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The use of tunable diode laser absorption spectroscopy for rapid measurements of the $\delta^{13}C$ of animal breath for physiological and ecological studies

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In this study we introduce the use of tunable diode laser absorption spectroscopy (TDLAS) as a technique for making measurements of the $\delta^{13}C$ of animal 'breath' in near real time. The carbon isotope ratios ($\delta^{13}C$) of breath CO_2 trace the carbon source of the materials being metabolized, which can provide insight into the use of specific food resources, e.g. those derived from plants using C_3 versus C_4 or CAM photosynthetic pathways. For physiological studies, labeled substrates and breath analyses provide direct evidence of specific physiological (e.g. fermentative digestion) or enzymatic (e.g. sucrase activity) processes. Although potentially very informative, this approach has rarely been taken in animal physiological or ecological research. In this study we quantify the utilization of different plant resources (photosynthetic types – C_3 or C_4) in arthropod herbivores by measuring the $\delta^{13}C$ of their 'breath' and comparing it with bulk tissue values. We show that breath $\delta^{13}C$ values are highly correlated with bulk tissues and for insect herbivores reflect their dietary guild, in our case C_3-specialists, C_4-specialists, or generalists. TDLAS has a number of advantages that will make it an important tool for physiologists, ecologists and behaviorists: it is non-invasive, fast, very sensitive, accurate, works on animals of a wide range of body sizes, per-sample costs are small, and it is potentially field-deployable. Copyright © 2009 John Wiley & Sons, Ltd.

In this study we introduce the use of tunable diode laser absorption spectroscopy (TDLAS) as a technique for making measurements of $\delta^{13}C$ of animal 'breath' in near real time. The measurement of carbon isotope ratios ($\delta^{13}C$) of breath CO_2 provides insight into the carbon source of the materials being metabolized, which, in turn, can inform us about the use of specific food resources. 'Breath tests' thus offer a significant potential for studying animal physiology, behavior and ecology. For physiological studies, for example, labels can be administered or fed to animals that allow for measurements of metabolic substrate use (e.g. lipids versus carbohydrates) or that assess digestive function (e.g. fermentative or non-fermentative). To date, this approach has been used on a limited basis to determine fuel and resource use of birds and bats.[1–6] Measurements of breath $\delta^{13}C$ of free-living animals can provide quantitative insight into resource use, e.g. the consumption of plants utilizing different photosynthetic pathways, C_3, C_4, or CAM (e.g. Wolf and Martínez del Rio[7]). Breath tests have an advantage over isotopic analysis of body tissues because they can provide information about the most recently consumed diet as well as the integrated diet over longer time periods, depending on the nutritional status of the subject.[2,8,9] Because they are non-destructive, breath tests can be repeated on the same individual and allow studies on rare or endangered species.

Although the idea of using this approach in animal studies has been of interest for some time, technological constraints have limited its use. Until recently, measurements of breath $\delta^{13}C$ from the field required capturing breath CO_2, storing samples in evacuated containers, and subsequently measuring $\delta^{13}C$ on a gas bench using isotope ratio mass spectrometry (IRMS). Several emerging technologies now make breath sampling a faster and more effective method for assaying resource use of animals. The cost of a TDLAS instrument is approximately one-half that of an IRMS instrument and per-sample costs are minimal (<$0.01 US, at least three orders of magnitude lower than the sample costs at current rates using IRMS). In addition, these instruments operate under modest vacuums and can be started up and shut down in minutes. They also can potentially be operated in the field with limited logistical support. TDLAS has recently been embraced by ecosystem ecologists and plant physiologists[10,11] but the potential power of this approach has so far been ignored by zoologists. Here, we demonstrate how an instrument (TGA100,

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Campbell Scientific Inc.) designed for measurements of atmospheric CO_2 and trace gas analysis[12–14] can be used for rapid, accurate and near real-time 'breath tests' on a community of grasshoppers (Orthoptera).

The Sevilleta LTER in central New Mexico (USA), part of the National Science Foundation's Long-Term Ecological Research Network, is home to approximately 50 species of grasshoppers and they are considered the dominant herbivores in this ecosystem. This Chihuahuan Desert ecosystem is comprised of a plant community that primarily uses two distinct photosynthetic pathways (C_3 and C_4) and, because C_3 and C_4 plant tissues differ greatly in their carbon isotope ratios, these resources leave a specific isotopic imprint in the tissues and 'breath' CO_2 of consumers as they are metabolized.[15] 'Breath tests' thus provide a direct link between producers and consumers and thereby allow quick identification of dietary specialists and generalists and their individual reliance on differing plant photosynthetic types.

