Acoustic phenotyping reveals sublethal pesticide stress in bees using BEEhaviourLab

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Abstract

Behavioural responses often provide the earliest detectable signs of physiological stress yet quantifying them at scale remains challenging. Acoustic behaviours such as buzzing are central to insect locomotion and communication but are rarely measured systematically, and their potential as sensitive indicators of environmental stress remains largely unexplored. Here, we test whether changes in insect acoustic behaviour can reveal sublethal effects of pesticide exposure. Using a newly developed automated behavioural platform (BEEhaviourLab), we recorded synchronised video and audio from insects, enabling simultaneous, high-throughput quantification of locomotor activity and buzzing behaviour across multiple individuals and species without the need for tagging. We applied this system to characterise the effects of the veterinary antiparasitic moxidectin on the bumble bee *Bombus terrestris*. Acute contact exposure produced dose-dependent reductions in locomotion and buzzing behaviour at concentrations below lethal thresholds. Acoustic measures detected behavioural disruption with sensitivity comparable to video-based activity metrics, demonstrating that buzzing behaviour provides a sensitive and previously unused indicator of neurotoxic stress. More broadly, scalable multimodal behavioural phenotyping enables subtle behavioural disruption to be detected across large experiments, opening new opportunities to incorporate acoustic endpoints into studies of animal behaviour, environmental stress, and ecological risk assessment.

Keywords

Sublethal toxicity; Behavioural phenotyping; Acoustic behaviour; Automated insect tracking; Pollinators; High-throughput screening

1. Introduction

Behaviour often provides the earliest detectable signs of physiological disruption [1,2], yet quantifying behavioural change at scale remains challenging [3]. In toxicology and environmental risk assessment, most regulatory tests still rely on lethal dose estimates obtained from a small number of model species [4]. These assays provide reproducible benchmarks for chemical hazard but reveal little about how chemicals alter behaviours that determine fitness and reproduction, leaving many ecologically relevant effects undetected [5]. This gap largely reflects the difficulty of measuring behaviour in a standardised and scalable way: behavioural responses are inherently variable and multidimensional, making them difficult to translate into quantitative endpoints that can be compared across individuals, experiments, and species [6].

Among behavioural endpoints, locomotion provides a particularly informative indicator of neurophysiological stress [7,8]. Many neurotoxic compounds disrupt sensory processing or motor control, leading to measurable changes in activity, coordination, or movement patterns [9,10]. Sublethal exposure can translate into large fitness consequences by limiting foraging efficiency, navigation, and predator avoidance [11–13]. Locomotion in flying insects is tightly linked to thoracic flight muscle activity, which also generates acoustic signals through vibration of the thorax and wings. In bees, these vibrations produce characteristic buzzing sounds that accompany locomotion, flight preparation, thermoregulation, defensive signalling, and floral sonication [14–16]. Acoustic behaviour has been particularly well studied in honey bees, where vibrational and airborne signals additionally play roles in communication [17] and have been used to infer colony-level state, including the prediction of swarming events [18,19].

Acoustic signals reflect motor output and neuromuscular function and therefore have the potential to provide sensitive indicators of physiological stress while enabling simple, scalable behavioural measurement. To test this, we developed BEEhaviourLab, a low-cost platform that combines synchronised video and acoustic recording with automated behavioural analysis. The system supports long-duration experiments across many individuals without physical tags or manual scoring, enabling quantitative comparison of locomotor and acoustic activity under controlled exposure conditions.

As a model case, we examined the effects of the widely used veterinary pesticide moxidectin, a macrocyclic lactone commonly applied in livestock management. Residues of these compounds can persist in dung, creating environmentally realistic exposure pathways for non-target insects [20–22], including bees [21,23]. Macrocyclic lactones bind glutamate- and γ-aminobutyric acid (GABA)-gated chloride channels in invertebrates [24], suppressing synaptic transmission and disrupting behaviours linked to sensory processing and motor control [22,25–27]. Despite evidence that these compounds can disrupt insect behaviour and occur at environmentally relevant concentrations, their effects on the behaviours that underpin pollination, such as movement and activity, remain largely unknown in bees.

