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(Article begins on next page)

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Acoustics related to the queen presence in honey bee (*Apis mellifera ligustica L.*) colonies

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***Abstract*—**The presence of a fertile queen is essential for the stability and productivity of a honey bee colony. However, her *status* is generally assessed through visual hive inspections, which are invasive, time-consuming, and performed at non-continuous intervals. For this reason, within the framework of the so-called precision beekeeping, there is growing interest in remote monitoring systems. The analysis of hive acoustics represents a promising approach for detecting variations in colony state. In this study, the potential of acoustic analysis to discriminate against the presence or absence of the queen in colonies of *Apis mellifera ligustica* Spin. was evaluated. Audio recordings were collected inside the hive during two experimental summer and autumn trials. The queen bee absence was simulated by isolating them in the secondary queen-rearing compartment of a French-like breeding hive. The acoustic signal was analyzed using Short-Time Fourier Transform (STFT) and spectral peak tracking within three selected frequency bands (80-140 Hz, 200-270 Hz, and 380-455 Hz). The results show a consistent increase in peak frequency within the middle band (200-270 Hz) following queen isolation, with a shift of approximately 20 Hz observed in three out of four colonies. In contrast, the lower and higher frequency bands investigated did not show systematic differences between simulated queenright and queenless conditions. These findings suggest that monitoring the peak frequency in the middle band may provide useful information on colony/queen *status* and represent a promising basis for the development of lightweight acoustic tools for queen monitoring in precision beekeeping. The ongoing study involving a larger number of colonies is however necessary to confirm the robustness and generalizability of such acoustic indicator.

Keywords—hive sound, queenbee, Precision Livestock Farming, Frequency band analysis

I. INTRODUCTION

The right functioning of the honey bee colony underpins both hive productivity and the provision of essential ecosystem services, particularly crop and wild plant pollination [1], [2]. Its functioning partly depends on the presence of a fertile and physiologically active queen, as she regulates reproduction and social organization through pheromonal and behavioural signals [3]. Her loss, especially when associated with biotic or abiotic factors, represents a critical event that can rapidly alter the internal dynamics of the hive, promoting demographic imbalances, the appearance of laying worker bees, behavioural disorganization, and a drastic reduction in productivity, potentially leading to colony collapse [4], [5], [6]. Traditionally, the queen's presence or absence is identified through visual inspections of the hive, either by visually inspecting the hive. However, these practices are invasive, time-consuming, and can be carried out occasionally, especially in large-scale professional beekeeping operations [7], [8], [9]. Diagnostic delays, often exceeding a week between inspections, can compromise the possibility of replacing the lost [5], [10] with desired genetic material. It is known that behavioural and physiological signals associated with queen loss can emerge within the first 24 hours, including changes in flight activity, worker behaviour and colony acoustic signals [11], [12], [13]. At the same time, these signals are not directly observable without continuous monitoring systems, and factors such as inter-colony variability, environmental conditions, and the lack of robust thresholds reduce the reliability of indirect approaches and the generalizability of predictive approaches [14], [15], [16]. Fortunately, recent years have seen increasing emphasis on the development of continuous, reliable, and non-invasive monitoring approaches for assessing colony status, supporting the broader concept of precision beekeeping [17], [18], [19], [20]. However, despite the growing interest in such monitoring strategies, the availability of reliable and easily implementable tools for the early detection of colony status, and more specifically queen presence, remains limited, and their effectiveness under real operative conditions is still under investigation [8], [9]. Recent studies have explored the use of acoustic signals as a non-invasive tool for continuous monitoring of colony status, with the aim of enabling the early detection of critical events [17]. This approach relies on the idea that the sound of a hive can be interpreted as a global signal, integrating the combined effects of individual and collective bee activities and reflecting functional and organizational changes within the colony [16], [21], [22]. The development of these remote monitoring systems can therefore enable the continuous acquisition of hive sound through microphones installed inside hives and integrated into IoT-based platforms [25], [26], [27]. In these studies, acoustic signals are generally analysed using processing pipelines that include signal segmentation, transformations in the time and frequency domains, and the extraction of acoustic descriptors such as Mel-frequency cepstral coefficients (MFCCs), which are widely used for the automated classification of the hive state [16], [28]. Several studies have investigated hive acoustic signals in relation to the presence or absence of the queen. In Howard et al. (2013), acoustic recordings from queenright and queenless colonies were compared using time- and frequency-domain analyses, revealing differences in

