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METHODS & TECHNIQUES

Measurement of oxygen consumption in *Tenebrio molitor* using a sensitive, inexpensive, sensor-based coulometric microrespirometer

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ABSTRACT

Coulometric respirometry is a highly sensitive method for measuring O_2 consumption in small organisms but it is not in widespread use among physiologists. Here, we describe a coulometric microrespirometer based on a digital environmental sensor inside a sealed glass chamber and controlled by an Arduino™ microcontroller. As O_2 is consumed, exhaled CO_2 is removed, causing pressure to decrease in the chamber. The sensor detects the decreased pressure, and the controller activates electrolytic production of O_2, returning pressure to the initial value. O_2 consumption is calculated from electrolytic charge transfer. The effects of developmental stage, body mass and temperature on O_2 consumption of *Tenebrio molitor* beetles were easily measured by the apparatus. This straightforward design is a significant innovation in that it provides continuous data regarding environmental conditions inside the experimental chamber, can be fabricated easily, and is adaptable to a wide range of uses.

KEY WORDS: Beetle, Electrolytic, Insect, Metabolism, Microcontroller, Respirometry

INTRODUCTION

Measuring metabolic rates provides essential quantitative insight into such diverse fields as climate change (Seebacher et al., 2015), food production (Bjøge et al., 2018), environmental quality (Villasenor et al., 2011) and human health (Rising et al., 1992). Metabolism can be measured directly from the heat produced by an organism, or indirectly from O_2 consumption or CO_2 production. If the assumptions of the methods are satisfied and the equipment is adequately sensitive, all of the above methods can accurately measure metabolism (Lighton, 2019). However, most available methods either require expensive and/or cumbersome apparatus (Hansen et al., 2004; Odell, 1998) or do not maintain constant recording conditions (Burggren et al., 2017).

Coulometric respirometry (also known as electrolytic respirometry) is a long-established method for measuring the rate of O_2 consumption ($\dot{V}_{O_2}$) in small organisms (Heusner et al., 1982; Hoegh-Guldberg and Manahan, 1995; Tartes et al., 1999; Werthessen, 1937). In coulometric respirometry, O_2 consumed by an organism is replaced by electrolytic O_2 production, with the amount of O_2 generated calculated from the charge used for electrolysis (Fig. 1A). In operation, a coulometric respirometer consists of an airtight chamber that contains CO_2-absorbent medium, so that pressure in the chamber drops as O_2 is consumed and CO_2 is produced (and removed). When pressure decreases below a threshold, current is applied across an electrolyte solution to produce O_2, thereby returning chamber pressure to the starting level. The charge used to generate the O_2 can be measured precisely, allowing calculation of the number of moles of O_2 (and therefore the volume) produced. This method is very sensitive and maintains stable O_2 and low CO_2.

Despite its advantages and long history, coulometric respirometry has not been adopted widely among physiologists, possibly because of technical challenges. Chamber pressure and current through the O_2 generator must be measured with high accuracy and precision, the chamber must be gastight, and temperature and pressure must remain highly stable throughout recording. Therefore, the construction of coulometric respirometers has been complex, using multiple chambers for pressure compensation, liquid-filled manometers as pressure sensors, and precisely calibrated capacitors to monitor charge transfer (e.g. Hoegh-Guldberg and Manahan, 1995; Tartes et al., 1999).

Miniature electronic sensors provide new resources for sensing and recording environmental variables. Here, we describe the design and construction of a coulometric respirometer based on a small, inexpensive Bosch BME 280 environmental sensor mounted inside a standard glass stopper that can be connected to a wide range of glass chambers. An Arduino™ microcontroller monitors the sensor, serves as a current source for the O_2 generator, and relays environmental and current data to a computer. The modular design greatly simplifies the construction and use of the respirometer and provides simultaneous data regarding pressure, temperature and humidity inside the chamber.

The microrespirometer was used to measure $\dot{V}_{O_2}$ in larvae and adults of the mealworm beetle *Tenebrio molitor*. $\dot{V}_{O_2}$ of the two stages could be distinguished from one another, and the values for both stages matched those published previously using independent methods. The device was also able to measure the effect of temperature on $\dot{V}_{O_2}$ of adult *T. molitor*. Because the equipment is inexpensive, portable, measures multiple environmental variables and can be assembled from inexpensive off-the-shelf components, it is a significant improvement over previous designs, and should lead to more widespread adoption of this versatile and sensitive technique.