Using BEEhaviourLab, we quantified insect activity using synchronised video and acoustic measurements across multiple species. Video-based tracking captured clear differences in locomotor behaviour and circadian organisation in honey bees (*Apis mellifera*), bumble bees

(*Bombus terrestris*), ivy bees (*Colletes hederae*), and hoverflies (*Eristalis tenax*). Acoustic behaviour proved particularly informative in bees, especially the social species *A. mellifera* and *B. terrestris*, where buzzing closely mirrored locomotor activity and reproduced circadian structure. In bumble bees, acoustic measures of buzzing also detected behavioural disruption following moxidectin exposure with sensitivity comparable to conventional video-based locomotion metrics. Together, these results demonstrate that BEEhaviourLab enables scalable multimodal behavioural phenotyping across insect taxa and identify acoustic behaviour as a powerful and previously underused indicator of stress in bees.

2. Methods

2.1. BEEhaviourLab equipment

BEEhaviourLab is a modular, distributed platform for automated, high-throughput recording of insect behaviour under controlled experimental conditions. The system can scale from a single recording unit to many parallel nodes, each operating independently but coordinated through a central control computer (Figure 1A-B). Each node integrates synchronised video recording (Raspberry Pi NoIR v2 camera), audio capture (RØDE smartLav+ microphone), and environmental monitoring (temperature and humidity via a DHT22 sensor). Illumination is provided beneath the transparent perspex recording arena using LED strip lights, which can operate in white (daylight) or red (night-mode) settings. The physical enclosure is constructed from laser-cut perspex and assembled using plastic fasteners or adhesive (Figure 1A). A Raspberry Pi 4B microcontroller (4 GB RAM) manages recording and sensor acquisition.

Experimental parameters are defined in a human-readable configuration file, allowing users to specify metadata (e.g. species, treatment, replicate), recording duration, inter-recording intervals, number of recording segments, and video and audio capture settings (Figure 1C). This approach enables identical experimental protocols to be deployed across multiple nodes with minimal manual intervention. Nodes are accessed remotely via SSH over Wi-Fi from the central control computer, which initiates recordings and coordinates data acquisition across the system. Each recording segment is automatically compressed and stored with structured metadata before being transferred to the central computer for storage and downstream analysis.

All hardware designs and Raspberry Pi control software are fully open source and available on GitHub (BEEhaviourLab/BEEhaviourLab-apparatus). The modular architecture allows users to adapt the system for different experimental needs, including adding sensors, modifying acquisition parameters, or extending recording and control functionality.

2.2. Automated insect tracking

Insects in BEEhaviourLab video recordings were automatically detected and tracked using YOLO version 8 “nano” (YOLOv8n, Ultralytics), a lightweight object-detection architecture suitable for rapid fine-tuning and deployment on standard computing hardware. To support reuse and adaptation, we provide a step-by-step tutorial for retraining the model on user-defined insect datasets (GitHub: BEEhaviourLab/BEEhaviourLab-YOLO-training).

Training and validation data were derived from video frames extracted from BEEhaviourLab recordings. Frames were manually annotated in Label Studio (labelstud.io) with two object classes: *bee* and *feeder*. Two YOLOv8n models were trained for 100 epochs: model 1 was trained on 155 annotated frames from recordings of bumble bees (*Bombus terrestris*); and model 2 was trained on a multi-species dataset comprising 455 annotated images across four insect species: honey bee (*Apis mellifera*), ivy bee (*Colletes hederae*), bumble bee (*Bombus terrestris*), and hoverfly (*Eristalis tenax*).

Initial tracking across frames was performed using the BoT-SORT algorithm [28], which is designed for scenarios where the number of tracked objects may vary over time. The algorithm includes mechanisms to recover identities when detections are temporarily lost (e.g. due to occlusion), but in our recordings it frequently generated new IDs when individuals reappeared. Because the number of insects in the arena was fixed, we applied an additional post-processing step after BoT-SORT tracking. For each frame, detections were matched to a fixed set of identities (IDs 1…N) using the Hungarian algorithm [29], minimising Euclidean displacement from positions in the previous frame. When detections were temporarily missing, the last known position was propagated forward and an empty record emitted, maintaining constant population size and preventing the creation or deletion of identities.

Model performance was evaluated on validation datasets consisting of recordings from independent experimental cohorts, ensuring no overlap between training and evaluation data. Performance metrics included recall (proportion of true objects detected), precision (proportion of detections that are correct), mAP50 (mean average precision calculated using detections with at least 50% overlap between predicted and true bounding boxes), mAP50-95 (mean average precision averaged across overlap thresholds from 50% to 95%), and the F1-score (harmonic mean of precision and recall)

Locomotor activity was quantified from the tracked positions of each individual insect across frames. Pixel coordinates were converted to physical distance using a calibration factor derived from the known dimensions of the recording arena, allowing movement to be expressed in mm regardless of recording resolution. Movement speed was calculated as the frame-to-frame displacement of each insect within the arena and used as a simple, robust measure of activity for comparing behavioural responses across treatments and time periods.