EXPERIMENTAL

We captured grasshoppers by hand (184 individuals of 15 species) at the Sevilleta LTER in central New Mexico between September 18 and November 1, 2006. Body mass ranged from 24 mg to 9.2 g (wet mass). The animals were transported to Los Alamos National Laboratory (Los Alamos, NM, USA) for isotopic breath analysis the day after capture, and were subsequently frozen at $-18°C$ for whole-body tissue isotopic analysis. For logistical reasons, TDLAS measurements always took place the day following capture. Grasshoppers thus were fasted for the 24 h preceding measurements and, based on their body size and temperature, we assume that they were post-absorptive.[16] To determine the effect of this fasting period, we subjected a group of 100 *Trimerotropis pallidipenis* to a controlled feeding and fasting experiment. We chose this species for its abundance and tolerance of captivity that allowed us to obtain good sample sizes. The animals were kept at temperatures comparable with what they had experienced previously in the field and fed a diet consisting of romaine lettuce, oats, and apples. These plants utilize C_3 photosynthesis and therefore have an isotopic signature of about $-26‰$.[17,18] After 2 weeks on this diet, the isotopic composition of 'breath' was analyzed from animals freshly fed, or fasted for varying time periods (20 animals in each group, fasting for 2 h, 6 h, 1 day, or 3 days).

The carbon isotope ratios in expired CO_2 were determined by TDLAS, a method to determine the concentration (in parts-per-billion) of target gases using infrared (IR) absorption. A diode laser is tuned to a particular narrow emission band by controlling the temperature and current applied to the laser. The wave number of the laser is selected to match an individual absorption line of a specific target molecule (CO_2 in this case) and the laser emission is repeatedly scanned across the entire width of the absorption spectrum of the molecule. The line width of the laser emission is typically much smaller than the molecular absorption line width and this allows the instrument to be very selective among components of a gas mixture. This is particularly true at low pressure where the absorption lines are narrower. The IR

radiation emitted by the laser traverses a sample cell containing the target gas before focusing on a detector. The sensitivity of the analyzer is dependent on the absorption strength of the line chosen and on the absorption path length.

The TDLAS system used in this study was a TGA100 trace gas analyzer (Campbell Scientific Inc., Logan, UT, USA) that uses a tunable lead-salt diode laser, cooled by liquid nitrogen. The laser is simultaneously temperature and current controlled to produce linear wavelength scans centered on selected absorption lines of the target gases. IR radiation produced by the laser is collimated and passed through a 1.5 m long sample cell. The pressure in the sample cell was kept low (<19 mbar) to keep individual absorption line widths narrow and to minimize interference from other gas components. A beam splitter directs most of the energy through a focusing lens to the sample detector and reflects a portion of the beam through a second focusing lens and a reference cell to the reference detector. A prepared reference gas of known CO_2 concentration flows through the reference cell. The sample concentration of the target gas is inferred by comparing the sample absorbance with the reference absorbance. The reference signal is used as a template for the shape of the absorption spectrum and allows the sample concentration to be determined without measuring the temperature or pressure of the sample gas or the spectral positions of the scan samples. The reference signal also provides feedback for an internal control algorithm that locks the center of the spectral scan at the center of the desired absorption line. The detector signals are amplified and converted in the analyzer electronics and then digitally processed to calculate the concentration of the target gas in the sample cell. The space between the laser and the front of the sample cell, as well as the sample detector housing, was purged with nitrogen gas to avoid absorption by CO_2 from ambient air.