MATERIALS AND METHODS

Experimental animals

Tenebrio molitor L. were obtained locally (Tropical Lagoon Aquarium, Silver Spring, MD, USA), and reared on rolled oats,

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Received 10 January 2022; Accepted 27 March 2022

Journal of Experimental Biology (2022) 225, jeb243966. doi:10.1242/jeb.243966

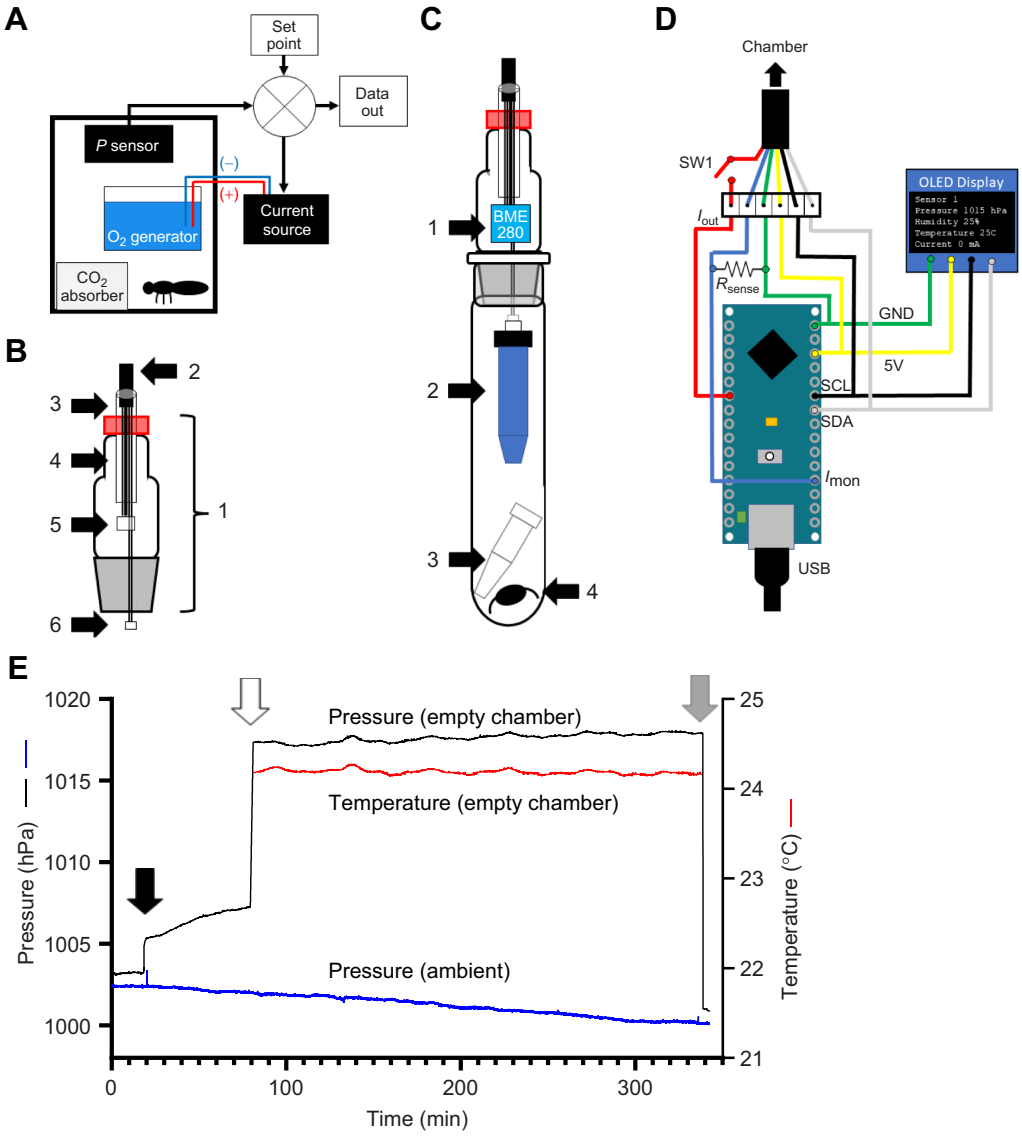

Fig. 1. Experimental apparatus and outputs. (A) The coulometric respirometer is a sealed chamber, with a CO_2-absorbent material (CO_2 absorber), an electrolytic O_2 generator and a pressure sensor (*P* sensor). Pressure data from the sensor is compared with the set point at the controller (circle with cross). O_2 is consumed and exhaled CO_2 is absorbed, causing pressure to decrease. When pressure drops below the ON threshold, the controller sends current across the anode (+) and cathode (–) of the O_2 generator until pressure reaches the OFF threshold. Data from the controller are sent to a computer. (B) Glass 19/22 stopper with thermometer adapter (1) that provides an airtight entry for wires into the chamber. A six-conductor cable (2) is fed into a 6 mm o.d., 5 mm i.d. glass tube (3) and sealed in place with epoxy (4). Inside the chamber, four of the wires connect a four-pin connector (5) for the BME 280 sensor, and a two-pin connector (6) for the O_2 generator. (C) The assembled chamber, showing the BME 280 sensor (1), the O_2 generator (2), the soda lime canister (3) and the experimental subject (4). (D) Connections of the respirometer controller, based on an Arduino Nano Every. Four wires of the six-conductor cable to the chamber were dedicated to I^2C control of the BME 280 sensor: 5 V power (5V, yellow), ground (GND, green), serial clock (SCL, black) and serial data (SDA, gray). The I^2C bus was connected in parallel to an OLED display on the controller. The O_2 generator was powered by a 5 V digital output of the Arduino (I_{out}, red), which could be gated manually by a toggle switch (SW1) on the controller. Current across the O_2 generator was measured across a 10 Ω sense resistor (R_{sense}) and monitored by one of the analog to digital converter (ADC) channels (I_{mon}, blue). The Arduino connected to a USB serial port of a computer. (E) Records from an experiment at 25°C, showing ambient pressure inside the incubator (blue line) and pressure in an unoccupied chamber serving as a control (black line). Pressure rises slightly when the chamber is sealed (black arrow), then increases steadily as humidity rises as a result of evaporation from the O_2 generator. Activation of the O_2 generator increases chamber pressure to the OFF threshold of 1017 hPa (white arrow). At the end of the experiment, the chamber is unsealed (gray arrow) and pressure returns to ambient. Note the small fluctuations in chamber pressure caused by small variations in temperature (red line), and the small negative change in barometric pressure during this experiment.

