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Metabolic Rate Of Honeybees At The Hive Entrance

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Abstract

Beekeeping in the Inland Pacific Northwest is difficult because of the area's seasonal extremes and unpredictable climate. Thermoregulation is one aspect of bee physiology that may be more energetically costly in such a climate. I looked at how metabolic rate of bees varied with ambient and hive temperatures of three hives during the summer of 2014. The prediction was as ambient temperatures increased the need for oxygen consumption would decrease. Honeybees were captured at their hive entrance and the metabolic rate (oxygen consumption) was measured using a modified respirometer. Ambient temperatures were recorded during the cooler morning hours when metabolic measurements were made. Data loggers recorded interior temperatures towards the hive centers and near the hive entrances every two hours from May 1st to September 1st. Oxygen consumption of the outbound honeybees varied from 21.330µl/100mg/hr to 147.750 µl/100mg/hr, and the ambient temperatures varied from 17°C to 30°C at the field site. The oxygen consumption decreased as temperatures increased in an inverse linear relationship (m= -5, P=.013, r^2=.295).The hive center and hive entrance temperatures showed no significant relationship with metabolic rate (P=.530, P=.859, respectively). The data loggers did show that temperatures in the hive center were moderated relative to air temperatures, consistent with hive thermoregulation found in similar studies. These findings support the view that honeybees regulate their metabolic rates for optimal foraging and hive thermoregulation.

Keywords: thermoregulation, temperatures, foraging

1. Introduction

Honeybees (*Apis mellifera)* are known worldwide for their potential as pollinators and providers of compounds useful to humans[1]. Modern honeybees are found in various climates and environments in both the southern and northern hemispheres[2,3]. Northern latitudes have longer winters increasing the need for stored hive honey required to maintain adequate hive temperatures[1].

Honeybees are heterothermic arthropods starting out as strict stenothermic larvae completely dependent on the adult bees for heat[2,4,5]. However, by the time honeybees develop into adults they are capable of alternating between ectothermic resting states or an endothermic state ready for flight[6]. This adaptation is quite different from a strict ectothermic insect where even a small change in their environment impacts the organism's ability to metabolize energy[7]. Honeybees can use this adaptation of being heterothermic to offset climate variation allowing honeybees to persist in a wide variety of ecological locations[8].

Hive thermoregulation is one activity where honeybees use endothermic activities to provide adequate temperatures for brood and adult bees[3,6,8] Honeybees shiver their thorax muscle to generate heat to maintain critical hive temperatures[8-10]. These thoracic muscles play another important role in regulating hive temperatures as bees use their wings to direct air flow[9]. This combination of heating and fanning in the hive gives honeybee's unique control of the hive environment[9]. The energy for honeybee metabolism is derived from plant nectar or pollen brought back to the hive from foraging activities during the seasonal nectar flows[1,3,11].

Nectar and pollen make up the hive honey that provides the stored energy for honeybees to care for brood and survive winters in temperate climates[1,11]. Hive thermoregulation is one aspect of honeybee physiology that can be more energetically costly in climates were nectar flow is unpredictable. Seasonal variations in nectar and pollen, combined with foraging actives in lower temperatures, have been suggested to have direct impact to honeybee thermoregulation and oxygen consumption[4,11]. Honeybees have an inverse relationship between metabolic rate and temperature as seen in most endotherms[4,10]. Honeybees, unlike strict ectothermic organisms, can use this adaptation to be successful foragers in seasonal variations by alternating between energy saving ectothermic activities to energetic endothermy[5,8]. To study these changes in honeybee activity metabolic measurements are more accurate when ordinary behavior is not restricted by capture and containment for laboratory evaluation[11]. This investigation was adapted to measure metabolic rates in the honeybee's natural environment.

The purpose of this experiment was to 1) measure the metabolic rate of honeybees as they launched from the hive entrance and 2) measure the temperature of the hive interior during seasonal nectar flow. The prediction was that metabolic rates would vary greatly between the honeybee's metabolic rate and temperatures in an inverse relationship typical to endothermic organisms. The hive interior hypothesis was that temperatures would vary with daily ambient and seasonal temperatures during the nectar flow and correlate to honeybee metabolic rate leaving the hive entrance.