spectral distribution and suggesting the informative contribution of specific frequency bands. Several studies have highlighted that specific frequency bands of the hive acoustic signal are particularly sensitive to queen loss. Significant changes have been observed in the low-frequency range (100-400 Hz), including reductions in spectral energy and alterations in temporal dynamics associated with queenless conditions [12], [16]. In Nolasco et al. (2018), audio signals acquired within the hive were represented using MFCCs and analysed through supervised classification models, showing that queen presence or absence can be discriminated, albeit with a marked reduction in performance when models are validated on colonies not included in the training phase. Similarly, Kanelis et al. (2023) analysed continuous recordings before and after artificial queen removal, observing measurable changes in the signal in both time and frequency domains, with particularly evident differences in low-frequency components and a partial reversibility of the acoustic profile following queen reintroduction. More recent studies have applied machine learning and deep learning models to time/frequency representations of hive sound for queen presence classification, achieving good experimental performance even on architectures compatible with embedded hardware, although still in contexts limited by the number of colonies and the duration of observations [15], [29], [30]. In parallel, anomaly detection approaches applied to hive sound have demonstrated the ability to identify significant deviations from a reference acoustic behaviour, suggesting that structural alterations of the signal, potentially also associated with queen loss, may emerge as unsupervised anomalies [31], [32], [33]. Overall, acoustic analysis emerges as a potentially promising tool for monitoring queen presence or absence within the hive. However, the existing literature highlights limitations related to inter-colony variability, dependence on experimental conditions, and limited model generalizability in real operational settings, underscoring the need for further validation on diverse datasets [9], [16], [34]. Moreover, most of the existing studies focus on comparisons between queenright and queenless colonies without exploring how the acoustic profile evolves across the day. To date, few contributions have investigated the during the day dynamics of hive sound under different colony conditions, despite the potential value of such information for improving diagnostic reliability and identifying early deviations in colony state.

In light of these considerations, the present study aims to evaluate the potential of acoustic analysis as a non-invasive tool for discriminating queen presence or absence in colonies of *Apis mellifera ligustica* Spin. Specifically, the study seeks to assess whether the sound signal recorded inside the hive exhibits measurable and consistent variations between queenright and queenless conditions, induced artificially, in a controlled experimental context, also considering their dynamics between daytime and nighttime periods.

II. Matherials and Methods

A. Apiary and selected hives

For this study, *Apis mellifera ligustica* Spin. colonies, provided by a queen bee breeder listed in the National Register of Italian Bee Breeders [35], were used and housed in two French-type dual-compartment hives for queen rearing. Each hive consisted of a size standard compartment for colony maintenance, and a smaller compartment that was used to host the queen during the simulated orphaning procedures. The apiary the experimental colonies belonged to was located at the Experimental-Educational Farm "Nello Lupori" (University of Tuscia, Viterbo, Italy) (42.4271454° E; 12.0796127° N; 302 m a.s.l.) [36]. The study was structured into two experimental trials conducted during different periods in 2025 with the aim of evaluating the potential seasonal effect on the acoustic characteristics of the colonies. The first trial was carried out from July 18 to 26, during the peak beekeeping season; the second was conducted from October 3 to 13 (one additional day compared to the previous trial due to adverse weather conditions), following pre-winter varroa mite treatments. In the first test, colonies A and B were monitored. In the second test, due to the natural queen loss (and subsequent collapse) of colony B, it was replaced with a third *A. m. ligustica* colony, designated as colony C.