wheat germ and vegetable scraps at 22°C. In the absence of preliminary data regarding population variation, we used sample sizes from previous studies (Bjøge et al., 2018: n=6 groups of 2 larvae; Hansen et al., 2004: n=8; Odell, 1998: n=6) to establish a sample size of 12 animals per treatment. Beetles and larvae were removed from the culture, weighed, and placed immediately in the recording chamber. Adults weighed 96.2±2 mg (mean±s.e.m., n=61), with no difference between any of the experimental groups (one-way ANOVA, $F_{4,56}$=0.1526, P=0.961). Large (121±4 mg) late-instar larvae were selected for larval experiments.

Respirometer construction

Materials, sources, and costs of the major components of the coulometric microrespirometer are provided in Table S1.

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Recording chamber

The body of the chamber used for most experiments consisted of a glass tube 22 mm in outer diameter by 100 mm length, with a fitting for a 19/22 stopper (Precision Glassblowing, Centennial, CO, USA). Because the pressure changes are small, and the experiments require hours of recording, the chamber needed to be gastight. Plastics are permeable to O_2 at a magnitude that would confound measurement of $\dot{V}_{O_2}$ of a single insect (Norton, 1957). Glass is impermeable to O_2, and its transparency allows visual monitoring of the subject.

Chambers were sealed with 19/22 glass stoppers with fittings for 6–7 mm thermometers (Synthware A521922; Fisher Scientific; Fig. 1B), providing a path for wires into the chamber. Six-conductor cable was sealed with epoxy (Loctite E-30CL, Henkel Corporation, Rocky Hill, CT, USA) into a short (∼5 cm) section of 6 mm o.d., 5 mm i.d. borosilicate glass tubing (Fisher Scientific), which formed an airtight seal with the thermometer fitting. Inside the stopper, four of the wires terminated in a four-pin 2.54 mm pitch socket into which the sensor (Bosch BME 280, DIYmall, Guangdong, China) was connected via a right-angle header.

The BME 280 provided data regarding temperature (accuracy 0.5°C; resolution ±0.01°C), pressure (accuracy ±1.0 hPa; resolution 0.18 hPa), and humidity (accuracy 3% relative humidity, RH; resolution 0.008% RH). When acquiring all three channels with 1× oversampling, response time is <8 ms.

