Honey bee colonies maintain CO$_2$ and temperature regimes in spite of change in hive ventilation characteristics

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Abstract – CO$_2$, a byproduct of respiration, is toxic at high concentrations so regulation of CO$_2$ within the honey bee hive is an important colony function. In this study, we measured hive CO$_2$ concentrations at 1-s intervals while ventilation characteristics of the hive were changed every few days, and we analyzed the data for effects of increased ventilation on colony behavior and thermoregulation. Average CO$_2$ concentrations were significantly higher, by > 200 ppm, when hives had screened bottom boards (higher ventilation) compared to hives with solid bottom boards (lower ventilation) at the same time. Daily CO$_2$ concentration amplitudes, hourly temperature, daily temperature amplitudes, nor hourly hive weight changes were not significantly affected by the changes in hive ventilation. In a second experiment, we found average CO$_2$ concentrations at the top center of the upper hive box, on top of the frames, were significantly lower than concentrations at the center of a solid bottom board underneath frames, which was expected due to the higher density of CO$_2$ relative to air. Bee colonies have been reported to cycle air, with shorter periods of 20 to 150 s and longer periods of 42–80 min, but a periodogram analysis of the CO$_2$ concentration data found no evidence of important CO$_2$ cycle periods other than a strong 24-h period. Bee colonies maintained strong daily cycles of CO$_2$ concentration, with average maximum concentrations > 11,000 ppm, even in conditions of increased ventilation, indicating that managing CO2 concentration is a complex colony behavior.

bee colony behavior / thermoregulation / continuous monitoring / social insects

1. INTRODUCTION

Honey bee colonies present unique opportunities for the study of colonial insect behavior. In order to maintain desired conditions within the hive, honey bees need to behave collectively in ways that isolated or solitary bees do not such as by regulating temperature (Gates 1914; Stabentheiner et al. 2010) and humidity (Human et al. 2006), and using allogrooming to protect the colony against diseases and pests (Evans and Spivak 2010). Bee hives can be disassembled and modified, and sensors to measure temperature, humidity, CO$_2$, and other variables can be installed at any point, without harming the bee colony. Sensors installed in bee hives can reveal information on colony behavior and health without the need for frequent colony disturbance.

Advances in sensor technology have resulted in smaller, cheaper, and more accurate sensors that make monitoring colonies easier and reveal new facets of colony behavior (Meikle and Holst 2015). For example, continuous hive weight data show changes in foraging activity due to nectar flows or pesticide exposure, as well as changes in colony resources due to robbing.
or reproductive swarming (Meikle et al. 2008, 2016, 2018b). Another example is internal hive temperature. Brood development, particularly pre-pupae and pupae, requires temperatures of 34–36 °C (Stabentheiner et al. 2010; Wang et al. 2016), but continuous temperature monitoring also shows daily cycles of temperature and metabolic activity driven by exterior (ambient) conditions (Southwick and Moritz 1987) and that colonies thermoregulate in the absence of brood (Gates 1914, Meikle et al. 2016). How intensely bee colonies thermoregulate is a function of subspecies (W-Worswick 1987), within-colony genetic diversity (Jones et al. 2004), phenological status (Stalidzans and Berzonis, 2013), and pesticide exposure (Meikle et al. 2018a, 2022). Temperature data are also affected by the colony size and the location of the sensor (Meikle et al. 2016). Sensors in or near the mass of bees (the “cluster”) at the core of the colony will be affected less by exterior conditions than those further from cluster, although distance to cluster can change as clusters change size and move during the year (Szabo 1989).

Carbon dioxide (CO₂), a byproduct of respiration, is toxic at high concentrations so regulation of CO₂ within the honey bee hive is an important colony function. Aspects of CO₂ concentration control in the hive may reveal, like temperature control, information on colony health. Hive CO₂ concentration increased in honey bee colonies exposed to neonicotinoid pesticide (Meikle et al. 2021). Bahreini and Currie (2015) manipulated CO₂ concentration in bee colonies under controlled conditions and speculated that high CO₂ concentration may affect Varroa densities. Seeley (1974) monitored the number of fanning bees when CO₂, O₂, and N₂ concentrations were manipulated; only CO₂ influenced fanning behavior. Fanning behavior has been associated with cycles of air movement out of the hive that have been reported to have shorter durations of about 22 s during the day, and about 150 s at night, and longer durations of 42–80 min (Southwick and Moritz 1987). If such cycles of air movement affect CO₂ concentration, then the frequency of those cycles may also be a useful parameter to monitor.