B. Queens' manipulation and hive sound recording

At the beginning of the experiment, a V71 Vormooi digital voice recorder with an integrated microphone (Vormooi, China), was placed in the main compartment of each hive, between the frames containing the queen, at approximately 16 cm below the inner cover. Acoustic recordings were performed at a 16 kHz sampling rate, selected as a compromise between signal resolution and file size, with a selectable bitrate up to 1536 kbps. After 72 h, orphaning was simulated by transferring the queen to the secondary compartment of the hive, attended by a suitable number of workers covering about three frames, thereby inducing a queenless condition in the colony. The queen was kept separated from the main colony for 96 h. At the end of the queenless phase, the microphone was removed and the queen was reintroduced into the colony. To avoid recording sounds generated by hive handling procedures (*e.g.*, microphone placement or queen searching), audio recording was temporarily paused until all handling operations were completed. Sound records were stored as WAV files.

C. Sound recordings manipulation and analysis

The frequency spectrum of the audio recordings was analyzed to assess whether queen loss induces measurable changes in the spectrogram shape. Specifically, the analysis investigated whether characteristic frequency peaks shifted in correspondence with bee activity associated with queen absence. To mitigate the effects of environmental noise and microphone sensitivity, peaks were computed only within the frequency ranges most relevant to bee acoustics, thereby reducing the influence of external sounds.

These ranges were identified from the overall spectrum computed over the entire recording period and across all hives. The spectrogram was obtained via the Short-Time Fourier Transform (STFT). For each recording, the magnitudes of the frequency bins were averaged over time and then normalized. The analysis was limited to the 50–2000

Hz band to suppress low- and high-frequency noise, since bee-generated sounds are predominantly concentrated within this interval [11], whereas components outside it were mainly attributable to noise or sources external to the hive. The resulting average spectral shape is shown in Fig. 1.

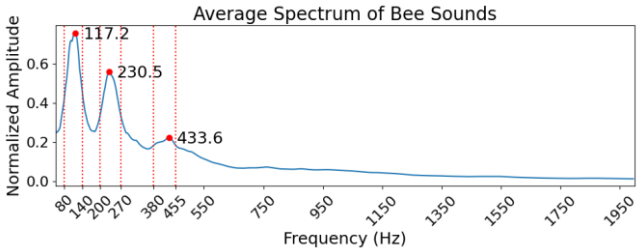


Fig. 1. Average spectrum of audio recordings. Red points indicate the peaks values, while red dotted lines highlight the bands selected.

According to [11], from the overall spectrogram are visible the sound of "Piping" (100-200 Hz and 300-400 Hz) and "Wangle Dance" (200-300 Hz). From this shape, three main frequency bands are selected, as listed in Table I.

TABLE I. FREQUENCY BANDS SELECTED

Low Band (Hz)	80 - 140
Middle Band (Hz)	200 - 270
High Band (Hz)	380 - 455

After band selection, the next step was to analyze the peak positions over time. To this end, the audio was segmented into five-seconds windows (Fig. 2). For each window, the spectrum was further extracted using STFT and the bin magnitudes were averaged over time. A Savitzky–Golay filter was then applied to smooth the spectrum and improve peak detection (Fig. 2).

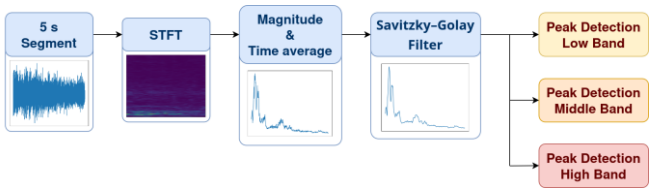


Fig. 2. Overview of the Peak Detection processing pipeline.

Peaks were computed within the three bands for both the conditions: *i.e.*, queen present (queenright state) or queen absent (queenless state). Peak frequencies were collected over one hour, and the hourly mean was computed to track peak evolution throughout the day, separating by daytime and nighttime using local sunrise and sunset times, enabling the analysis of diurnal differences in colonies' behavior.