IR absorbance is proportional to molecular density but also dependent on temperature. It is, therefore, important to maintain the reference and sample gases at the same temperature. The TDL is housed in an insulated fiberglass case with a thermostatically controlled heater and two fans that circulate the air inside the housing to maintain a constant temperature. An insulated cover was placed over the analyzer enclosure. The reference and sample gases each flowed though a length of tubing inside the enclosure to bring them to the same temperature before they entered the absorption cells. In this study, the dual isotopologue mode of the TGA100 was used. The stable isotopologues $^{12}CO_2$ and $^{13}CO_2$ have unique absorption lines that can be selected and measured using a jump-scan technique.[19] The reference gas used for these measurements contained approximately 10% $^{12}CO_2$ and 0.1% $^{13}CO_2$ and was maintained at a flow rate of 10 mL min^{-1} through the reference cell. In the dual isotopologue mode, the spectral scan wavelength alternates 500 times a second between two adjacent absorption lines, in our case one for $^{13}CO_2$ and the other for $^{12}CO_2$, that are then averaged separately and give nearly simultaneous mole fraction measurements for each isotopologue. We recalculated the values as isotope ratios in delta notation, relative to the Vienna Pee Dee Belemnite (VPDB) standard. The carbon

isotope ratio of sample gas CO_2 ($\delta^{13}C$) is then $\delta^{13}C = R_s/R_{VPDB} - 1$, where R_s and R_{VPDB} are the $^{13}C/^{12}C$ ratios of the sample and the VPDB standard. $\delta^{13}C$ is reported in parts per thousand (‰) VPDB.

All measurements were calibrated against two primary calibration cylinders with total CO_2 concentrations ($[CO_2]_t$) of 350.275 and 541.591 ppm, and $\delta^{13}C$ of -8.44‰ VPDB and -16.16‰ VPDB, respectively (measured by National Oceanic and Atmospheric Administration, Earth System Research Laboratory, Silver Spring, MD, USA, for CO_2 and the Institute for Arctic and Alpine Research, Boulder, CO, USA, for $\delta^{13}C$). The primary calibration cylinders were used to calibrate two working standards with concentrations of the two isotopologues of $[^{12}CO_2] = 359.170$, $[^{13}CO_2] = 3.904$, and $[^{12}CO_2] = 560.025$, $[^{13}CO_2] = 6.089$ ppm. The working standards were measured for 1 min each out of every 8 min during grasshopper breath measurements. A two-point gain (G) and offset (O) correction, specific to each isotopologue, was performed for each calibration cycle (see Bowling et al.[12] for a discussion of correction methods). For $\delta^{13}C$ of grasshopper breath we took 5-s averages during which time CO_2 concentration within the optical cell was between 350 and 550 ppm, i.e. within the calibration range of the cylinders. We note here that by using calibration gases with higher or lower concentrations, an extended measurement range (150 to several thousand ppm) can be achieved. Animals were placed in an airtight chamber (modified BD 60-mL plastic syringe) of variable volume (15 to 60 mL) that was flushed with dry CO_2-free medical air at 100 mL min^{-1}. The sample system was plumbed with ¼″ Bev-A-Line^{TM} tubing and a bypass line supplied dry CO_2-free air to the instrument during the periods when animals were off-line. Four animal chambers were arranged in parallel with the bypass line in order to increase sampling rates. Sample chamber selection was controlled manually by two sets of ball valves. Only a single chamber or the bypass line was open to the instrument during any period. Sample and bypass air were pulled by the internal pump of the instrument and passed through a low-flow Nafion counterflow water trap (PD625 dual configurations, Campbell Scientific Inc.), before entering the sample cell of the instrument at a flow rate of approximately 100 mL min^{-1}, and was controlled by a critical flow orifice. The pressure within the sample cell was typically 22.10 ± 0.04 mbar during measurement of the standards, and 22.11 ± 0.04 mbar during measurement of the sample and reference lines.

The animal chambers were initially flushed with dry CO_2-free air and exhausted to the room after animals had been introduced into the chamber so that atmospheric CO_2 did not contaminate breath measurements. The rates of CO_2 production at room temperature (approximately 25°C) of larger animals (body mass > 2 g) were sufficient to allow direct measurements using this flow-through setup. For smaller animals, we initially flushed the chamber with dry CO_2-free air and then sealed it for a period of 1 to 5 min, allowing CO_2 concentrations in the chamber to increase to within the calibrated range of the instrument.

Because the instrument is designed for continuous flow-through measurements of relatively stable CO_2 concentrations, we also tested the measurement accuracy when the range of CO_2 concentrations was rapidly changing and large (400–600 ppm) as might be expected during measurements of animal breath. We injected 15 to 60 mL of calibration gases with CO_2 concentrations of 350, 550 and 750 ppm into dry, CO_2-free carrier gas (air) to produce a pulse of gas similar to that produced by live animals. For all analyses, we use only data collected when CO_2 concentrations were within the calibrated range of 350 to 550 ppm.