The remaining two conductors extended past the sensor, ending in a two-pin socket to connect with the O_2 generator tube. The anode and cathode of the generator consisted of Pt and Cu wires, respectively, soldered into a two-pin connector (Fig. 1C). The wires were secured with epoxy into holes in the cap of a 2 ml screw-cap centrifuge tube, drilled to allow the escape of O_2. The generator tube was filled with saturated $CuSO_4$ solution, capped, and the pins from the generator connected to the sockets from the cable. Voltage from the controller (see below) across the O_2 generator caused liberation of O_2 at the anode based on the following reaction:

$$2CuSO_4 + 2H_2O \leftrightarrow 2H_2SO_4 + 2Cu + 2O_2. \quad (1)$$

One molecule of O_2 was liberated at the Pt anode per four electrons, metallic Cu was deposited on the Cu cathode, and H_2SO_4 remained in solution. The gas produced in this way has been shown to be 100% O_2 (Hoegh-Guldberg and Manahan, 1995).

The wires exiting the chamber terminated in a waterproof six-pin circular connector (Switchcraft Inc., Chicago, IL, USA), which connected to the controller via a six-conductor cable.

Controller

The controller was based on an Arduino Nano Every (Fig. 1D; Arduino LLC, Wilmington, DE, USA), housed in a plastic relay box (Bud Industries, Willoughby, OH, USA). The Arduino was programmed to read the pressure, temperature and humidity outputs of the BME 280 via an I^2C bus, which also controlled an OLED display in the controller box (code is provided in Supplementary Materials and Methods).

The Arduino was programmed to maintain pressure between 1015 and 1017 hPa, slightly above the ambient barometric pressure (985–1015 hPa). A 5 V output was triggered when pressure dropped below a pre-set value (ON threshold 1015 hPa) and was turned off when pressure reached a second pre-set value (OFF threshold, 1017 hPa). Thus, at the beginning of the experiment, the O_2 generator was activated to pressurize the chamber to 1017 hPa (Fig. 1E). When enough O_2 was consumed by the beetle to decrease pressure below the ON threshold, the Arduino provided current to the O_2 generator and pressure increased until it reached the OFF threshold, thus maintaining pressure and P_{O_2} within a 2 hPa range. Pressure was maintained at a slightly hyperbaric level (≤20 hPa above ambient) so that the same setpoints could be used across experiments, and to facilitate detection of leaks. Current output to the O_2 generator was gated by a toggle switch on the controller to prevent premature activation of the O_2 generator, and depletion of $CuSO_4$, during setup.

The current through the O_2 generator was monitored by one of the analog to digital converters (ADCs) built into the Arduino, across a 10 Ω sense resistor (R_{sense}). The value of R_{sense} determines the voltage signal to the ADC, so accurate determination of R_{sense} is critical for accurate measurement of current through the generator. Current through the $CuSO_4$ solution was determined by the voltage across the two electrodes and the sum of the resistance of the $CuSO_4$ solution (∼50 Ω) and R_{sense}. The combined resistance across the generator and sense resistor was constrained by the requirement for a minimum 2 V potential to sustain electrolysis.

The Arduino sent comma-delimited data (identity of the sensor, time in ms, chamber pressure, chamber temperature, chamber humidity, and current through the O_2 generator) to a serial port of the computer every 500 ms. The same information was displayed on an OLED display in the controller for quick reference. Input from multiple controllers was routed to the computer via a USB hub, and data were logged using puTTY (putty.org).

Experimental design

Most experiments were carried out under constant temperature conditions in a biological incubator (Percival 130VL, Perry, IA, USA). Fresh soda lime pellets were placed in a small, perforated centrifuge tube in the bottom of the respirometer chamber to absorb exhaled CO_2, *T. molitor* were weighed and added, and the O_2 generator was connected. Seals were thoroughly cleaned before each experiment, silicone grease (Dow Corning) was applied, and stoppers were inserted into the chambers. Chambers were placed into the incubator, allowed to equilibrate for 15 min with the thermometer port unsealed, and then sealed for another hour to allow the temperature and humidity in the chamber to stabilize (Fig. 1E). At this point, O_2 generators were switched on and the chambers were pressurized until they reached the OFF threshold, initiating the experiment. In a few cases, a chamber failed to pressurize because of a damaged seal, and was removed from the experiment. As described previously (Hoegh-Guldberg and Manahan, 1995), maintaining stable temperature was critical to the function of the apparatus, as even small variations in temperature (<0.15°C) resulted in detectable fluctuations in the pressure record (Fig. 1E). After 4–5 h of recording, O_2 generators were turned off, chambers were unsealed, and recording ceased.