III. RESULTS AND DISCUSSION

A. Middle-Band Peak Frequency Tracking

The peak distribution for the **Middle Band** is shown in Fig. 3 and Fig. 4. Each point represents the hourly mean of the detected peak frequency. Discontinuities indicate hours in which no peaks were detected within the selected band. The corresponding average spectral shape is reported below each plot: the black dotted line represents the average over the entire queenright or queenless period, whereas the colored lines represent the averages computed separately over daytime and nighttime intervals.

The July recordings for hives A and B are shown in Fig. 3(a) and Fig. 3(b), respectively, while the October experiments conducted on hives A and C are depicted in Fig. 4(a) and Fig. 4(b). A seasonal difference is evident in the day–night peak positions. In summer, peak frequencies are higher during daytime, whereas this gap is almost absent in autumn.

With respect to queen presence, all four experiments show an increase in peak frequency after queen removal. Under queenright conditions, middle-band peaks typically remain around 220-230 Hz. In three out of four hives, a clear increase is observed, with peaks shifting to an average of approximately 250 Hz. Hive B shows a less pronounced change, as shown in Fig. 3(b): during the initial recordings, peaks vary between 220 and 240 Hz. After queen removal, an increase is still detected, but with smaller magnitude.

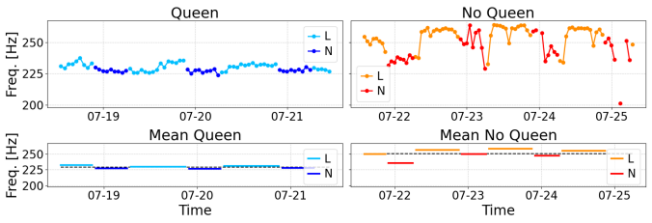
B. Low- and high-band frequency peak tracking

For the **Low Band** and **High Band**, the peak distributions do not show marked differences between the queenright and queenless conditions. However, the July recordings still highlight a day–night effect, particularly in the **Low Band**, where peak frequencies tend to increase during daytime. Fig. 5 shows the peak distributions for the **Low Band** across the four hives.

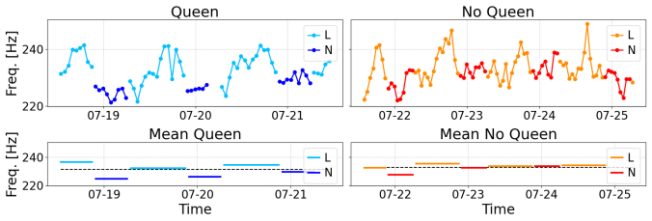
C. Discussion of peak-frequency behavior

The experiments were conducted in two different seasons, enabling the observation of distinct colony behaviors. In summer, colony population and overall activity are typically higher, and the diurnal analysis shows how the spectrum evolves during daylight hours compared to nighttime, when a larger fraction of bees is inside the hive. Overall, the nighttime spectrum appears more stable and smoother than the daytime spectrum. Seasonal effects also influence peak-frequency behavior, likely due to reduced external activity during colder periods. The **Middle Band** (200-270 Hz) emerges as the most informative range for detecting queen loss. Three hives out of four exhibit a frequency shift of approximately 20 Hz, while hive B shows a smaller shift (about 5 Hz), which is nonetheless detectable.

Overall, these results support an association between the "Warble" frequency (within the 225-285 Hz range) and the colony status. Monitoring this frequency band may therefore provide useful information on queen presence and, more generally, on colony condition.