The goals of this study were to determine (1) whether we could detect changes in CO₂ within the hive that could conceivably correspond to the rapid air movement at the entrance of the hive reported previously by other workers; and (2) whether the average hive CO₂ concentration would be significantly reduced by the use of a ventilated bottom board, which in turn would imply that hive CO₂ concentrations are due at least partly to hive design rather than just colony behavior. We monitored hive CO₂ concentration as we altered the ventilation characteristics of honey bee hives by blocking or opening a screened bottom board every 5–7 days, and we tried to detect cycles of CO₂ concentration on time scales that varied from long (> 24 h) to very short (< 20 s) at that point in the hive. Data were collected once per second from a sensor placed in a position (top center of the uppermost box) that did not interfere with bee movement and was easily accessed by researchers without disturbing the colony. We compared those results with continuous data on hive temperature and weight collected at the same time. We conducted a second experiment to compare CO₂ data on the top of the frames with data gathered underneath the frames on the bottom of a hive, in order to determine whether there were aspects of CO₂ management under the frames that could not be detected by the sensor at the top of the frames.

2. MATERIALS AND METHODS

On June 20th 2020, 12 colonies of Italian honey bees (Apis mellifera ligustica) with marked queens originally acquired on April 1st, 2020 (Marquette Apiaries, Winchester, CA), were selected at an apiary in Tucson, AZ. Criteria for selection were that colonies were of similar size, queenright, and did not show significant signs of disease or Varroa damage. Each hive consisted of two painted, 10-frame, wooden Langstroth boxes (each 43.7 l capacity) with a migratory wooden lid, no ventilation holes in the upper box, and was visually estimated to contain at least two frames of brood and 1.5 kg adult bees (see Delaplane et al.
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Each hive had a wooden reducer limiting the entrance size to 12 cm width × 1 cm height. Hives were placed on electronic scales (Tekfa model B-2418 and Avery Weigh-Tronix model BSAO1824-200) (max. capacity 100 kg, precision ± 20 g; operating temperature – 30 to 70 °C) installed on top of cinder blocks in an apiary with a gravel bed. The scales were linked to 16-bit dataloggers (Hobo UX120-006 M External Channel datalogger, Onset Computer Corporation, Bourne, MA) with weight recorded every 5 min. Hives were 0.5–1 m apart. The apiary was covered with a roof and protected on two sides from wind.

Temperature sensors (iButton Thermochron, resolution ± 0.06 °C, accuracy ± 0.5 °C, accessed using 1-Wire Drivers × 64, version 4.05) enclosed in plastic cassettes (Thermo Fisher Scientific, Waltham, MA) were stapled to the center of the top bar on the middle frame in the bottom box and set to record every 15 min. CO₂ probes (model GMP251, Vaisala Inc., Helsinki, Finland) were placed on top of the center frames in the top box of each hive and linked to HOBO UX120-006 M dataloggers set to record at 1-s intervals. CO₂ probes calibrated for concentration of 0–20% were chosen in order to ensure that the concentration in the hive did not exceed the maximum calibration for the probe; CO₂ concentrations as high as 3.90% (39,000 ppm) have been reported (Bahreini and Curry 2015). Calibration certificates for all probes showed that the difference between reference and observed CO₂ concentrations was always inferior to 0.01%. However, given the wide range of the CO₂ probes, low recorded values should be interpreted with caution. To reduce error, all probes were placed next to each other in ambient conditions for 1 h and those values used in probe calibration. To compare data from probes calibrated to lower values, in a separate trial, 2 hives were selected (queenright, at least 7 frames of brood and 3 kg adult bees) and 2 probes were placed on the top center: one probe calibrated to 0–20% CO₂ and the other calibrated to 0–10,000 ppm CO₂. Data were collected every 15 s, and periodogram analyses were conducted for comparison between probe types.

An experiment (“Experiment 1”) was initiated on June 24th, 2020. All hives were fitted with screened bottom boards that had a removable corrugated plastic insert in a slot just below the screen (IPM screened bottom board, Mann Lake Ltd). Each hive was randomly assigned to one of two treatment groups, A or B. On June 26th, the corrugated plastic inserts in group B were removed to expose the screen (“open”) while the inserts in group A were left in place (“closed”). On June 30th, the inserts were replaced in group B and were removed in group A. On July 4th, the inserts were replaced in group A and were removed again in group B. The same replacing and removing procedure was conducted again on July 8th, 12th, and 16th, always changing the treatment group with the open bottom boards (Figure 1). The experiment was terminated on July 20th. Ambient temperature data were obtained from https://ag.arizona.edu/azmet/az-data.htm.