2.3. Audio analyses

Audio recordings were processed using a custom pipeline to detect segments containing buzzing or flight sounds. Recordings were resampled to 16 kHz and bandpass-filtered (200–8000 Hz) to focus on bee-relevant frequencies. Frame energy was calculated using 0.1 s windows with 0.05 s overlap, and a robust threshold (median + k×MAD, k = 5) was used to classify frames as containing sound or silence. Short segments (<0.1 s) were removed, and gaps (<0.15 s) were filled to reduce spurious detections. For each 60 s recording, we calculated the proportion of time containing buzzing sounds.

2.4. Tracking performance across insect species

To evaluate the flexibility of the automated tracking pipeline across taxa, four insect species were recorded in BEEhaviourLab: honey bee (*Apis mellifera*, n = 21), ivy bee (*Colletes hederae*, n = 3), hoverfly (*Eristalis tenax*, n = 5), and bumble bee (*Bombus terrestris*, n = 33). Honey bees, ivy bees, and hoverflies were wild-caught immediately prior to recording and released afterwards. Bumble bees were obtained from commercial colonies (BioBest, Westerlo, Belgium; via Agralan, Swindon, UK). Individuals were housed one per cage.

Recordings were conducted under a 12 h light:12 h dark cycle (lights on 06:00–18:00). Video recordings consisted of 5 min segments collected at 25 min intervals over a 48 h period, yielding 96 recordings per cage. These data were used to evaluate detection and tracking performance across species differing in body size, morphology, and movement patterns.

2.5. Moxidectin toxicity in bumble bees

To demonstrate the application of BEEhaviourLab for sublethal toxicity assessment, we quantified behavioural effects of moxidectin on *Bombus terrestris* over a 48 h period following contact exposure. Individual bees received a 1 µl droplet applied to the dorsal thorax containing moxidectin (Cydectin Pour-On formulation, 0.5% w/v) diluted in DMSO. Exposure doses were 0 (vehicle control; n = 30), 0.782 (n = 33), 7.82 (n = 36), 78.2 (n = 33), 782 (n = 30), and 7,820 (n = 45) ng per bee. Following exposure, bees were placed in clear perspex recording cages (three bees per cage) and provided with 1 M sucrose via a syringe feeder.

Recordings were conducted under a 12 h light:12 h dark cycle (06:00–18:00 light). Video and audio were recorded for 1 min every 14 min over a 48 h period, yielding 192 recordings per cage. Bees exposed to the highest dose (7,820 ng) experienced complete mortality within 1–12 h and were excluded from subsequent behavioural analyses. At the end of the experiment, sucrose consumption, body mass, and cage-level mortality were recorded. Surviving bees were euthanised by freezing overnight.

2.6. Statistical analyses

Statistical analyses were performed in R Studio [30]. Behavioural data were analysed using generalized linear mixed-effects models implemented in glmmTMB [31], with cage identity included as a random intercept in all models to account for repeated measures. Model diagnostics were assessed using simulation-based residual checks in DHARMa [32] and model-based estimates and contrasts were obtained using emmeans [33]. Model predictions and effect sizes are reported with 95% confidence intervals derived from the fitted mixed-effects models using Wald approximations based on the estimated standard errors.

Locomotor behaviour was analysed using a two-part mixed-effects framework separating behavioural state from movement intensity. Bees were classified as moving or inactive using a data-derived speed threshold based on the low-speed mode of the speed distribution. Movement probability was modelled using a binomial GLMM with a logit link, while movement speed conditional on activity was modelled using a Gamma GLMM with a log link. For the moxidectin

experiment, log-transformed dose and time were included as covariates; for the cross-species dataset, species and time were included as fixed effects. Similarly, audio-derived behavioural measures were analysed using zero-inflated beta GLMMs, separating the probability of complete inactivity from the distribution of non-zero proportions. Dose and time were included as covariates with cage identity as a random intercept.

Circadian patterns of activity were analysed using harmonic mixed-effects models including 24 h sine and cosine terms. The presence of circadian rhythmicity and its modulation by treatment or species were evaluated using likelihood-ratio tests.