To examine the effects of alternative configurations, larvae were tested in groups of five in a chamber consisting of a 50 ml, two-neck, round-bottom flask, with each neck having a 19/22 joint (total chamber volume ∼100 ml). For these chambers, the sensor was contained in a stopper similar to that used above, but with the incoming wires sealed into the stopper with epoxy. Pressure in the chamber was equilibrated using a stopcock in the second neck of the flask. For these experiments, temperature was controlled using a circulating water bath/chiller (Amersham Biosciences Multitemp III), with the flasks secured by ring stands and clamps in an acrylic trough (Glass Cages, Dickson, TN, USA). The tubes containing soda lime and the O_2 generator were the same as those used above.

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Analysis

O_2 consumption was measured from the first pulse triggered by the ON threshold after pressurization, to the beginning of the final current pulse (Fig. 2A, arrow). In this way, only pulses for which the O_2 was completely consumed by the beetle were included in the analysis. Current pulses were detected manually in Excel (Microsoft), and charge (Q) was calculated as the product of the mean amplitude of a current pulse and its duration. The total charge transfer for a given beetle was calculated as the sum of all pulses. The volume of O_2 generated was calculated from the equation:

$$V_{O_2} = Q\,V_m/4F, \quad (2)$$

where Q is the charge transfer measured as described above, V_m is the molar volume of O_2, which was calculated for each temperature using the ideal gas equation ($PV=nRT$), and F is the Faraday constant (96,485 C mol^{-1}; Heusner et al., 1982). O_2 consumption rate ($\dot{V}_{O_2}$) was calculated by dividing the volume of O_2 consumed by the recording time.

The following values were used to calculate the expected pressure change from a single pulse from the O_2 generator using the ideal gas equation: n (number of moles of O_2 per pulse)=$Q/4F$; V (chamber volume)=0.0318 l, measured in multiple chambers by subtracting the mass of an empty chamber from one filled with water; T=298 K; and R (gas constant)=0.08205 l atm K^{-1} mol^{-1}.

The temperature coefficient, Q_{10} was derived from the equation:

$$Q_{10} = \left(\frac{\dot{V}_{O_2,b}}{\dot{V}_{O_2,a}}\right)^{10/(T_b-T_a)}, \quad (3)$$

where $\dot{V}_{O_2,a}$ and $\dot{V}_{O_2,b}$ are the O_2 consumption rates at temperatures T_a and T_b, respectively. Q_{10} was calculated by plotting log-transformed $\dot{V}_{O_2}$ versus temperature, multiplying the slope of the linear regression by 10 and taking the antilog of the result (Lighton, 2019).

Data plots, regression analyses and statistics were performed in Graphpad Prism (Graphpad Software, San Diego, CA, USA). Regression lines were tested for significant difference from zero using F-tests. In all cases, data fitted the assumptions of parametric statistics using Bartlett's test, so differences between groups were tested using one-way ANOVA followed by Tukey's test for multiple comparisons. Data are reported in the text as means±s.e.m.