A second experiment (“Experiment 2”) was conducted June 7th–11th, 2021. Four colonies were selected at the apiary in Tucson, AZ, that were similar in size, and obtained from the same supplier, as the colonies in Experiment 1. One CO₂ sensor was placed in the same location as in Experiment 1, i.e., on top center of the upper box, while another sensor was placed in the center of the bottom board under the frames. Data were gathered every second for 89 h. Only CO₂ data were collected.

2.1. Data analysis

Data on within-hive CO₂ concentration were collected each second, but comparison of data from outside of the hive with data collected inside the hive revealed inherent periods due to the behavior of the sensor, not the colony. Those periods, of about 10–15 s and with amplitudes of about 50 ppm, represented noise in the analog output. To eliminate the noise from the sensor, data were reduced to 15-s average values. The averaged data were subjected to a periodogram analysis (RStudio version 1.2.5033). A periodogram analysis decomposes a signal, in this case CO₂ concentration, into discrete frequencies of sinusoidal components and
provides an estimate of the contribution of each frequency to the signal in the form of the power spectral density (R Development Core Team 2020). The power spectral density was divided by the product of the variance and \((N-1)\), where \(N\) is the number of observations in a sample, to produce the proportion sums of squares contributed by each frequency. The total sums of squares contributions are equal to one for the analysis of each sample, so the relative importance of each frequency is easy to judge. Periodograms are expressed in the frequency domain rather than the time domain.

To examine changes in daily amplitudes, CO\(_2\) (30-min average values) and temperature data were transformed as within-day detrended data, calculated as the difference between the 24-h running average and the raw data. Sine curves were fit to daily datasets of the detrended data (see Meikle et al. 2016) in C+++(Qt Creator 4.1.0), and daily amplitudes of the fitted curves were obtained and used as response variables.

Experiment 1 data were subjected to a repeated measures MANOVA (Proc Glimmix, SAS Inc. 2002) to evaluate the fixed effects of treatment (open bottom board or closed), time (hour or day, depending on the analysis), and their interaction, on hourly estimates of (1) log hourly average CO\(_2\) concentration; (2) the five periods of CO\(_2\) cycles with the highest contributions to the sums of squares; (3) daily amplitudes of CO\(_2\) concentration and of temperature; (4) hourly average hive temperatures; and (5) hourly hive weight changes. Hive number was used as a random effect, an ar(1) autoregressive covariance structure was used, and degrees of freedom calculated using the Kenward-Roger method. A similar MANOVA was conducted with log hourly average CO\(_2\) concentration in Experiment 2, in which the fixed effects were sensor location (top or bottom of hive), time of day, and their interaction.

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### Figure 1

Diagram of the design for the first experiment. Each column of boxes (hives) shows the approximate position of the hive in the apiary. Each date shows when the inserts were changed for each hive.

| Hive Group | Hive no. | 26 June | 30 June | 4 July | 8 July | 12 July | 16 July | 20 July |
|------------|----------|---------|---------|--------|--------|---------|---------|---------|
| B          | 1        |         |         |        |        |         |         |         |
| B          | 2        |         |         |        |        |         |         |         |
| A          | 3        |         |         |        |        |         |         |         |
| B          | 4        |         |         |        |        |         |         |         |
| A          | 5        |         |         |        |        |         |         |         |
| A          | 6        |         |         |        |        |         |         |         |
| B          | 7        |         |         |        |        |         |         |         |
| A          | 8        |         |         |        |        |         |         |         |
| A          | 9        |         |         |        |        |         |         |         |
| B          | 10       |         |         |        |        |         |         |         |
| B          | 11       |         |         |        |        |         |         |         |
| B          | 12       |         |         |        |        |         |         |         |

- Hive changed to closed (solid) bottom board
- Hive changed to open (screened) bottom board
3. RESULTS

During Experiment 1, data on CO₂ were downloaded at four time points, and during those downloads, some equipment failures were detected. As a result, all 12 hives were included in data from June 26th to 30th; 9 hives (4 from group A and 5 from group B) were included from June 30th to July 9th; 9 hives (5 from group A and 4 from group B) were included from July 9th to 14th; and 12 hives were included from July 14th to 20th. One hive scale also malfunctioned (in group B) so those data were discarded, leaving weight data for 6 hives in group A and 5 hives in group B. All temperature data were collected. In Experiment 2, all CO₂ data were collected.

