{10} and body mass are used to develop equations that predict thoracic temperature during flight for both sphingids and saturniids.

INTRODUCTION

Because of its rapidity and the extremely high rates of mass-specific oxygen consumption involved, the pre-flight warm-up of heterothermic moths offers a spectacular example of endothermic control of body temperature. Although it has attracted physiological study intermittently for at least 50 years (see, for example, Dotterweich, 1928), none of the prior studies of this phenomenon or associated events of flight and post-flight cooling, have yielded data on instantaneous rates of oxygen consumption. This lack has impeded analysis of the energetics of the events linked to the rapid changes in body temperature associated with each episode of flight in heterothermic moths.

Instantaneous measurements of rates of oxygen consumption are of interest in themselves and also facilitate understanding of moth energetics by making possible (1) precise estimates of aerobic metabolic scope, (2) an independent check of the

John Lighton discusses the 1981 paper by George Bartholomew, David Vleck and Carol Masters Vleck entitled: 'Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths'.

A copy of the paper can be obtained from http://jeb.biologists.org/content/90/1/17.short

Some papers address important phenomena using existing methods; others describe new methods without adding substantial new research insights. A select few combine significant research findings with methodological breakthroughs. This paper (Bartholomew et al., 1981) belongs solidly in that last category, among elite company. As Bert Heinrich pointed out in a previous JEB Classic (Heinrich, 2007), it has long been known that some flying insects, including many moths, use rapid, tight contractions of their flight motors to elevate their thoracic temperatures with impressive rapidity before taking flight. All well and good, but what are the metabolic costs of 'priming the pump' of thermal energy required in such insects to gain the muscular performance necessary for flight, within a brief time span of a couple of minutes? That was unknown, and for good reason. By 1981, technology had advanced enough to measure the energetics of pre-flight warm-up using flow-through respirometry, but not in a helpful way. This is because flow-through respirometry usually surrounds the animal being monitored with a sealed chamber through which air flows at a known rate. The oxygen consumption rate ($\dot{V}_{O_2}$) is calculated from the change in O_2 concentration caused by the animal at that flow rate. But here's the kicker. The volume of the chamber and the rate of air flow through it conspire to blunt and distort the kinetics of the metabolic signal, making it impossible to track the rapid changes in $\dot{V}_{O_2}$ that accompany pre-flight warm-up.

What causes this blunting and distortion? A hefty chamber volume is required to house a large moth, and a low flow rate through that chamber is necessary to distinguish the moth's metabolic signal, i.e. the change in O_2 concentration in the air flowing from the chamber, from analyzer noise and drift. According to Bartholomew et al. (Bartholomew et al., 1981), the volume of their chamber was 700 ml. Air flowed through it at a rate of 125 ml min^{-1}. This translates to a time constant – the time required for a step change in O_2 concentration flowing from the chamber, caused by a step change in $\dot{V}_{O_2}$ within the chamber, to shift to just 63% (1–1/e) of its final or equilibrium value – of 700/125 or 5.6 min. But as figs 2 and 3 in the manuscript so dramatically show, typical warm-up bouts lasted for approximately half that time. Clearly, their setup's time constant was so abysmally sluggish that accurate estimates of peak $\dot{V}_{O_2}$ during pre-flight warm-up were impossible. Increasing the flow rate was not an option; their O_2 analyzer, though the best available at that time, was already stretched to its resolution limit, while the chamber could not shrink further without impacting the moth's behavior. Epic failure. Or so it seemed.

The impact of this classic paper (Bartholomew et al., 1981) derives in part from giving us the first valid measurements of real, moment-by-moment $\dot{V}_{O_2}$ during pre-flight warm-up in two different families of large moths, but more by doing so using a clever mathematical trick, now widely used throughout the science of metabolic measurement, to reduce the data distortion caused by the time constant in flow-through respirometry.

Anyone who knew Bartholomew (and as his last graduate student I knew him well, having survived multiple intense field seasons with him at Barro Colorado Island in Panama, where this study took place) can picture his frustration, even anger, at the limitation that the chamber's time constant imposed. They can also picture his bulldog determination to find a way around the fact that he and his co-authors needed – demanded! – the instantaneous equilibrium value of the O_2 concentration (F_{eq}) in the air flowing from the chamber, uncontaminated by the sluggish time constant, so that moment-by-moment, or 'instantaneous', $\dot{V}_{O_2}$ could be calculated. There had to be a solution.