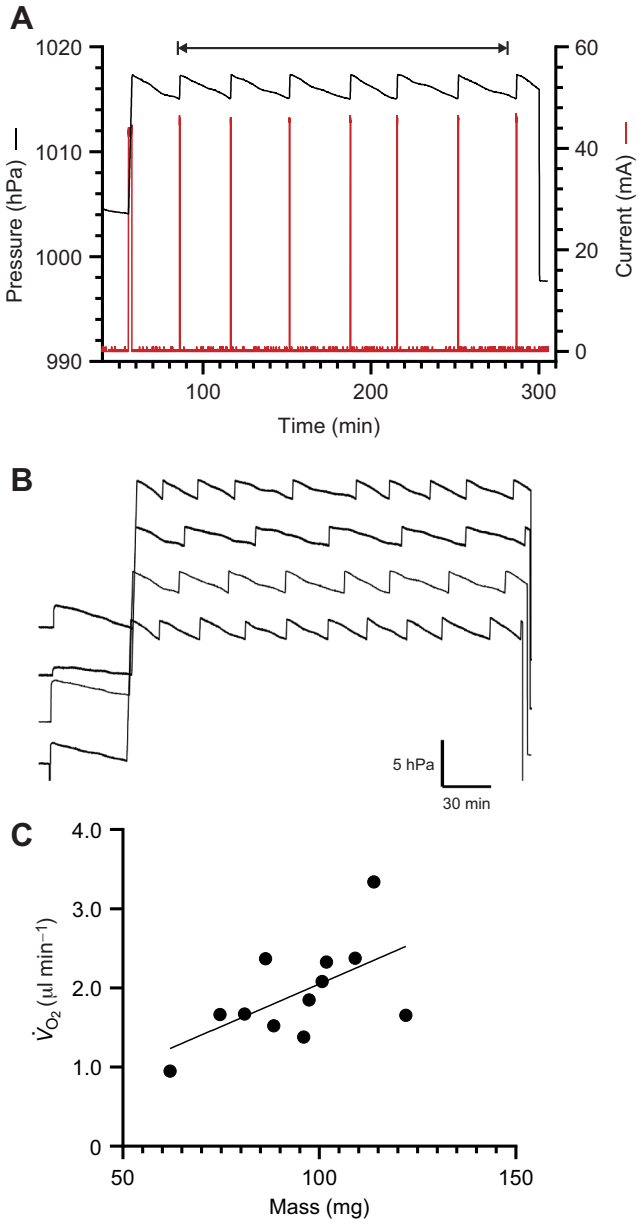

Fig. 2. Respirometer in operation. (A) Pressure (black) and current (red) records from a chamber containing a single beetle at 25°C. The first, longer, current pulse pressurizes the chamber from an ambient pressure of ~1004 hPa to the OFF threshold of 1017 hPa. As the beetle consumes O_2, pressure decreases slowly to the ON threshold of 1015 hPa, activating the O_2 generator and returning pressure to 1017 hPa, resulting in cyclic pressure fluctuations. The horizontal arrow indicates the time for which charge transfer was calculated. (B) Simultaneous records from four chambers at 25°C. Note the variation between beetles. Traces were offset by 5 hPa for clarity. (C) Correlation between live mass and O_2 consumption ($\dot{V}_{O_2}$) for adult *Tenebrio molitor* at 25°C. Linear regression showed a modest but statistically significant effect of mass on $\dot{V}_{O_2}$ (y=0.0216x–0.103; r^2=0.351; $F_{1,10}$=5.404, P<0.05; n=12).

RESULTS AND DISCUSSION

Once assembled, equilibrated and sealed, chambers were pressurized to 1017 hPa using the O_2 generators. Respiration of a single adult *T. molitor* at 25°C caused a steady, slow decrease in pressure until it reached the ON threshold (1015 hPa), when the O_2 generator was activated and pressurized the chamber to the OFF threshold (1017 hPa). Experiments lasted 4–5 h, and this cycle of slow decrease and rapid return produced a sawtooth pattern (Fig. 2A). Current through the generator ranged from 45 to 55 mA, depending on the relative positions of the electrodes, and each pulse lasted between 17 and 22 s. Because the generator produced pure O_2, and pressure remained above ambient at all times, chambers remained slightly hyperoxic for the entire experiment. Although there were small baseline fluctuations due to slight temperature variations, pressure in a control chamber that contained no beetle was stable for the duration of all experiments (Fig. 1E).

To assess the accuracy of coulometric measurement, we used the ideal gas law to calculate the expected pressure change due to hydrolysis of $CuSO_4$ (see Materials and Methods) and compared it with the measured change in pressure during the cycling of the respirometer. In one experiment using five chambers at 25°C, each current pulse produced 0.953±0.07 C (n=39 pulses), which should produce enough O_2 to increase pressure by $1.90\times10^{-3}\pm1.41\times10^{-4}$ atm per pulse. The measured pressure change caused by each pulse was 2 hPa (=1017 hPa−1015 hPa), or 1.97×10^{-3} atm. Therefore, the value predicted from charge