2. Methods

A major component to this investigation was to measure honeybee's metabolic rate at the entrance of a Langstroth hive in a typical field setting. Langstroth hives are the industry standard for both commercial and hobby beekeeping[12,13]. Langstroth hives are generally divided into a top and bottom box with a solid top and a screened bottom board. The entrance to the hive is located at one end of the screened bottom board. The Langstroth hives were placed on four CMU construction blocks (20cm x 20cm x 40cm) set on their ends to keep hives off the ground and reduce predation.

2.1 Field Site And Specimens

The hives used in this experiment were located in Cheney Washington N47.42295° W117.56632° elevation 2293 feet, and located in the Intermountain Semi-desert sagebrush steppe division[14]. Three new hives were established in early-spring of 2014 (March/April); two side by side hives owned and managed by a local hobbyist and one hive owned by this researcher. The local hobbyist obtained honeybees from a commercial beekeeper in Spokane, Washington. The third hive is a feral hive that populated an empty Langstroth box baited with previous season honey and was located approximately 11.2 km west of the side by side hives. The colonies were allowed to prepare for summer foraging by supplemental feeding during the early spring months (March/April). A 1:2 water/sugar solution via in-hive feeders were in place until a noticeable nectar flow was observed (April/May). This investigation commenced measurements on May 21st and continued until August 21st. Metabolic rates were measured during the morning hours of 0700 to 1000 and on different dates.

2.2 Field Metabolic Rate Kit (FMRK)

A Field Metabolic Rate Kit (FMRK) was constructed by placing a plastic KOH screen with reservoir into a 40mL glass chamber open at one end. A Kimble Serological 1mL in 1/100th glass pipette was inserted through a hole in the No.4 rubber stopper. The stopper and pipette assembly was used to seal the open end of the 40 mL glass chamber and constitutes a single "Measuring Chamber." All the components of the Measuring Chamber came from the Carolina Small Animal Metabolism Classroom Kit #6822022 manufactured by the Carolina Biological Supply Company (Burlington, NC). The measuring chamber was secured to a portable tray and a 3cm plexi-glass cover used to prevent wind from altering pipette readings in the field.

2.3 Temperature Data

Interior hive temperatures were measured by placing four DS1921G Thermacron iButton Temperature Loggers (Whitewater, WI) inside the hives. One iButton was placed close to the entrance in the bottom hive box and one was

placed in the top back of the upper hive box in the two side by side hives. The four iButtons were set to collected temperatures every two hours starting on April 21st and were removed on September 6th. A standard alcohol thermometer from the EWU Biology stock room was used in the FMRK for reading temperatures when measuring the metabolic rate of the honeybees.

2.4 Measuring metabolic rate of honeybees

The FMRK was secured in a shaded area to prevent a solar reaction with the KOH in a sealed chamber. A single 200mg cotton ball was placed inside the plastic screened KOH reservoir and saturated with 1mL of 15% KOH prepared by the EWU Biology stock room. The saturated cotton ball and screened reservoir was placed inside the glass chamber. The glass pipette was pushed through a No. 4 rubber stopper. A small amount of water was placed on the pipette at the rubber stopper to create a tighter seal between the pipette and the hole in the rubber stopper. Another drop of water was placed on the narrow end of the rubber stopper right before inserting into the glass chamber to help seal that connection.

Single honeybees were captured at the entrance to a hive using a 50mL plastic vial. The vial and specimen was weighed (± 0.01g) three times and an average recorded. The vial was subtracted from total weight and the weight of the honeybee recorded. The specimen was transferred to the 40mL glass chamber and the pipette/stopper assembly installed to close the Measuring Chamber. The Measuring Chamber and one thermometer, was placed in the FMRK on wooden cradles. A small amount of indicator dye (red food coloring) was injected into the tapered end of the pipette. The position of the dye was noted in one minute intervals beginning when the indicator dye at the tapered end of the pipette reached the .90mL graduated mark. When the indicator dye reached the -.1mL graduated mark (when 1mL of oxygen had been consumed) the total time was recorded and the honeybee released at the hive entrance. The cotton ball in the KOH screen reservoir and pipette were replaced so more specimens could be measured. Equipment was cleaned with water between measurements and prior to storage. The FMRK was returned to the laboratory where all the components and tray were cleaned with mild soap and water then allowed to dry before reuse. Statistical analysis was performed with VassarStats linear regression and two-sample t-test for independent or correlated samples.