We determined the relative contribution of C_4-derived resources to the diet of our study species based on the isotopic composition of its breath with a simple two-endpoint mixing model: $\delta13C_{breath} = p(\delta_1 + \Delta) + (1 - p)(\delta_2 + \Delta)$, where p is the relative contribution of C_4 plants to the diet, δ_1 and δ_2 are the $\delta^{13}C$ values of the two endpoints, C_3 or C_4 plants, and Δ is a diet-to-breath discrimination factor. For the two endpoints of this model we used the values $\delta^{13}C_{C4} = -13.9$‰ and $\delta^{13}C_{C3} = -27.2$‰ (see Results section). Incorporation of dietary isotopes into a consumer's tissue and eventually breath is rarely perfect, and the difference between the isotopic composition of resources and a consumer's breath is the 'discrimination factor' ($\Delta = \delta^{13}C_{breath} - \delta^{13}C_{resource}$). In our model we use a community-wide value of -1‰ for $\Delta^{13}C_{breath}$. Based on the average $\delta^{13}C_{breath}$ values, we classified species as C_3-specialists or C_4-specialists when their diet consisted to > 90% of either C_3 or C_4 plants. These cut-off values correspond to $\delta^{13}C_{breath} < -26.9$‰, respectively, $\delta^{13}C_{breath} > -16.3$‰. All other species were considered generalists.

In addition to the $\delta^{13}C$ of expired CO_2, we also determined the whole-body $\delta^{13}C$ of the animals from a homogenized sample of dry body tissue at the Stable Isotope Laboratory of the University of New Mexico by continuous flow elemental analyzer/isotope ratio mass spectrometry (EA-IRMS) using a Costech ECS 4010 elemental analyzer (Costech International S.p.A., Cernusco, Italy) coupled to a Thermo-Finnigan Delta Plus mass spectrometer (Finnigan MAT GmbH, Bremen, Germany). Laboratory standards (valine $\delta^{13}C = -26.3$‰ VPDB) were included in each run to correct the raw values obtained from the mass spectrometer. The precision of these isotope analyses is approximately ± 0.1‰ standard deviation (SD) based on long-term variation in the valine standard. All values are presented as mean ± 1 SD.

To identify the food resources available to consumers during the measurement period, we used estimates of net primary production (NPP) from sampling sites within 2 km of our study area on the Sevilleta.[20] All NPP data and methodology are available in the Sevilleta archival dataset SEV156.[21] The study was designed to monitor the effects of prescribed burning on grasslands; for our purpose we used only the data of the unburned control plots. We also collected tissues from 59 plant species on our study site to identify the $\delta^{13}C$ values of the local vegetation. Carbon isotope ratios of the plant samples were also determined by EA-IRMS. Plants comprised two photosynthetic types, annuals and perennials, using the C_3 photosynthetic pathway and grasses using C_4 photosynthesis. A few species of cacti (CAM type) were also found at our study site, but were excluded from this analysis because they occurred in low densities, accounted for a very small proportion of NPP, and are not usually used as a food resource by grasshoppers.
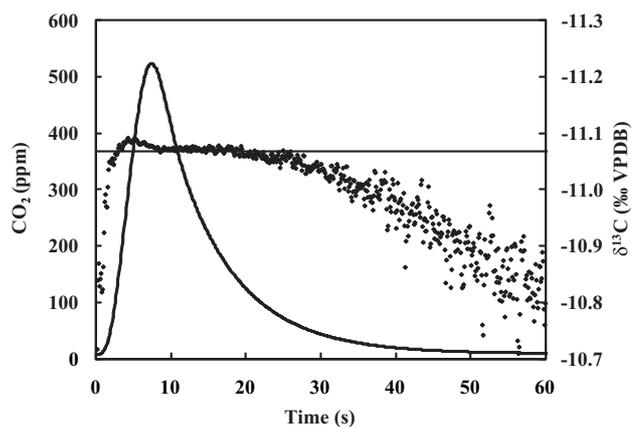
Figure 1. TGA100 measurement of a pulse of 50 mL standard gas of 750 ppm CO_2 and known $\delta^{13}C$ (thin horizontal line) over CO_2-free carrier gas. Peaked black line = $[CO_2]$ (left axis). Diamonds: $\delta^{13}C$‰ VPDB (right axis). Measured $\delta^{13}C$ equals known $\delta^{13}C$ after an initial overshoot. The $\delta^{13}C$ fit between measured and actual values deteriorates when $[CO_2] < 200$ ppm.