To summarise multimodal behavioural effects, we calculated a sublethal toxicity index based on cycle-averaged predicted deviations from control behaviour across locomotor and acoustic metrics. Effects were scaled by the standard deviation of control behaviour, producing an index expressed in units of control behavioural variability (SD). A threshold of one control SD was used to estimate the dose at which sublethal behavioural effects first emerged.

3. Results

3.1. A single multi-species detector enables accurate, tag-free tracking

We evaluated whether a single computer-vision detector could support tag-free tracking across diverse pollinator taxa (Figure 1). We selected four ecologically relevant and experimentally tractable insect pollinators spanning gradients in body size, morphology, social organisation (eusocial and solitary), and flight dynamics: *Bombus terrestris* (eusocial bumble bee; mean length 15.6 mm), *Apis mellifera* (eusocial honey bee; 13.3 mm), *Colletes hederae* (solitary bee; 12.2 mm), and *Eristalis tenax* (solitary hoverfly with a distinct dipteran body plan and flight kinematics; 10.4 mm).

A YOLOv8n model trained exclusively on *B. terrestris* images (model 1, 155 annotated images) achieved high detection performance for bumble bees (precision, recall, and F1 > 0.97; Table 1) but generalised poorly to other taxa. Performance was lowest for *C. hederae* (precision and recall < 0.50) and was moderately reduced for *A. mellifera* and *E. tenax*, particularly under motion blur. We therefore trained a second model (model 2) using 455 annotated images from all four species (bumble bee: 155 images; other species: 100 images). This multi-species model achieved consistently high performance across taxa (mean average precision > 0.98 for all species; Table 1), demonstrating robust detection across differences in body size, morphology, and movement dynamics. Together, these results show that incorporating morphological diversity during training enables a single lightweight detector to support accurate, tag-free tracking across pollinator taxa within a shared experimental framework.

3.2. Multimodal tracking reveals species-specific activity patterns

Using the multi-species detector, we quantified locomotor activity over 48 h (Figure 2A). Species differed markedly in both the probability of movement and locomotion speed when active. Bumble bees showed the highest probability of movement (P = 0.76, 95% CI 0.68–0.82), whereas honey

bees had the highest average speeds when active (2.16 mm s^{-1}, 95% CI 1.92–2.43). Hoverflies were both less likely to move and slower when active. Activity declined slightly over time, but this effect was small relative to interspecific differences. A harmonic mixed-effects model revealed a strong 24-h periodic component overall, with significant species differences in both circadian amplitude (sin24: $\chi^2 = 58.0$, $p = 1.6 \times 10^{-12}$) and waveform (cos24: $\chi^2 = 162.1$, $p < 2 \times 10^{-16}$). Honey bees and ivy bees showed high-amplitude diurnal rhythms, whereas bumble bees remained more active across the diel cycle, producing a weaker circadian rhythm. Hoverflies showed comparatively weak and irregular circadian structure.

Acoustic activity also differed between species (Figure 2D). Buzzing behaviour was quantified by classifying short audio frames as containing buzzing or not and calculating the proportion of time with buzzing within each 60 s recording (see Methods). Bumble bees showed the highest levels of acoustic activity, whereas ivy bees and hoverflies produced lower levels of detectable buzzing. Among recordings containing buzzing (Figure 2E), bumble bees exhibited the highest mean activity (0.208, 95% CI 0.163–0.261), significantly exceeding ivy bees (0.102, 95% CI 0.063–0.161; Holm-adjusted $p = 0.039$, EMM) and marginally exceeding hoverflies (0.111, 95% CI 0.070–0.173; $p = 0.058$, EMM). Honey bees showed intermediate activity (0.141, 95% CI 0.117–0.169) and did not differ significantly from the other species after correction. Species also differed in the probability that an entire recording contained no buzzing (zero-inflation component; Figure 2F). Bumble bees had a lower probability of complete inactivity (0.067, 95% CI 0.052–0.086) than honey bees (0.127, 95% CI 0.112–0.144; $p < 0.001$) and ivy bees (0.224, 95% CI 0.178–0.278; $p < 0.001$). Structural zeros were rare in hoverflies, making zero-inflation estimates unstable. Across species, acoustic activity increased modestly over time ($\beta = 0.071 \pm 0.018$ SE, $p < 0.001$).