3. Results

The oxygen consumption decreased as temperatures increased in an inverse linear relationship (m= -5, P=.013, r^2=.295, y = -5.029x + 208.560,). Measurements included eight experimental runs using a total of 20 replicates. Ambient air temperatures at the FRMK at time of metabolic measurements ranged from 17°C to 30°C (Figure 1) Oxygen consumption for outbound honeybees varied in a wide range from 21.330 µl/100mg/hr. to 147.750 µl/100mg/hr. during seasonal nectar flow of July 7th to August 20th, (Figure 2). 29.5% of the variability of observed metabolic rate of outbound bees can be explained by the temperatures (Linear regression r^2=0.295).

In-hive temperatures were taken from May 1st to September 1st and recorded every two hours (Figures 3 and 4). Temperatures recorded at the hive center and hive entrance showed no significant relationship with metabolic rate (P=.530, P=.859, respectively, Figure 5) Mean hive interior temperatures varied from 16.54 °C to 30.94 °C (Figure 3) and mean hive temperatures at the entrance ranged from 9.19 °C to 29.67 °C (Figure 4). Only 2.25% of the variability of observed metabolic rate of outbound bees can be explained by hive interior temperatures (Linear regression r^2=0.023) and less than 1% of the variability of observed metabolic rate of outbound bees can be explained by hive entrance temperatures (Linear regression r^2=0.023). Therefore, interior hive temperatures did not affect the metabolic rate of outbound honeybees (Figure 5) and ambient temperatures had the greater impact on the outbound honeybees.

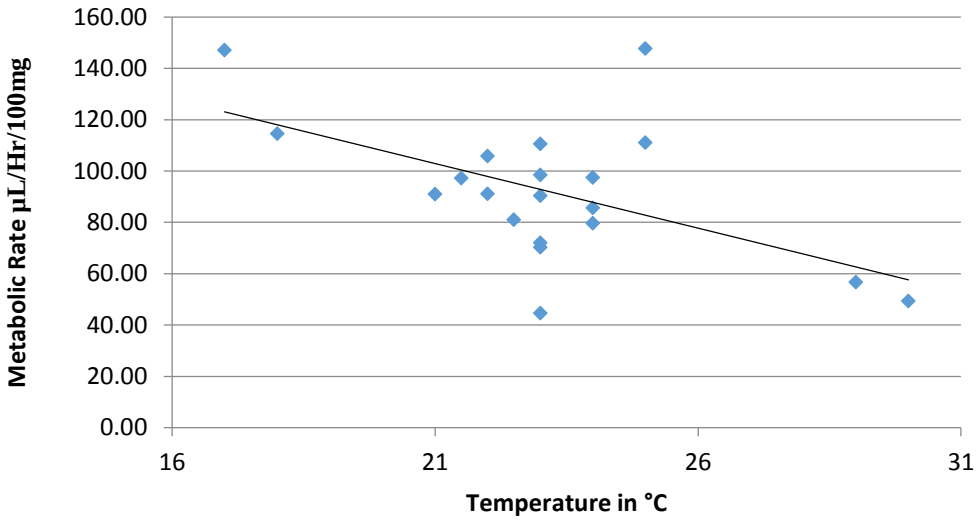

Figure 1. Inverse relationship between honeybee metabolism and ambient temperatures taken in the field. Each point in this graph represents an individual bee measured at the hive entrance.