Their solution, as it turns out, was simple and elegant. Boiling that solution down to its essentials, two equations [eqns 1 and 2 in Bartholomew et al. (Bartholomew et al., 1981)] describe the progress of O_2 concentration in the air flowing from the chamber to its final or equilibrium concentration, F_{eq}, after a step change in the $\dot{V}_{O_2}$ of the organism within the chamber. The first equation is the standard first-order washout equation, which describes the progress towards equilibrium of the O_2 concentration in the air leaving the chamber as a function of the volume of the chamber and the rate of air flow through it. The second equation is a more naive and functional definition of that progress towards equilibrium, which has the advantage of containing the sought-after F_{eq}. The key insight in this classic paper is that the two equations can be combined to invoke and materialize the coveted F_{eq}, freed from the time constant. And better still, the resulting equation is very simple in essence: take the rate of change of the O_2 concentration in the air flowing from the chamber, multiply it by a constant easily calculated from the first equation referred to above, add that product to the O_2 concentration in the air flowing from the chamber, and there before you in all its majesty is F_{eq}, from which 'instantaneous' $\dot{V}_{O_2}$ can be calculated using standard respirometric equations. Epic success.

Exceptional claims demand exceptional proof, which Bartholomew and the Vlecks obtained by puffing their breath briefly into the moth chamber (minus the moth) and demonstrating that the 'instantaneous transformation', as it became known, eliminated the leisurely washout curve of the untransformed data; shortly after the puff ended, it was as if the puff had never happened, while the untransformed data had still not equilibrated to zero even 10 min later (fig. 1 in their paper). Thus armed, they went on to investigate the energetics of their moths, and were easily able to quantify the moment-by-moment metabolic cost of pre-flight warm-up and flight, and their mass-scaling allometry, in sphingid and saturniid (indeed, any) moths for the first time.

Various variations of the 'instantaneous transformation' exist, as do some criticisms of the technique with their associated metacriticisms [for discussions, see Chaui-Berlinck and Bicudo and others (Chaui-Berlinck and Bicudo, 2000; Lighton, 2008; Lighton and Halsey, 2011)]. In general, excellent mixing is required within the chamber, and the 'effective volume' of the chamber [as defined in eqn 1 of Bartholomew et al. (Bartholomew et al., 1981)] is best determined by empirical iteration using a known transient signal rather than from its physical volume (Lighton, 2008). For extremely demanding applications such as room calorimetry, where 1 min resolution is demanded of a ~28,000 l room at a flow rate of ~80 l min^{-1} (time constant >5 h), other approaches such as wavelet analysis give excellent results (Brychta et al., 2009), but so does a completely orthodox approach based on Bartholomew et al.'s classic paper (Melanson et al., 2010).

Thus, to this day, every informed investigator who measures the metabolic cost of transient phenomena in a chamber-based flow-through respirometry system uses the instantaneous transformation or a functional equivalent. It is a keystone in my research and that of my colleagues, an incredibly versatile tool, essential for energetics studies but also capable of sharpening and matching the response characteristics of disparate analyzers [thus allowing useful novelties such as 'background baselining' and direct compensation for water vapor dilution (Melanson et al., 2010)], speeding the multiplexing of gas streams in metabolic phenotyping applications, improving the time resolution of direct calorimeters (because heat transfer kinetics are analogous to washout kinetics), and more.

It isn't surprising, then, that awareness of the importance of the 'instantaneous transformation' is increasing. Out of interest, I graphed the cumulative citations of Bartholomew et al. (Bartholomew et al., 1981) from 1982 to 2011. The result was curious: a straight-line increase at a rate of 8.6 citations yr^{-1} from 1982 to 1997 (r^2=0.99), followed by a breakpoint to a steeper linear increase from 1998 to 2011 of 15.3 citations yr^{-1} (r^2=0.99 again). What this change in the respirometric zeitgeist late last century may have been can be debated, but of the classic stature of this paper there can be no doubt.

10.1242/jeb.068288

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