3.3. Moxidectin exposure induces dose-dependent behavioural impairment in bumble bees

We next examined the effects of acute moxidectin exposure on bumble bee behaviour over 48 h following contact exposure to moxidectin or the vehicle control (Figure 3). Locomotion was analysed using a two-part mixed-effects framework that separates the probability of movement from movement speed conditional on activity. Increasing dose produced a strong, monotonic reduction in the probability of movement (log-dose effect: $\beta = -0.91 \pm 0.12$ SE, $z = -7.58$, $p < 0.0001$), declining from 0.66 in controls to 0.50 at the lowest dose (0.782 ng bee^{-1}) and to 0.13 at the highest dose (782 ng bee^{-1}, Figure 3B). Among bees that were active, locomotion speed also decreased with increasing dose ($\beta = -0.13 \pm 0.03$ SE, $z = -4.49$, $p < 0.0001$), but the magnitude of this effect was smaller (Figure 3C).

Acoustic measurements showed parallel dose-dependent effects on buzzing behaviour (Figure 3D). Increasing dose increased the probability that an entire recording contained no buzzing activity (zero-inflation component; Figure 3E; $\beta = 0.514 \pm 0.022$ SE, $z = 23.7$, $p < 0.0001$), with even the lowest dose increasing inactivity relative to controls and progressively larger increases at higher doses. Among recordings containing buzzing, activity declined more modestly with increasing dose (Figure 3F; $\beta = -0.232 \pm 0.057$ SE, $z = -4.10$, $p < 0.0001$) and over time ($\beta = -0.0495 \pm 0.0144$ SE, $z = -3.43$, $p < 0.0001$).

Moxidectin exposure also altered the temporal organisation of activity. Interactions between dose and both harmonic components were highly significant (sin24: $\chi^2 = 56.3$, $p = 1.8 \times 10^{-11}$; cos24: $\chi^2 = 36.1$, $p < 0.0001$). While the lowest dose produced little detectable change in circadian patterns, higher doses altered the amplitude and phase of activity cycles, with the strongest effects at the highest dose (782 ng bee^{-1}).

3.4. Multimodal behavioural integration identifies a sublethal effect threshold for moxidectin in bumble bees

To integrate behavioural responses across modalities, we defined a sublethal toxicity index combining dose-specific deviations from control in movement probability, locomotion speed, and buzzing probability. Components were scaled by natural between-individual variability observed in control bees. The resulting sublethal toxicity index increased monotonically with dose, indicating progressively larger behavioural disruption across modalities (Figure 4A).

We defined the lowest observed effect concentration as the lowest dose at which the multimodal toxicity index exceeded 1 (i.e., the mean predicted effect across components was at least one control SD from baseline). An index ≥1 therefore reflects a behavioural deviation exceeding natural baseline variability, establishing a standardized effect-size threshold for comparison with lethal responses. The index crossed this threshold at an estimated dose of 2.73 ng bee^{-1} (log-linear interpolation between tested doses), with larger deviations at higher doses. Contributions to the composite index were not uniform across components (Figure 4B), with reductions in movement probability contributing most strongly, followed by reductions in buzzing probability, and smaller contributions from changes in locomotion speed among active bees.

We quantified survival and feeding behaviour over the same 48 h period. Moxidectin caused a clear, dose-dependent increase in mortality, with a 48 h LD_{50} of 81.2 ng bee^{-1} (Figure 4C). Complete mortality occurred at the highest dose (7820 ng bee^{-1}) within 1–12 h; this dose was excluded from the behavioural assay. Feeding behaviour, measured as total sucrose consumption per cage, also declined with increasing dose, with reduced consumption detectable from 78.2 ng bee^{-1} onward (Figure 4D).

4. Discussion

This study shows that acoustic activity provides a practical behavioural signal for detecting sublethal neurotoxic effects in bees. Across multiple bee species, buzzing activity tracked locomotor behaviour and circadian organisation, indicating that acoustic signals reflect underlying behavioural state. In bumble bees, acoustic measurements detected dose-dependent behavioural impairment following moxidectin exposure with sensitivity comparable to conventional video-derived locomotion metrics. Integrating acoustic monitoring with automated video tracking therefore expands the behavioural signals that can be quantified in high-throughput phenotyping systems.