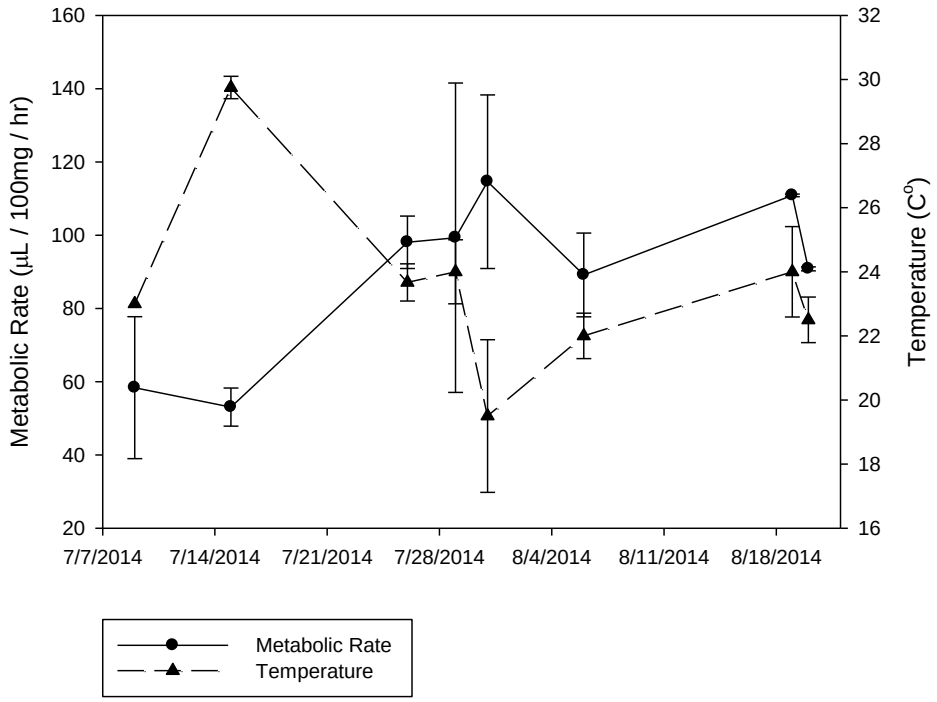

Figure 2. Metabolic rate measurements as honeybees leave the hive in an endothermic state ready for flight vary greatly from ambient temperatures. Each point represents the average of 3 to 5 bees measured on the same day. This graph shows the mean values of temperature and metabolic rate over the seasonal nectar flow.

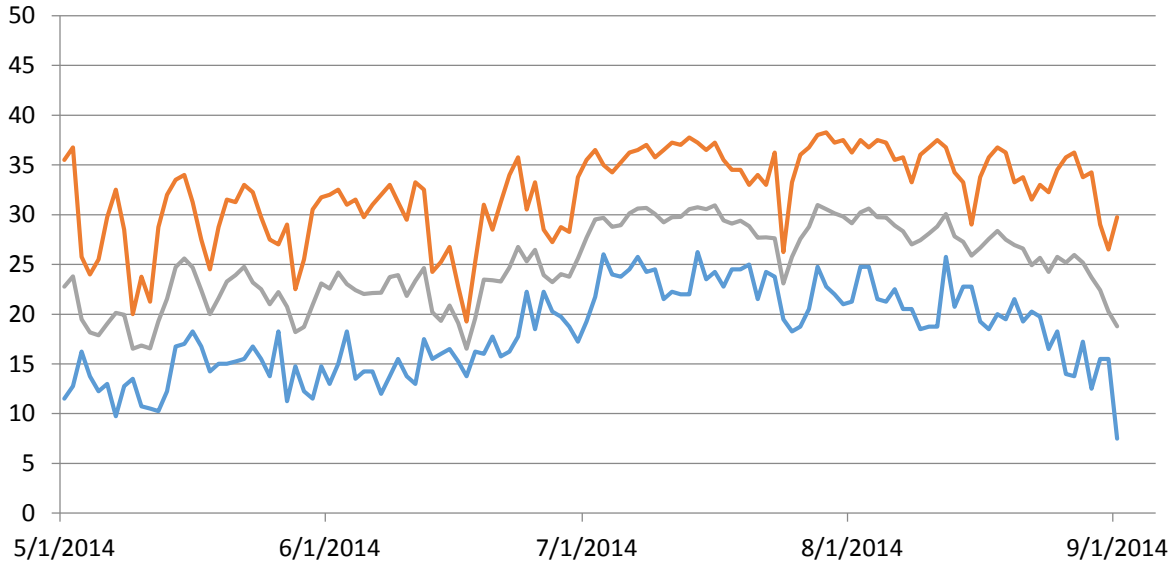

Figure 3. Temperatures of the deep hive interior measurements recorded by data loggers showing three ranges from minimum (lowest line) to maximum (highest line) with the mean in the middle. Interior temperatures varied greatly in the daily cycle with minimums and maximums close to the mean demonstrating greater control of deeper hive interior thermoregulation.