CO₂ data showed large changes on a daily basis (Figure 2), and within hive values ranged up to 23,000 ppm. Examination on a shorter time frame shows the daily pattern more clearly (Figure 3). Honey bee colonies clearly changed their management of CO₂ rapidly to changes in hive ventilation characteristics. Hives with open bottom boards had significantly higher average CO₂ concentrations, with an arithmetic average of about 2080 ± 50 ppm, than hives with solid bottom boards, on average about 1840 ± 30 ppm (Figure 4, Table 1). Hives with closed bottom boards uniformly had lower CO₂ concentrations with one exception—from 30 June to 1 July. During that period, high winds, gusting to 50 kph, kept bees from exiting the hive, and while that did not have a large effect on hives with ventilated bottom boards, CO₂ concentrations increased dramatically in hives with closed bottom boards. That was the only such event during the study. The daily amplitudes of CO₂ concentrations were not affected by treatment. However, it may be that that method is not sensitive enough to detect effects—the analysis yields a single value per hive per day, and the days in which bottom boards were switched were excluded from the analysis.

![Figure 2. Average log CO₂ concentration ± s.e. at the middle of the top of the hive. Vertical dashed line shows the start of the experiment, in which group A hives had closed bottom boards and group B hives had open bottom boards. Gray bars indicate time periods when group A had open bottom boards and group B had closed bottom boards; periods outside gray bars indicate the contrary setup. The number of hives per treatment group varied over time (see text for details).](image-url)
Periodograms of 5 min (300 s) samples, taken during the course of the experiment, show the contributions of various frequencies (Figure 5). The sensors themselves had signal periods of about 9–15 s (See Online Resource 1 Figure S1) so periods < 16 s were ignored. Inspecting data for periods above 15 s showed no appreciable peaks (defined here as those that account for at least 0.05 of the proportion sum of squares) until periods are at least 75–100 s, with the greatest amount of variance accounted for at the longest period (here 300 s). The direct relationship between period and proportion sums of squares suggested that they are harmonics of larger values.

Average CO₂ was slightly higher at the bottom location, which was expected (Figure 6). A periodogram of 5-min sets of data at 1-s intervals showed that CO₂ concentration measured on the bottom of the hive did not differ materially from that measured at the top (See Online Resource 1 Figure S2) so either the methods used here are not sensitive to such cycles, or they do not exist.
Figure 5. Average values ± s.e. of periodograms of 5 min samples of CO$_2$ concentration collected at 1-s intervals over 14–20 July 2020. (A) Periodogram for data collected during the day (defined here as 6:00:00AM to 5:59:59PM). (B) Periodogram of data collected during the night (defined here as 6:00:00PM to 5:59:59AM). No period less than 9 s explained more than 0.1% of the sums of squares and were omitted here. N=6 hives per treatment group.
Considering the periodogram of all the data from a larger data set, from July 14th to 20th (about 144 h), it was clear that the 24-h period explained the vast majority of the variance (Figure 7). The periodogram shows a very strong peak at 24 h, then peaks at the harmonics of \( \frac{1}{2} \) (12 h), \( \frac{1}{3} \) (8 h), \( \frac{1}{4} \) (6 h), \( \frac{1}{5} \) (4.8 h), \( \frac{1}{6} \) (4 h), etc. No other peaks were noteworthy. If a particular period exhibits a peak in explained variance but it is also a harmonic of another period that has a much stronger peak, further analysis may be needed to determine whether the first period offers new information. To examine the relationship between different periods and the factors affecting within-hive CO\(_2\) concentration, i.e., the presence or absence of a bottom board.

**Figure 6.** Thirty-minute average CO\(_2\) concentration ± s.e. collected at 1-s intervals for sensors placed on top of the hive frames (“Top”) and under the hive frames (“Bottom”). \( N = 4 \) hives. Data collected 7–11 June 2021.

**Figure 7.** Average values ± s.e. of periodograms of 144 h of CO\(_2\) data, collected at 1-s intervals and averaged to 15-s values. \( N = 6 \) hives per treatment group. Data collected 14–20 July 2020.
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(“Open or closed”), time (hour or day), and their interaction (“OC*time”), mixed model repeated-measures ANOVA were conducted on periods of 3600 s (one h), 1800s, 1200 s, 900 s, and 720 s (the $1/24$, $1/48$, $1/72$, $1/96$, and $1/120$ harmonics of 24 h) over the course of the experiment. While those periods are harmonics of a period with a very strong signal, they may also reflect independent periods of CO2 cycling within the hive. However, whether the bottom board was open or closed did not have a significant effect on any of the periods of interest (all $P > 0.06$). The bottom board treatment thus significantly affected hourly average CO2 concentration but not cycle periods of one hour or less.