RESULTS

In contrast to other studies using a TGA100, our samples (insect 'breath') entered the sample cell in pulses of high and changing CO_2 concentrations over a stable background of CO_2-free carrier gas. When mimicking such pulses with calibration gases of known CO_2 concentration and $\delta^{13}C$ (Fig. 1) we found that the carbon isotope ratios determined from these pulses (averaging over 2 s) equaled the known values ±0.04‰ SD for CO_2 concentrations down to about 200 ppm (Fig. 2). An initial overshoot of $\delta^{13}C$ values was observed that corresponds with rapidly changing CO_2 concentrations (Fig. 1). This overshoot was not included in the averages. The accuracy and precision of the measurements are partially a function of the temporal averaging of the data. For averaging times of 1 to 8 s, the accuracy, i.e. the
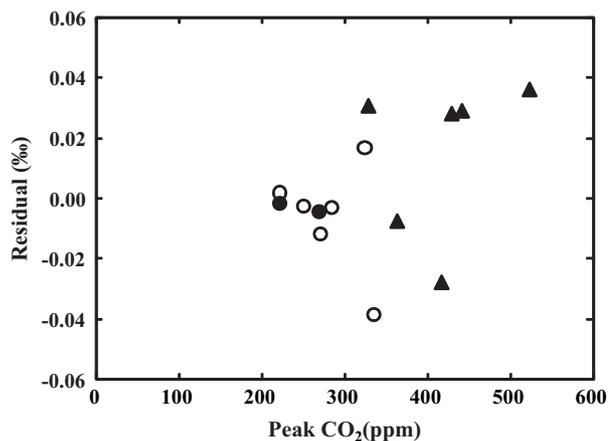

Figure 2. Difference between measured and known $\delta^{13}C$ values for pulses of known standard gases (filled circles: 350 ppm, −11.21‰; open circles: 550 ppm, −11.12‰; triangles: 750 ppm, −11.07‰). Peak CO_2 concentrations are lower than standard concentrations due to dilution with CO_2-free medical air that was used as carrier gas.
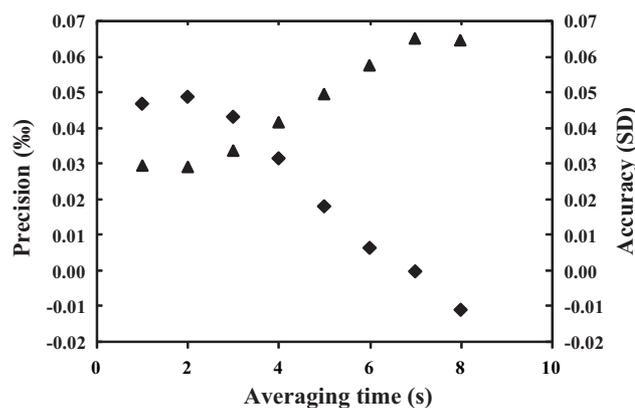
Figure 3. Accuracy (diamonds) and precision (triangles) of $\delta^{13}C$ measured from pulses of standard gases (n = 12) as a function of averaging time. For 'accuracy', $\delta^{13}C$ was averaged over 1 to 8 s and compared with the known value of the standard gas. 'Precision' is the standard deviation of the respective period (10 Hz sampling rate).

difference between known and measured $\delta^{13}C$, was between −0.01 and 0.05‰. Longer averaging times resulted in higher accuracy. Conversely, the precision of these measurements decreased with longer averaging time from 0.03 to 0.06‰ SD (Fig. 3). The possible averaging time depended on the volume and CO_2 concentration of the pulse – bigger volumes of higher CO_2 concentration generally allowed averaging over a longer time period. The decreasing precision with longer averaging time was mainly caused by small pulses (15 mL) of low CO_2 concentrations (200–300 ppm). Based on these data we chose an averaging time of 5 s from pulses > 350 ppm to determine the $\delta^{13}C$ from insect 'breath'. Repeated measurements of working standards produced standard deviations of 0.10 ppm for $[CO_2]$ and 0.26‰ for $\delta^{13}C$ over 5 s of measurement.