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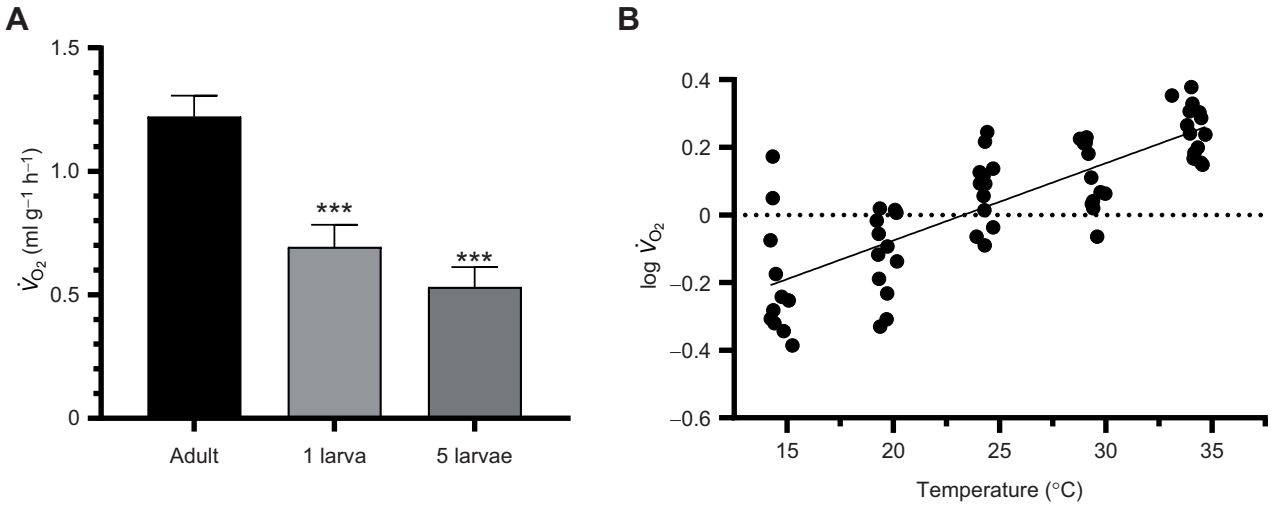

Fig. 3. Effect of developmental stage and temperature on $\dot{V}_{O_2}$ in *T. molitor*. (A) Mass-specific $\dot{V}_{O_2}$ at 25°C for adult beetles (n=12), single larvae recorded in the same apparatus (n=13) and larvae recorded in groups of five in a larger chamber regulated by a water bath (n=5 groups). Data are means±s.e.m. Larval $\dot{V}_{O_2}$ differed significantly from that of adults (one-way ANOVA with Tukey *post hoc* test $F_{2,27}$=14.24, P<0.001), but the two groups of larvae did not differ from one another (P=0.553). (B) Log-transformed $\dot{V}_{O_2}$ versus temperature, shown with the linear regression (y=0.0228x–0.533; r^2=0.639; $F_{1,59}$=104.4, P<0.0001) used to calculate Q_{10} as described in Materials and Methods. Each datapoint represents calculated $\dot{V}_{O_2}$ and the recorded temperature for a single beetle (sample size: 15°C, n=11; 20, 25 and 30°C, n=12; 35°C, n=14).

transfer was within 4% of the measured pressure change, showing that current across the O_2 generator accurately reflected O_2 production.

Fig. 2B shows an example of data recorded from four chambers, each containing one beetle, simultaneously. Because computers can accommodate many universal serial bus (USB) ports, the number of channels that can be recorded at any given time is limited only by the available hardware, such as chambers, controllers and physical USB connections. Fig. 2B also illustrates the variation between individuals, which results from differences in size (see below), activity level, age, feeding state, hydration state or a combination of these.

Average O_2 consumption rate ($\dot{V}_{O_2}$) for adult beetles at 25°C was 1.93±0.17 µl min^{-1} (=0.077±0.007 µmol min^{-1} or 48.4±3.4 µmol h^{-1}; Table S2). There was a modest but significant effect of body mass on $\dot{V}_{O_2}$ (r^2=0.351; $F_{1,10}$=5.404, P<0.05; Fig. 2C). To facilitate the following comparisons with larval data and previous studies, data were normalized to body mass and are presented as mass-specific $\dot{V}_{O_2}$ in ml O_2 g^{-1} h^{-1}.

At 25°C, $\dot{V}_{O_2}$ of adult *T. molitor* was 1.185±0.084 ml O_2 h^{-1} g^{-1} (n=12; Fig. 3A). At the same temperature, larval $\dot{V}_{O_2}$ was lower, whether recorded singly in the same apparatus as adults (0.693 ±0.086 ml O_2 g^{-1} h^{-1}; n=13) or in groups of five using larger chambers in a water bath (0.531±0.081 ml O_2 g^{-1} h^{-1}; n=5 groups). The difference between adults and both groups of larvae was significant (one-way ANOVA with Tukey *post hoc* test $F_{2,27}$=14.24, P<0.001), whereas the two groups of larvae did not differ significantly (P=0.553).