A comparison of probes calibrated for 0–20% CO2 with those calibrated for 0–10,000 ppm showed that probe outputs were similar (See Online Resource 1 Figure S3). A regression between the two probe types of spectral densities across periods from 1 to 162 h obtained from periodogram analyses showed high $r^2$ values (0.85 and 0.94 for data from hives 1 and 2, respectively, for that study), supporting the argument that the 0–20% probes had acceptable output for these purposes (See Online Resource 1 Figure S4).

The mixed-model analysis, using log-transformed temperature values, indicated that opening and closing bottom boards did not affect hive cluster temperature (Figure 8). Likewise, changing bottom boards had no measurable impact on hourly hive weight change (Figure 9). Hourly changes in hive weight reflected departure and return of the foraging population, with some weight loss due to food consumption and water loss (Meikle et al. 2016); for this reason, the time of day factor was significant (Table I). Experiment 1 was conducted outside of a nectar flow, so during the course of the experiment, colonies lost on average $4.25 \pm 0.22$ kg.

4. DISCUSSION

Southwick and Moritz (1987) used an anemometer to measure air flow in an experimental hive with 2000 bees kept in a laboratory, and observed “2.9 ‘breaths’ per minute” during the daytime, which would correspond to a period of about 22 s. At night, they reported about 0.4 “breaths” per minute, equivalent to longer cycles with periods of 150 s. Southwick and Moritz (1987) also reported previously published cycles of 42–80 min. Southwick and Moritz apparently obtained those longer cycle values from their analysis of data published elsewhere, e.g., Seeley (1974) and Kronenberg and Heller (1982). Given that we measured CO2 every second, with a sufficient level of sensitivity, it seems likely that we would be able to detect cycles of CO2 change that would correspond with those measured cycles of interior air movement. We used a periodogram analysis to express the observed patterns in CO2 concentration in terms of frequencies of periodic functions.

We chose the top center of the uppermost box as a location because a CO2 sensor there would not interfere with bee movement within the hive and it could easily be accessed by researchers without disturbing the colony. The sensors were 25 mm in diameter, which precluded placing them between frames. However, air exchange on top of the hive frames may occur at a slower rate than at other locations in the hive, and other factors, such as the distance from the hive entrance to the sensor location, may dampen the signals from air exchange cycles generated by the bees. We tested that by placing sensors both on top of the frames and resting on the (solid) bottom board, with the hive entrance on the same level and < 10 cm away. CO2 that is descending because of its higher density would need to pass by the sensor as it lands on the bottom board and eventually mixes with exterior air at the hive entrance. The proximity of the entrance and the movement of bees through the entrance on the bottom board would also mean much more ventilation.

Opening and closing bottom boards did not affect hive cluster temperature. We expected that using a screened bottom board would have reduced hive insulation, thus making maintaining ideal brood temperatures (about 35 °C) more difficult, which in turn would have increased colony-level metabolic activity and with it increased CO2 production. However, CO2 is more dense than air, which consists mainly of the smaller molecules
N₂ and O₂, and CO₂ molecules would have dropped down naturally through the air column, so we had expected that the screened bottom board arrangement would have been associated with lower CO₂ concentration compared to closed bottom boards. That CO₂ concentration increased as much as it did suggests that CO₂ management by honey bee colonies is more complex than simply reducing its concentration.

Cycles of change in CO₂ were driven almost entirely by a 24-h cycle, with comparatively little contribution from cycles with shorter periods. We found no evidence for cycles of CO₂ change, at either the top of the hive or near the hive entrance at the bottom, corresponding to cycles of air movement reported by other researchers (see Southwick and Moritz 1987). Signal frequencies inherent in the sensors used here may have

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**Figure 8.** Temperatures from 26 June to 20 July. (A) Within-hive temperature (°C) running average (upper graph) for the two treatment groups during the course of the experiment. Vertical dashed line shows the start of the experiment, in which group A hives had closed bottom boards and group B hives had open bottom boards. Gray bars indicate time periods when group A had open bottom boards and group B had closed bottom boards; periods outside gray bars indicate the contrary setup. N=6 hives per treatment group; (B) ambient temperature.
Figure 9. Hourly average hive weight change. Hive weight data were collected every 5 min, averaged across each hour, and these data show the difference between a given hour and the preceding hour. Vertical dashed line shows the start of the experiment, in which group A hives had closed bottom boards and group B hives had open bottom boards. Gray bars indicate time periods when group A had open bottom boards and group B had closed bottom boards, whereas periods outside gray bars indicate the contrary setup. \( N = 6 \) hives in group A and 5 hives in group B.

