Measuring the metabolic rate of *Drosophila*: What's involved?

A white paper by John Lighton, Ph.D.
Adjunct Professor, UNLV and President, Sable Systems International
Author of "Measuring Metabolic Rates: A Manual for Scientists" (Oxford University Press, 2008)

Metabolic measurement in *Drosophila melanogaster* is not as simple as it may appear. Numerous pitfalls await the unwary scientist entering the field of metabolic measurement in general, and for *Drosophila* measurements this holds true in spades. As a result, much of the published metabolic data in this field are problematic. The purpose of this white paper is to warn against prevalent misconceptions in the field of *Drosophila* metabolic rate (MR) measurement. I'll start each paragraph below with a statement, indicate whether the statement is true or false, and then explain the reasoning behind that judgment. I bring to bear the perspective of a research scientist who has spent much of his career making technically demanding gas exchange measurements on small insects – and who has seen more than one research program damaged by metabolic measurement misconceptions.

Manometric metabolic measurement techniques (Gilson, Warburg, etc.) work well and are accurate. **FALSE.** While these techniques, which measure O_2 consumption rates via pressure changes, are simple, intuitive and economical, they suffer from serious disadvantages. In order of significance, these are: (1) They give integrated measurements over a period of hours. Therefore it is impossible to avoid contaminating MR data with periods of elevated MR caused by bouts of voluntary activity. (2) They are intrinsically inaccurate for a variety of reasons, including inefficient CO_2 absorption by scrubber chemicals, and therefore give inconsistent results compared to more sophisticated techniques (Van Voorhies, pers. comm.). (3) They are intrinsically static, and cannot be used to determine the effects of dynamic variations in vitally important variables such as O_2 concentration (hypoxia/hyperoxia) or temperature. For example, Figure 1 shows the effect of a temperature ramp from 30 to 45°C (green line) on CO_2 emission by a single *D. melanogaster* (black line; baselines to either side). Note that the MR of the fly is measured in real time as the rate of CO_2 emission (VCO_2). Quite apart from the fact that temperature variations and their associated pressure changes would fatally compromise manometric measurements, data with this level of temporal detail are simply impossible to obtain manometrically. Indeed, workers who are used to using manometric techniques are typically unaware that such high temporal resolution is attainable, which unnecessarily limits the range of hypotheses they consider testable.

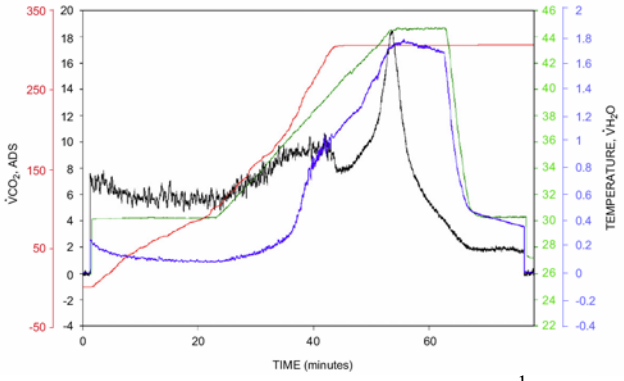

Figure 1. Real-time graph of VCO_2 (black; µl hr^{-1}), temperature (green; °C), water loss rate (blue; mg hr^{-1}) and activity (red) in a single *Drosophila melanogaster*. Source: Lighton, 2007.

Oxygen analysis is intrinsically "better" than CO_2 analysis. **FALSE.** This superstition stems from vertebrate physiology, where in some cases metabolic acidosis can falsely elevate CO_2 emission rates in poorly aerobic animals by shifting the CO_2-bicarbonate equilibrium towards CO_2. This phenomenon does not occur in *Drosophila*, even at the high MRs encountered in flight (e.g. Dickinson and Lighton, 1995). Because all CO_2 can be removed from an airstream prior to flowing it over a fly, the tiny increment in CO_2 caused by mitochondrial catabolism is relatively straightforward to measure with suitable equipment (Fig. 1). In the case of O_2 analysis, however, no analyzers capable of resolving individual *Drosophila* in real time exist; all O_2 analyzers must therefore use groups of flies for real-time measurements. This has two serious disadvantages. (1) Because it is impractical to transfer the 10 or more *D. melanogaster* that are required for a usable O_2 reading without using CO_2 anesthesia, the effects of such anesthesia on metabolic rate (which are pervasive and long-lasting; e.g. Nicolas and Sillans, 1989) will invalidate the subsequent measurements. (2) By definition, all information on inter-individual variability in MR is lost, leading to an impoverished understanding of this important parameter and to unacceptably weak statistical analyses. But perhaps the most im-