4.1. Scalable behavioural phenotyping across insect taxa

Automated behavioural phenotyping has transformed toxicology and neurobiology by enabling scalable behavioural screening across model organisms including *Caenorhabditis elegans* [34,35], zebrafish [10,36], and aquatic invertebrates such as *Daphnia* [37]. In insects, similar approaches have been developed for *Drosophila*, where automated platforms support high-throughput behavioural analysis and pharmacological screening [9,38,39]. However, comparable systems for standardised behavioural toxicology across insect pollinators remain limited.

Recent advances in bee monitoring have begun to address this gap. In-hive and semi-field tracking systems can generate detailed behavioural datasets spanning individual and colony-level processes [40], while laboratory platforms tracking tagged individuals within microcolonies enable detailed analysis of social behaviour [41]. These approaches provide important insights into colony dynamics and task allocation but are typically tailored to specific behavioural contexts.

BEEhaviourLab complements existing bee monitoring systems by providing a generalised platform for high-throughput behavioural phenotyping under controlled laboratory conditions. BEEhaviourLab is the first automated insect phenotyping platform to integrate acoustic monitoring alongside video tracking. This addition captures a behavioural dimension that has largely been overlooked in automated systems. In bees, buzzing is closely linked to key behavioural and physiological processes, including pollination, thermoregulation, defence, and social communication [16,17,42], and therefore provides a direct proxy for behavioural engagement and motor activity. Consistent with this biological role, buzzing activity in our experiments closely tracked locomotor behaviour, particularly in social species such as honey bees and bumble bees. Incorporating acoustic monitoring therefore expands the behavioural signals accessible to automated phenotyping and provides a scalable framework for studying behaviour in buzzing insects.

4.2 Sublethal effects of moxidectin reveal risks of veterinary pesticides to pollinators

Our results indicate that the veterinary antiparasitic moxidectin can cause substantial behavioural impairment in bumble bees at environmentally relevant concentrations [22]. Although macrocyclic lactones are widely used in livestock management and their ecological effects have been studied primarily in dung-associated arthropods [22,27], their impacts on pollinators remain poorly understood. The strong behavioural responses observed here therefore suggest that veterinary pharmaceuticals may represent an underappreciated source of risk to pollinator communities.

We observed clear sublethal effects of moxidectin on motor behaviour in bumble bees. Similar impairments to locomotor, circadian, and flight-related activity have been reported for other neuroactive pesticides affecting bees, including neonicotinoids [12,13,43,44]. In those systems, disruptions to basic motor activity scale to higher-level behavioural outcomes such as reduced foraging efficiency and altered resource collection, ultimately affecting individual and colony fitness. The motor impairments quantified here therefore likely represent early behavioural indicators of ecologically significant pesticide effects.

To quantify the onset of such behavioural disruption, we integrated locomotor and acoustic endpoints into a multimodal sublethal toxicity index. This index provides a practical framework for identifying the doses at which behavioural anomalies first emerge. Applying this approach revealed substantial behavioural impairment at an estimated dose of 2.73 ng bee^{-1}, more than an order of magnitude below the 48 h LD_{50} (81.2 ng bee^{-1}). This separation highlights the potential for multimodal behavioural phenotyping to identify ecologically meaningful pesticide effects at concentrations that would remain undetected using conventional mortality-based endpoints.

4.3. Limitations

Several limitations should be considered when interpreting these results. Tag-free insect tracking does not maintain individual identity across recordings, and trajectories were linked using nearest-neighbour continuity rather than an explicit motion model. As a result, identity swaps may occur during close interactions, and gaps in detection were handled conservatively by carrying positions forward rather than interpolating missing detections, which could introduce bias into downstream motion metrics. In addition, species-level circadian comparisons were confounded by differences in rearing history: bumble bees were laboratory-reared whereas other species were wild-caught, and laboratory conditions may influence circadian entrainment and behavioural flexibility. Experimental exposures were also acute and conducted under simplified laboratory conditions, enabling precise dose–response estimation but not capturing chronic exposure dynamics, social feedback, or task allocation present in natural colony environments. Finally, behavioural analyses focused on a limited set of locomotor and acoustic endpoints selected for robustness and cross-modality integration. Richer behavioural features, including posture changes, fine-scale motor patterns, and detailed acoustic signatures, remain to be explored in future work.