Table I Results for repeated-measures MANOVA for various response variables

| Exp | Response variable       | Factor                      | Num DF | Den DF | F Value | Pr > F |
|-----|-------------------------|-----------------------------|--------|--------|---------|--------|
| 1   | Log hourly average \( CO_2 \) | Open or closed              | 1      | 214.9  | 23.51   | < 0.0001 |
|     |                          | Hour                        | 568    | 4427   | 8.43    | < 0.0001 |
|     |                          | OC * hour                   | 568    | 4426   | 1.48    | < 0.0001 |
| 1   | Log daily \( CO_2 \) amplitude | Open or closed              | 1      | 119.3  | 3.23    | 0.0750  |
|     |                          | Day                         | 17     | 129.6  | 13.87   | < 0.0001 |
|     |                          | OC*day                      | 17     | 127.4  | 3.12    | 0.0001  |
| 1   | Average temperature     | Open or closed              | 1      | 353    | 0.00    | 0.9712  |
|     |                          | Hour                        | 576    | 5432   | 3.39    | < .0001 |
|     |                          | OC * hour                   | 576    | 5432   | 1.33    | < .0001 |
| 1   | Log daily temp. ampl     | Open or closed              | 1      | 149    | 0.48    | 0.4898  |
|     |                          | Day                         | 19     | 174.7  | 1.26    | 0.2189  |
|     |                          | OC*day                      | 19     | 172.3  | 1.55    | 0.0742  |
| 1   | Hive weight change       | Open or closed              | 1      | 1255   | 0.21    | 0.6438  |
|     |                          | Hour                        | 575    | 4848   | 6.01    | < 0.0001 |
|     |                          | OC * Hour                   | 575    | 4849   | 1.74    | < 0.0001 |
| 2   | Log hourly average \( CO_2 \) | Top or bottom               | 1      | 104.6  | 265.18  | < 0.0001 |
|     |                          | Hour                        | 88     | 475.8  | 16.38   | < 0.0001 |
|     |                          | TB*hour                     | 88     | 475.8  | 2.04    | < 0.0001 |

“Exp.” refers to the experiment (1 or 2), “Num DF” and “Den DF” refer to numerator and denominator degrees of freedom, respectively, “\( CO_2 \)” refers to carbon dioxide concentration, “temp.” refers to internal hive temperature, and “ampl.” refers to daily amplitude, as estimated by the fit of a sine wave to detrended data (please see text for details).
interfered with the detection of cycle periods less than 16 s, but our sensors would have detected any longer periods. Likewise, we observed no effects on hive temperature or weight change, indicating that, at least in early summer in Arizona, whether hives had screened and solid bottom boards had little effect on colony behavior.

Changing the bottom board from solid to screen changed hive ventilation characteristics but it no doubt changed other characteristics, such as light, acoustics, and concentrations of volatile organic compounds within the hive. Van Nerum and Buelens (1997) observed that bees actively maintained low (15%) O2, causing a reversible hypoxia and reduced metabolic rate among the bees that, they hypothesized, allowed them to conserve water and energy, live longer, and increase activity on short notice. It may be that the effects on CO2 concentration observed here were at least partly due to changes in those other hive characteristics. Southwick and Moritz (1987) observed that the presence and direction of light could affect the fanning behavior of worker bees, and that fanning increased during the daytime. While fanning behavior was not measured here, if the additional light in the hive due to the screened bottom board had any effect in this experiment, it was associated an increase, not a decrease, in CO2 concentration.

Daily changes in honey bee hive weight and temperature have been shown to convey information on colony growth and activity. Here, we observed that honey bee colonies maintained strong daily cycles of CO2 concentration, even when hive ventilation was increased and maintenance of those concentrations required more effort. These cycles, varying from an average minimum close to ambient up to an average maximum > 11,000 ppm during Experiment 1, may have parameters, such as maximum and minimum values, and rates of change at different times of the day (Ohashi et al. 2009) that can also be used to obtain information on bee colony health and activity.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

All authors contributed to the study conception and design. MW performed material preparation and data collection. WGM and AB conducted data analysis. WGM wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author on reasonable request.

CODE AVAILABILITY

R code used for analysis is available upon request.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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