$\dot{V}_{O_2}$ recorded by the coulometric respirometer was within the range described previously using different methods. For example, using direct calorimetry, Acar et al. (2004) reported heat production of approximately 5.5 µJ mg^{-1} s^{-1} (estimated from data from Acar et al., 2004) for adult beetle *Harmonia* at 25°C. A standard conversion factor of 20.2 J ml O_2^{-1} renders a value of ∼0.9 ml O_2 g^{-1} h^{-1}, essentially indistinguishable from the value determined in the present study for adult *T. molitor* (1.185±0.084 ml O_2 g^{-1} h^{-1}). Data from larvae in the present study (0.531–0.693 ml O_2 g^{-1} h^{-1}) are similar to those reported by Bjøge et al. (2018) (∼0.4±0.2 ml O_2 g^{-1} h^{-1}) for larval *T. molitor* using stop-flow respirometry. Therefore, the apparatus can measure $\dot{V}_{O_2}$ of individual *T. molitor*, the apparatus can distinguish the difference in $\dot{V}_{O_2}$ between two life stages, and the metabolic rates are consistent with those described previously.

As expected for ectotherms, $\dot{V}_{O_2}$ of *T. molitor* increased with temperature (Fig. 3B). There was a significant effect of temperature on $\dot{V}_{O_2}$ (one-way ANOVA, $F_{4,56}$=26.88, P<0.0001). *Post hoc* Tukey tests (Table S2) showed that $\dot{V}_{O_2}$ was significantly higher at 25 and 30°C than at 15 or 20°C (P<0.01), and $\dot{V}_{O_2}$ at 35°C was higher than that at 25 or 30°C (P<0.001). The temperature coefficient, Q_{10}, calculated from the slope of log-transformed $\dot{V}_{O_2}$ versus temperature, was 1.69 (Fig. 3B). This value falls within the broad range of acute thermal responses reported for arthropods (Seebacher et al., 2015), and demonstrates that the microrespirometer can measure changes in metabolism due to biologically meaningful manipulations.

In conclusion, we have fabricated and demonstrated the efficacy of a simple, inexpensive coulometric respirometer. With the exception of the custom glass chamber (which can be replaced by a commercially available flask), the entire apparatus can be assembled with simple tools from standard parts stocked by scientific supply companies and/or online retailers. In the present study, the lowest $\dot{V}_{O_2}$ of 1.1 µl min^{-1} was easily detectable (Fig. 3B; Table S2), and sensitivity can be increased by using a smaller chamber or longer recording times.

The design is modular and versatile. $\dot{V}_{O_2}$ can be quantified solely by measuring current through the O_2 generator, so knowledge of the precise volume of the chamber is not needed. Therefore, the respirometer can be used with any airtight chamber that is suitable for the organism and has a standard glass joint that can accommodate the sensor assembly (Fig. 1B). Data from multiple controllers can be acquired at once, with each controller recording pressure, temperature, humidity and current simultaneously.

As shown here, temperature can be controlled by a biological incubator or water bath. With minor modifications, such as adding a secure digital (SD) memory card to the controller box to serve as a data logger, the whole system can be battery powered and left

in the field for as long as adequate $CuSO_4$ remains in the O_2 generator. Based on the $\dot{V}_{O_2}$ of *T. molitor* beetles at 25°C (1.77×10^{-3} ml min^{-1}), 1 ml of saturated (1.27 mol l^{-1}) $CuSO_4$ will produce more than 28 ml O_2 which should last for at least 11 days. Once the chamber is pressurized, P_{O_2} remains slightly hyperoxic and varies by less than 0.002 atm during cycling of the O_2 generator. Therefore, experimental subjects the size of *T. molitor* can be maintained in the chamber for days at a time without concern about stress from hypoxia.

Given its sensitivity, versatility, ease of construction and the quality of data generated, the design presented here is a significant improvement upon previous designs and has the potential for broad application in the study of metabolism.

Acknowledgements
We thank the Universities at Shady Grove for the use of laboratory space and the environmental chamber, and students of the 2020–2021 USG Invertebrate Physiology Research Seminar for inspiration, help with the early stages of this work and some larval recordings.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: D.J.S.; Methodology: D.J.S., B.W.O.; Software: D.J.S., B.W.O.; Validation: D.J.S., B.W.O.; Formal analysis: D.J.S.; Investigation: D.J.S.; Resources: D.J.S., B.W.O.; Data curation: D.J.S., B.W.O.; Writing - original draft: D.J.S.; Writing - review & editing: D.J.S., B.W.O.; Visualization: D.J.S.; Supervision: D.J.S.; Project administration: D.J.S.; Funding acquisition: D.J.S.

Funding
This work was supported by a Department Reinvestment Initiative Fund from the Biology Department at the University of Maryland, College Park.

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