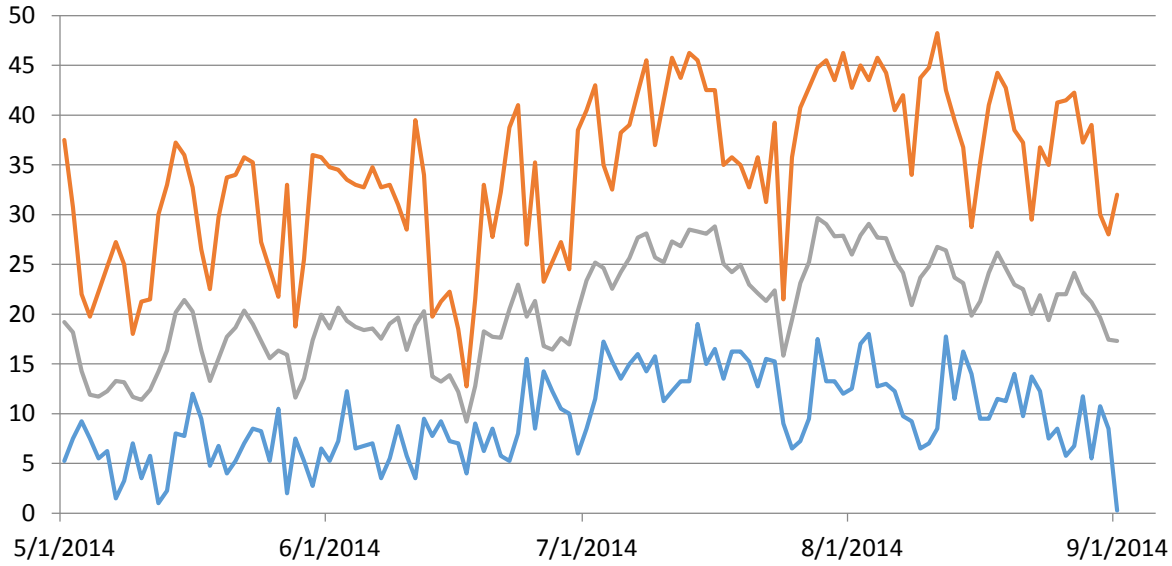

Figure 4.Temperatures of the hive interior close to entrance recorded by data loggers showing three ranges from minimum (lowest line) to maximum (highest line) with the mean in the middle. Daily cycles still vary greatly and the minimums and maximums have larger distance in the values demonstrating less thermo-regulative control close to the external environment.

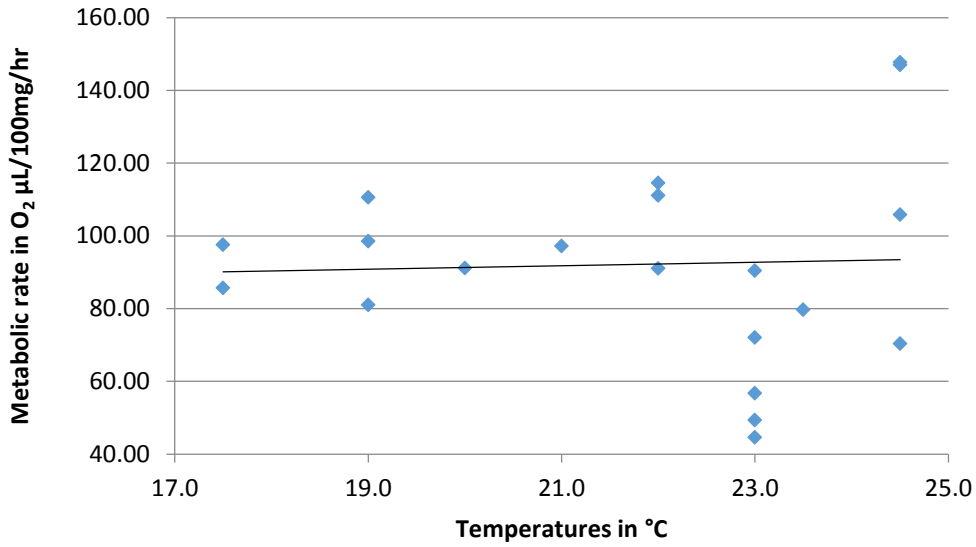

Figure 5. Metabolic rates measurements taken at the hive entrance and then compared to the deep hive interior temperatures demonstrated no significant relationship. Each point represent an individual bee measured at the hive entrance. The regression of metabolic rate on hive interior temperature (not shown) was similar and also not significant.