We measured the 'breath' of 184 individuals from the field (325 pulses) in a total of 25 hours of TDLAS use. This makes an average of 13 pulse measurements per hour with the manual sample system used in this study. We have been able to greatly increase sample throughput rate with a computer-controlled sampling valve system. Breath $\delta^{13}C$ measurements on wild-caught grasshoppers demonstrate great variability in the use of specific plant photosynthetic types (Fig. 4), as reflected in the species' average of breath $\delta^{13}C$ which ranged from −15.0 ± 0.5 to −28.7 ± 1.0‰ VPDB (Table 1). Based on these data, we were able to place species into three distinct groups: C_4-specialists, generalists, and C_3-specialists. Four grasshopper species (*Phlibostroma quadrimaculatum*, *Opeia obscura*, *Eritettix simplex*, *Ageneotettix deorum*) fed primarily on C_4 plants and had breath $\delta^{13}C$ which averaged > −17‰ VPDB. We also found 10 generalist species with average $\delta^{13}C$ values between −17 and −24‰ VPDB (*Melanoplus arizonae*, *M. lakinus*, *M. sanguinipes*, *Trimerotropis pistrinaria*, *T. pallidipennis*, *T. californica*, *Brachystola magna*, *Xanthippus corallipes*, *Arethea gracilipes*, *Arphia pseudonietana*). The third group, C_3-specialists, comprised only a single species, *Tropidolophus formosus*, known to specialize on plants in the family Malvaceae. When using a simple two-pool mixing model,[22] using $\delta^{13}C_{breath}$ as a proxy for
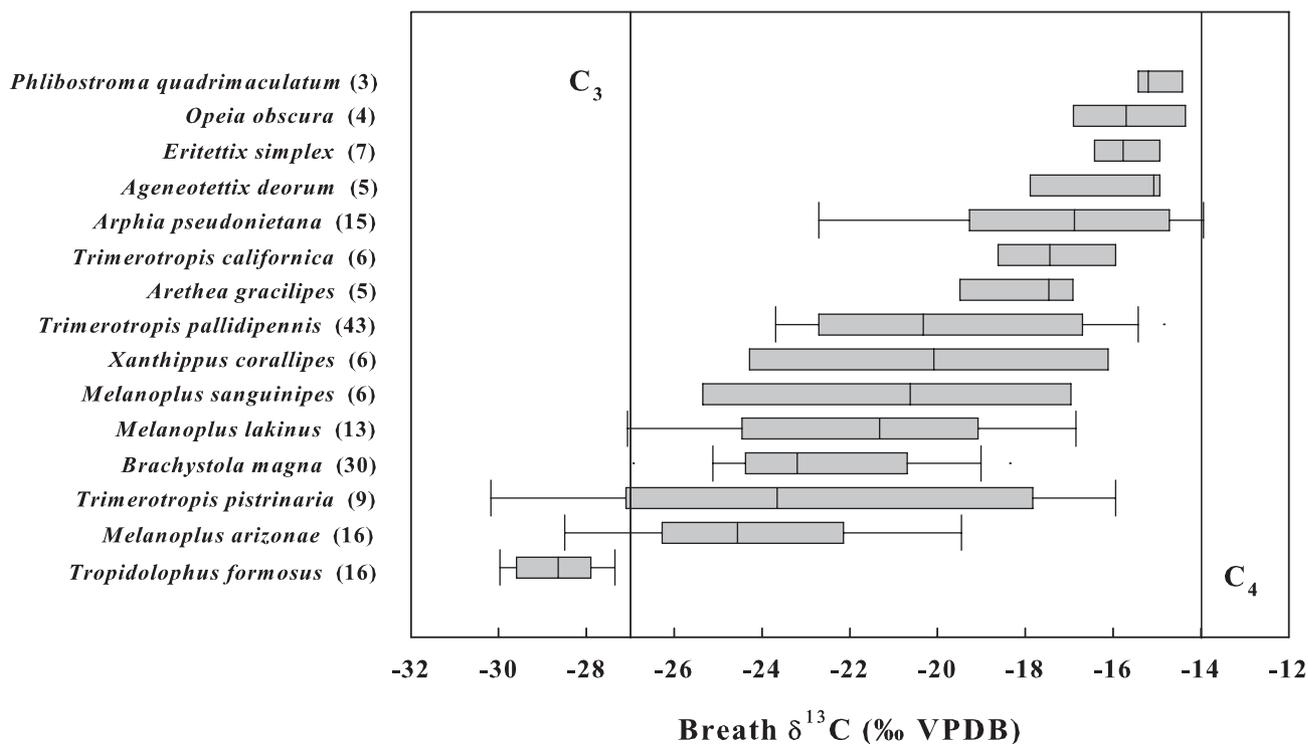
Figure 4. Breath carbon isotope ratios of 15 grasshopper species as determined by TDLAS (median, 5th, 25th, 75th, 95th percentiles). Sample size is given after species names. Vertical lines represent average carbon isotope ratios of C_3 (-27.2 ± 1.8‰; $n = 38$), respective C_4 plants (-13.9 ± 0.7‰; $n = 21$).