4.4. Conclusions and outlook

This study demonstrates that incorporating acoustic behaviour into automated phenotyping enables sensitive detection of sublethal neurotoxic effects. BEEhaviourLab provides a scalable framework for quantifying behavioural impairment and linking sublethal neurobehavioural disruption to conventional toxicity benchmarks. The platform also establishes a foundation for richer behavioural analysis. Continuous trajectory and audio data support behaviour segmentation, state-based modelling, and classification of behaviours such as feeding, resting, grooming, and abnormal motor activity. Coupled with machine learning approaches for behaviour annotation, these tools provide a pathway toward scalable and standardised behavioural phenotyping across insect species.

More broadly, automated platforms such as BEEhaviourLab support a growing shift toward incorporating behavioural endpoints into ecotoxicological screening. Standardised behavioural metrics measured across species and stressors create opportunities to quantify sublethal pesticide effects and to define biologically meaningful thresholds for environmental risk assessment. By making subtle neurobehavioural impairment measurable at scale, multimodal phenotyping systems can help bridge the gap between laboratory toxicity testing and the ecological consequences of chemical exposure.

5. Author contributions

Conceptualization: R.H.P. Methodology: R.H.P., J.S., O.N.F.K., and C.N. Software: R.H.P., O.N.F.K., and C.N. Investigation: J.C.Z.K., A.S., and K.W. Data curation: R.H.P. and O.N.F.K. Formal analysis: R.H.P. and O.N.F.K. Visualization: R.H.P. Supervision: R.H.P., G.A.W., and S.R. Project administration: R.H.P. Funding acquisition: R.H.P. Writing (original draft): R.H.P. Writing (review and editing): All authors.

6. Funding

Funding for this project was provided by Schmidt Sciences through the Schmidt AI in Science Fellowship awarded to R.H.P. and C.N., and by the Crankstart Foundation through the Crankstart Internship Programme awarded to A.S. Additional support for J.C.Z.K was provided by the Royal (Dick) School of Veterinary Studies.

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8. Figures

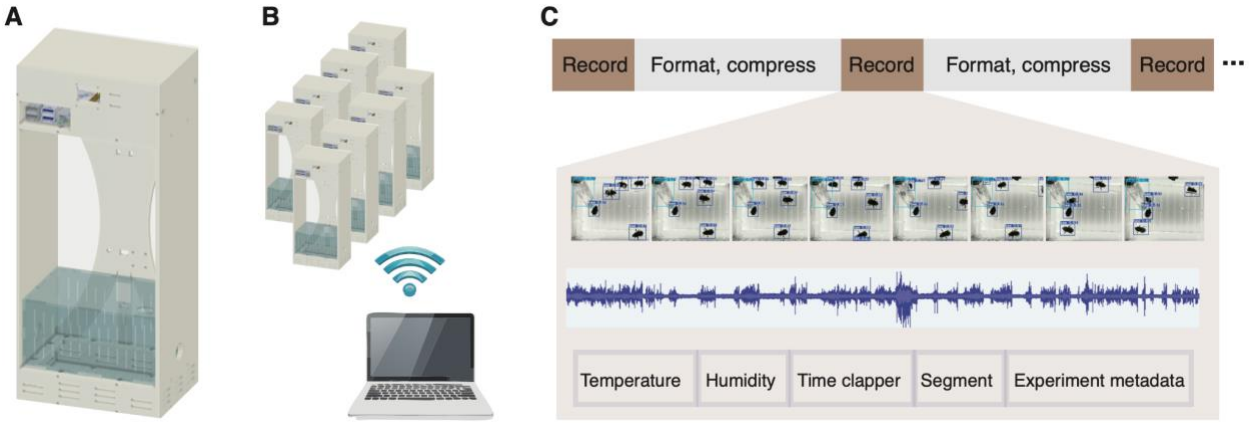

Figure 1: High-throughput insect behavioural tracking using BEEhaviourLab.

(A) Schematic of a single BEEhaviourLab unit, showing the Perspex enclosure (white) and transparent recording arena (clear). (B) Multiple units operate in parallel and communicate wirelessly with a central node computer. (C) User-defined recording protocols enable flexible specification of recording duration (brown), inter-recording intervals (grey), and number of recording segments. During inter-recording intervals, video, audio, sensor data, and experimental metadata are formatted, compressed, and transferred to the central node via Wi-Fi.