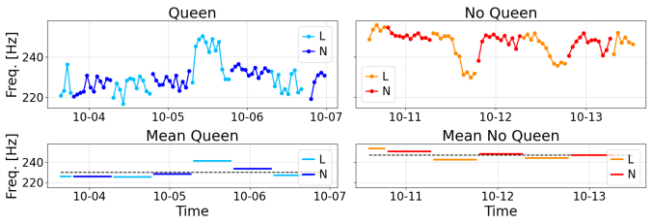


(a) Hive A, Middle Band, recorded in July

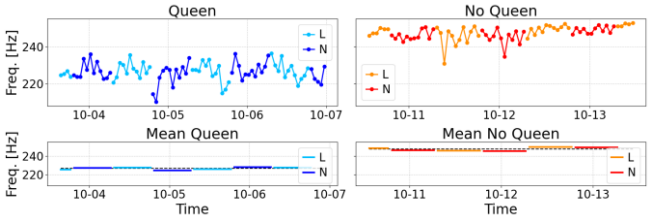


(b) Hive B, Middle Band, recorded in July

Fig. 3. Frequency peak positions for the hives before (**Queen**) and after (**No Queen**) queen removal. Light colors denote daytime hours (**L**), while dark colors denote nighttime hours (**N**). Mean along the entire period and along light/night segment is displayed below.



(a) Hive A, Middle Band, recorded in October



(b) Hive C, Middle Band, recorded in October

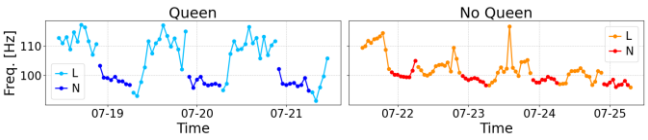
Fig. 4. Frequency peak positions for the hives before and after queen removal. Mean along the entire period and along light/night segment is displayed below.

IV. Conclusions and Future Perspectives

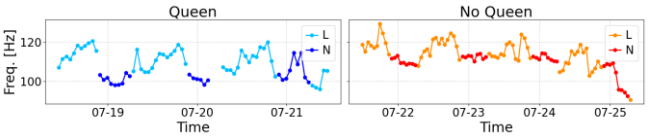
This study proposes an innovative approach to investigate honey bee colony status through continuous in-hive audio recording. By leveraging spectrogram-based features and analyzing peak distributions within frequency ranges relevant to bee sound, the results indicate that audio can provide significant information about colony condition. In particular, within the 200–270 Hz band, a consistent change is observed after queen isolation, characterized by an increase in peak frequency. This behavior is detected in all the experiments on three different bee colonies and across two seasons, supporting an association between queen absence and a shift in middle-band peak frequency. Moreover, the recording protocol adopted in this work proves suitable for comparative analysis across colonies.

On the other hand, extracting STFT-based features is computationally lightweight compared to alternatives such as MFCCs and deep learning models commonly used in the literature for queen absence detection. This makes the proposed approach a promising starting point for the development of embedded algorithms and tiny machine-learning solutions deployable on low-power devices, enabling continuous and non-invasive monitoring directly inside the hive.

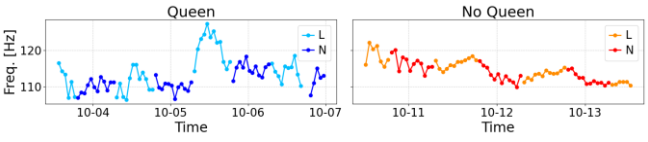
Future work will expand the dataset through additional recordings using the same experimental setup, under both similar and different conditions (e.g., different bee Spins or microphones), to further validate robustness and generalizability.



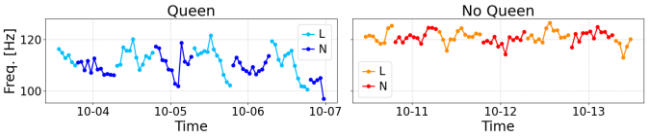
(a) Hive A, Low Band, recorded in July



(b) Hive B, Low Band, recorded in July



(c) Hive A, Low Band, recorded in October



(d) Hive C, Low Band, recorded in October

Fig. 5. Frequency peak positions for the hives before and after queen removal.

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