$\delta^{13}C_{diet}$, and assuming a discrimination between $\delta^{13}C_{diet}$ and $\delta^{13}C_{breath}$ of -1‰, these values represent a relative contribution of C_4 plants to the diet of >90% for C_4-specialists, 0% for the C_3-specialist, and a mix containing 32 to 84% of C_4 plants for the generalist species. Breath data correlate very well with the values obtained from whole-body tissue analysis (Fig. 5; $n = 184$; $R^2 = 0.86$). The species' means of tissue samples and breath CO_2 had an average offset of -0.9‰ ($n = 15$; $R^2 = 0.96$, Table 1; paired t-test: $p < 0.001$). Because lipids are depleted in ^{13}C compared with carbo-

hydrates,[23] we conclude that our grasshoppers were metabolizing carbohydrates at the moment of 'breath' analysis instead of catabolizing lipid body stores. To determine the magnitude and time course of a shift from carbohydrate to lipid catabolism we measured the 'breath' δ^{13}C of a subset of 100 *T. pallidipennis* that were fed a C_3-based diet for 2 weeks. The 'breath' δ^{13}C after this treatment was -25.9 ± 0.8‰ for freshly fed individuals ($n = 20$) and decreased significantly to -27.5 ± 1.0‰ ($n = 20$, $p < 0.025$) after a fast of 3 days. Fasting periods of shorter duration resulted in decreased 'breath' values compared with those of freshly fed animals (2 h: -25.8 ± 0.9‰, 6 h: -26.1 ± 1.0‰, 1 day: -26.9 ± 1.0‰), but these differences were not significant.
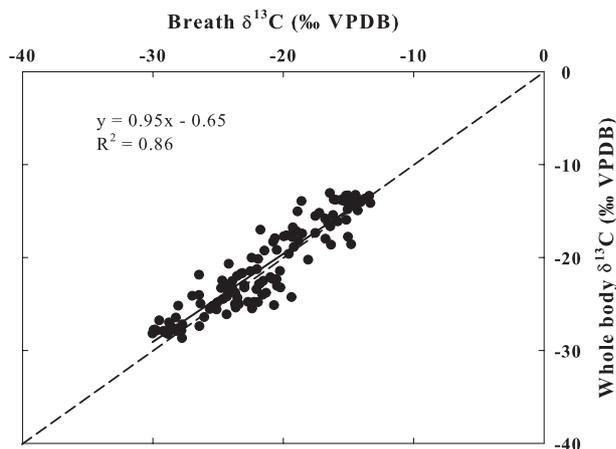

Figure 5. Carbon isotope ratios from a grasshopper community as determined from animal 'breath' by TDLAS and from homogenized samples of body tissues by IRMS. Solid line: least squares regression (statistics are given in upper left corner); stippled line: x = y. Animals were fasted for 24 h prior to measurements.

Table 1. Average carbon isotope ratios of breath and body tissues for 15 grasshopper species from the Sevilleta LTER