portant limitation of O_2 analysis is the fact that it simply cannot be used to study metabolic responses to altered oxygen concentrations, because the alterations in pO_2 overwhelm the metabolic signal from the flies, even if they are measured in groups. Even though metabolic responses to hypoxia, for example, are so important, it is an unfortunate fact that workers in the hypoxia field who use O_2 analyzers for measuring MR are methodologically crippled. Contrast this to Figure 2, which demonstrates the effect of sudden-onset hypoxia followed by normoxia on metabolic flux rates measured as VCO_2. The metabolic reactions to anoxia and reperfusion would be impossible to track with an O_2 analyzer, but can easily be recorded with a top-of-the-line CO_2-based respirometry system. With such systems, water loss rate of individual flies can also be tracked in real time, opening a valuable window into the status of the fly's neuromuscular control systems (Lighton and Schilman, 2007).

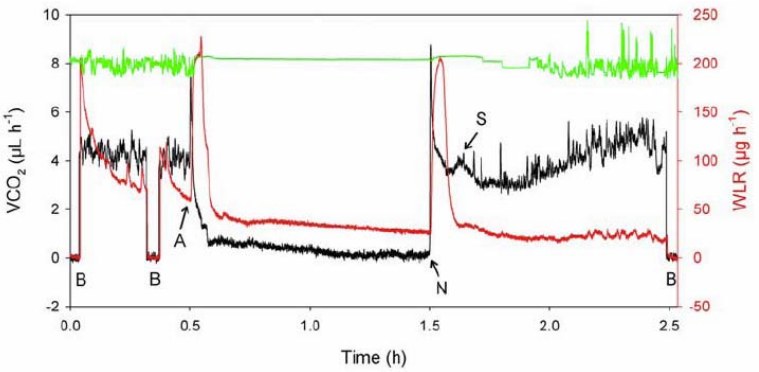

Figure 2. Real-time graph of VCO_2 (black), activity (green), and water loss rate (red) in a single *Drosophila melanogaster.* A =- initiation of anoxia; N = restoration to normal (20.95%) O_2 concentration. B = baselines. Source: Lighton and Schilman, 2007.

Second by second tracking of metabolic flux rates in individual flies is practically impossible. **FALSE.** The equipment required for real-time metabolic tracking is not cheap, but it is affordable for most laboratories. Since 1992, Sable Systems International has been supplying leading scientists around the world, including Nobel prize-winners, with systems capable of automatically measuring multiple flies in succession in real time. A large number of peer-reviewed papers in top journals cite these systems. They are the world standard in this consummately demanding field.

I can get acceptable results with a cheaper system. **TRUE and FALSE.** True in the sense that Sable Systems International can supply accurate systems that are inexpensive but non-manometric, and that are usable for many research applications, although they acquire integrative measurements over an hour or so and thus lack real-time capability, unlike the more expensive systems. False in the sense that cheap systems with poor quality analyzers, short warranties and inferior technical support from parties with no experience in insect respirometry do exist and are being aggressively marketed, and not surprisingly, they may yield unusable or, worse, misleading results. It is worth remembering that the cost of a respirometry system is miniscule against the overall costs of a research laboratory, which must publish reliable and replicable results in the peer-reviewed literature in order to maintain grant support – which is priceless.

Sometimes it is best to see the benefits of real-time metabolic measurement in, well, real time. For that reason, I am willing to personally demonstrate the technology free of charge to any interested researcher who is willing to make the trip to Sable Systems' headquarters in Las Vegas, Nevada. You can even acquire data for publication, or for inclusion in a grant application as seed data.

Thank you for your time. Feel free to contact me personally at john.lighton@unlv.edu or lighton@sablesys.com if you are interested in seeing real-time *Drosophila* MR measurement for yourself, or if you have any other questions regarding metabolic measurement in *Drosophila*.

Literature cited

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