4. Discussion

These results showed that the metabolic rate of honeybees at the hive entrance is inversely related to outdoor air temperature[4],[10]. This data is consistent with previous studies showing that this type of endothermic activity is used to provide thermal homeostasis in the hive[6]. Data loggers in the hive interior measured smaller diurnal fluctuations than data loggers near the hive entrance, suggesting thermoregulation by the hive. Yet, the hypothesis that the metabolic rate and hive thermoregulation was related was not supported by my findings. Hive thermoregulation is preformed collectively by thousands of individual bees. Because adult bees vary in terms of the hive tasks they perform in a given day, the relationship between hive thermoregulation and the metabolic rate of an individual foraging bee is difficult to model[6]. A preliminary study preformed in the summer of 2013 demonstrated the large increase in the metabolic rate of honeybees after ingesting hive honey (Davis *unpublished data*). After being allowed to feed the results showed the metabolic rate of honeybees is dependent on available hive honey, derived from local flora nectar.

The critical balance between endothermic demands on honeybees and the availability of flora nectar is directly tied to the hives' geographical location[3,5,11]. This investigation was performed in a region where the nectar flow is limited by northern latitude short summers. Shortened flora availability is one aspect of honeybee physiology that is especially energetically challenging in this climate and has the most influence on individual honeybee's metabolic rates[11].

Beekeeping in the Pacific Inland Northwest faces two further challenges. First, with limited seasonal nectar flow the common beekeeping practice in this region is to supplement nectar by diluting a processed sugar with water into either a liquid or solid form to feed the honeybees. The major concern with this practice is honeybees require a large diversity of nectar sources to create the honey constituents needed to up-regulate detoxifying and immunity genes[1]. This is complicated by the wide range of processed sugars used to supplement the honeybees none, of which have been studied well enough to provide viable data.

The second aspect to challenging beekeeping in this region is the large number of packaged bees brought into the region each spring by honeybee enthusiasts. Packaged bees include a viable queen capable of laying over a thousand eggs per day while preparing her brood for the spring nectar flow[12,15]. A large and sudden increase to the local population of honeybees at the time when the overwintering hives are just recovering from winter may diminish the success of beekeeping in this geographical region. Further studies are needed to better understand the impact that increased populations of packaged honeybees have on the total resources available; and how to supplement honeybees with the correct nutrients for when nectar resource are low.

Many of the destructive honeybee pathogens heard in the media worldwide are common to the beekeeper of this region. One common honeybee disease is the tracheal mite *Acarapis woodi.* This internal parasite was shown in a study to restricted gas exchange and reduce foraging abilities that require higher metabolic rates[16]. Reduction of oxygen consumption is one value that can be readily measured in the field and could be used to better understand the pathogen.

Nosema ceranae, a microspirdian, and deformed wing virus (DWV), simultaneously infect honeybees and is another well know pathogens that is implicated in honeybee colony losses[17]. The frequency of *Nosema ceranae* is increasing and is now considered one of the major threats to global honeybee survival[18]. The observable properties of the disease is well understood, yet, the genetics information is limited and only currently being studied[18]. I suggest that metabolic rate measurements of honeybees with novel parasites can be used as a tool during the earlier stages of these pathogens to circumvent the collapse of the colony.

Metabolic rate measurements like the ones used in this experiment have the potential to be used as an indicator for monitoring honeybee health and this experiment was able to show how this indicator can be used directly at the hive entrance. This study further suggests establishing honeybee metabolic rate standards to a specific region so that the data could be used to monitor hives at location to determine if the hive is healthy. Metabolic rate measurements can also be extended to be a component of understanding the types of sugars and nutrient supplementation required for beekeeping in this region during low nectar periods.

Funding for promoting honeybee health in this direction has potential return to both hobbyist and commercial beekeepers. This in turn has direct influence on the agricultural sector which would benefit from these studies by increasing production from a healthier pollinator. Local beekeeping organizations and State Beekeepers Associations can provide avenues for alternate funding resources that could be used to gather regional data.

Honeybees face many challenges in today's world and climate. Studies have shown where the diversity of flora has reduced worldwide[19]. Honeybees face this challenge while being constantly exposed to compounds that are counterproductive to health and sustainability[19]. Novel parasites that have propagated from anthropogenic causes exacerbate the honeybee colonies taking advantage of the stressors on the individual bee and the hive collective[19]. Effective monitoring of honeybees is mandated by the human dependence on this pollinator's integral connection to the human food chain[19].

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