Species	N	$\delta^{13}C_{breath}$ ($\pm$SD)‰	$\delta^{13}C_{body}$ ($\pm$SD)‰
Phlibostroma quadrimaculatum	3	-15.0 ± 1.3	-13.8 ± 0.4
Opeia obscura	4	-15.6 ± 1.3	-13.7 ± 0.6
Eritettix simplex	7	-15.9 ± 1.1	-13.7 ± 0.2
Ageneotettix deorum	5	-16.1 ± 1.8	-14.6 ± 1.0
Arphia pseudonietana	15	-17.2 ± 3.0	-15.5 ± 1.5
Trimerotropis californica	4	-17.5 ± 1.5	-17.4 ± 1.2
Arethea gracilipes	5	-18.0 ± 1.3	-17.7 ± 1.0
Trimerotropis pallidipennis	43	-19.8 ± 3.2	-19.3 ± 3.3
Xanthippus corallipes	6	-20.2 ± 4.1	-19.4 ± 4.3
Melanoplus sanguinipes	6	-21.1 ± 4.7	-20.8 ± 4.0
Melanoplus lakinus	13	-21.6 ± 3.4	-21.1 ± 3.6
Brachystola magna	30	-22.7 ± 2.5	-23.0 ± 2.6
Trimerotropis pistrinaria	9	-22.8 ± 5.1	-21.3 ± 5.2
Melanoplus arizonae	16	-24.3 ± 3.0	-24.4 ± 1.5
Tropidolophus formosus	16	-28.7 ± 1.0	-27.9 ± 0.3

The 59 plant species that we collected made up 88% of total NPP (123 ± 11.2 g_{dry} m^{-2}) during the fall (July–September) of 2006.[21] Of these, plants using the C_4 photosynthetic pathway had an average $\delta^{13}C$ of -13.9 ± 0.7‰ VPDB (range -15.0 to -12.7; n = 21). This group consisted almost totally of C_4 grasses, and accounted for 59% of total NPP in this time period, most of which (37%) was contributed by a single species, *Bouteloua eriopoda* (black grama grass, $\delta^{13}C = -14.6$‰). C_3 annual and perennial plants, in contrast, accounted for only 29% of total NPP during this period and had $\delta^{13}C$ values averaging -27.2 ± 1.8‰ VPDB (range -31.0 to -22.5; n = 38).

DISCUSSION

Our study shows that TDLAS is a suitable method for the rapid analysis of 'breath' $\delta^{13}C$. Implemented in laboratory or field studies, 'breath tests' can contribute significantly to physiological and ecological research. With minimal temporal and logistical effort we were able to quickly determine dietary specialists and generalists in a grasshopper community and their respective reliance on plant photosynthetic types. When extended over a longer period, such studies can monitor changes in resource utilization in response to seasonal or climatic changes. With recent technological progress, such tests are now economically and logistically feasible, thus allowing large-scale applications that were formerly not possible.

The TGA100 allows rapid measurements at high precision (0.3‰ SD, see Fig. 3), at a wide range of CO_2 concentrations, depending mainly on the calibration gases being used. It thus allows for studies on a wide range of differently sized study subjects. It facilitates generating high sample sizes as done in this study, or time-course measurements requiring fast responses.[12,13] The need of the instrument for frequent calibration against standard gases (ca. 10 L weekly) requires the use of several gas tanks, and the laser needs to be cooled with liquid nitrogen. This makes the TGA100 not a truly portable instrument. However, the instrument is clearly field-deployable in a mobile laboratory, e.g. in a trailer that can be taken into the field.[14] The system provides real-time, uncalibrated output of CO_2 and $\delta^{13}C$ values that are typically within 5 ppm and 1.0‰ of calibrated values; therefore, first approximations of animal breath $\delta^{13}C$ can be made in real time. This is not only convenient but also allows immediate trouble-shooting in the case of system malfunction. Calibration of the data takes minutes and improves precision by up to an order of magnitude. Although the purchase cost of a TDLAS system is relatively high (~$120 000), the actual costs per sample are minimal (<$0.01 US, at least three orders of magnitude lower than the sample costs at current rates using IRMS), which makes the technique very attractive for long-term ecological studies, like the study of climate-change effects on animal communities. With the simple sampling system that we used for this study (manually switched ball valves and plastic syringes) we were able to measure 'breath' $\delta^{13}C$ of animals with a body mass as small as 24 mg. We now have a more sophisticated computer-controlled sampling system that allows the processing of large sample sizes (~200 per day) and the measurement of even smaller individuals, possibly down to individual ants.

Our estimates suggest that 1000 or more individuals could be measured in five 10-h days, which virtually allows the study of whole communities in days. In our opinion, the advantages clearly outweigh the disadvantages of the technique, namely the relatively high initial purchase costs and the need for several calibration gases. Here, we have presented just one example of how TDLAS can be applied in ecological research. 'Breath tests' have a huge potential for ecological and physiological studies and we think that TDLAS greatly facilitates their application in the field.

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