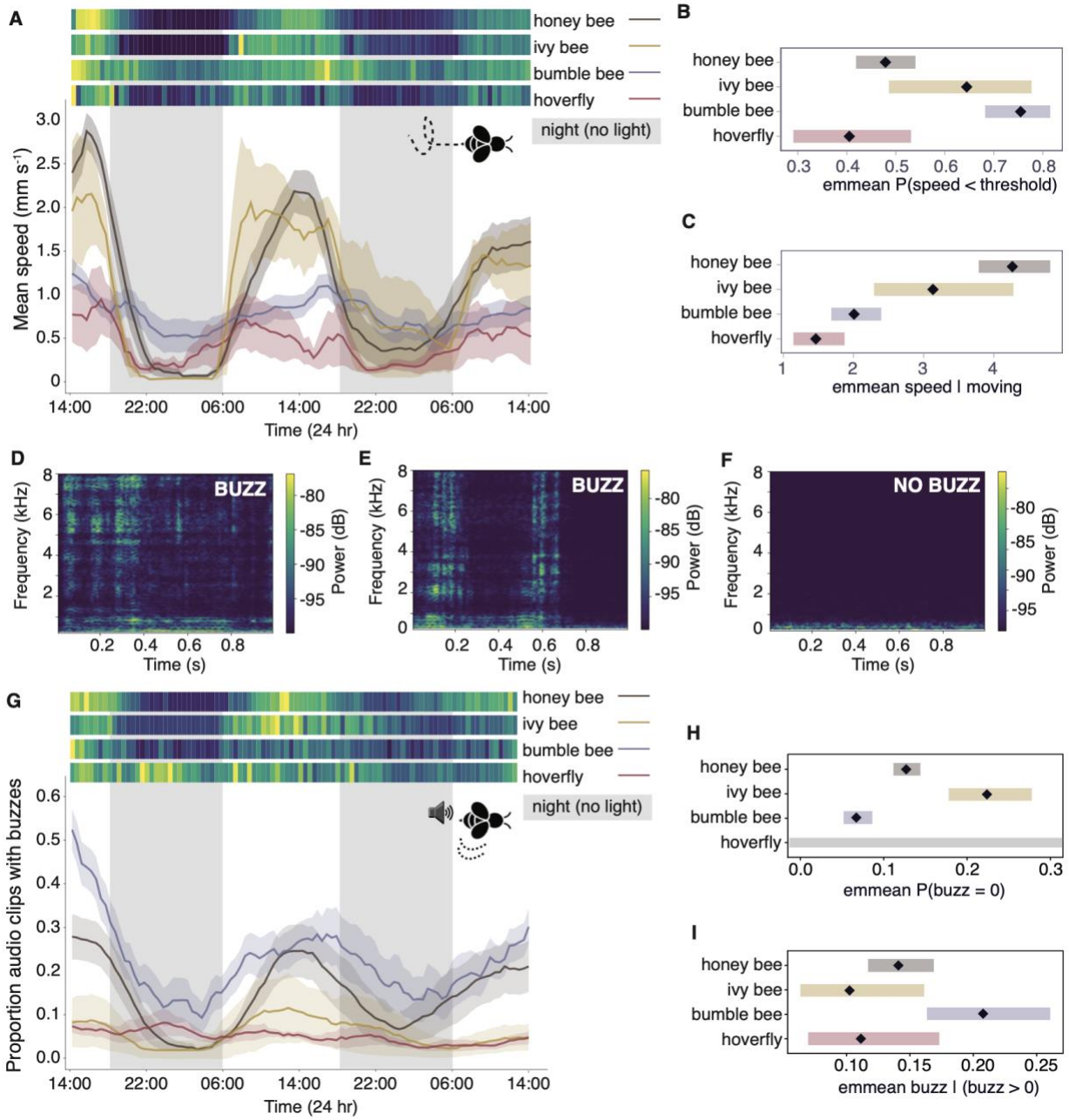

Figure 2: Cross-species locomotor and acoustic activity patterns.

(A) Locomotion speed over 48 h by species; heatmaps are scaled to species-specific maxima, with lines showing mean ± SD across individuals. (B) Estimated marginal means (EMMs) for probability of movement. (C) EMMs for locomotion speed conditional on movement. (D–F) Example spectrograms showing audio clips with buzzing (D–E) or no buzz (F). (G) Proportion of clips containing buzzes over 48 h by species; heatmaps scaled to species-specific maxima, lines show mean ± SD. (H) EMMs for probability of no buzzing (hoverflies excluded due to absence of complete zeros). (I) EMMs for proportion of clips with buzzes, conditional on non-zero observations.

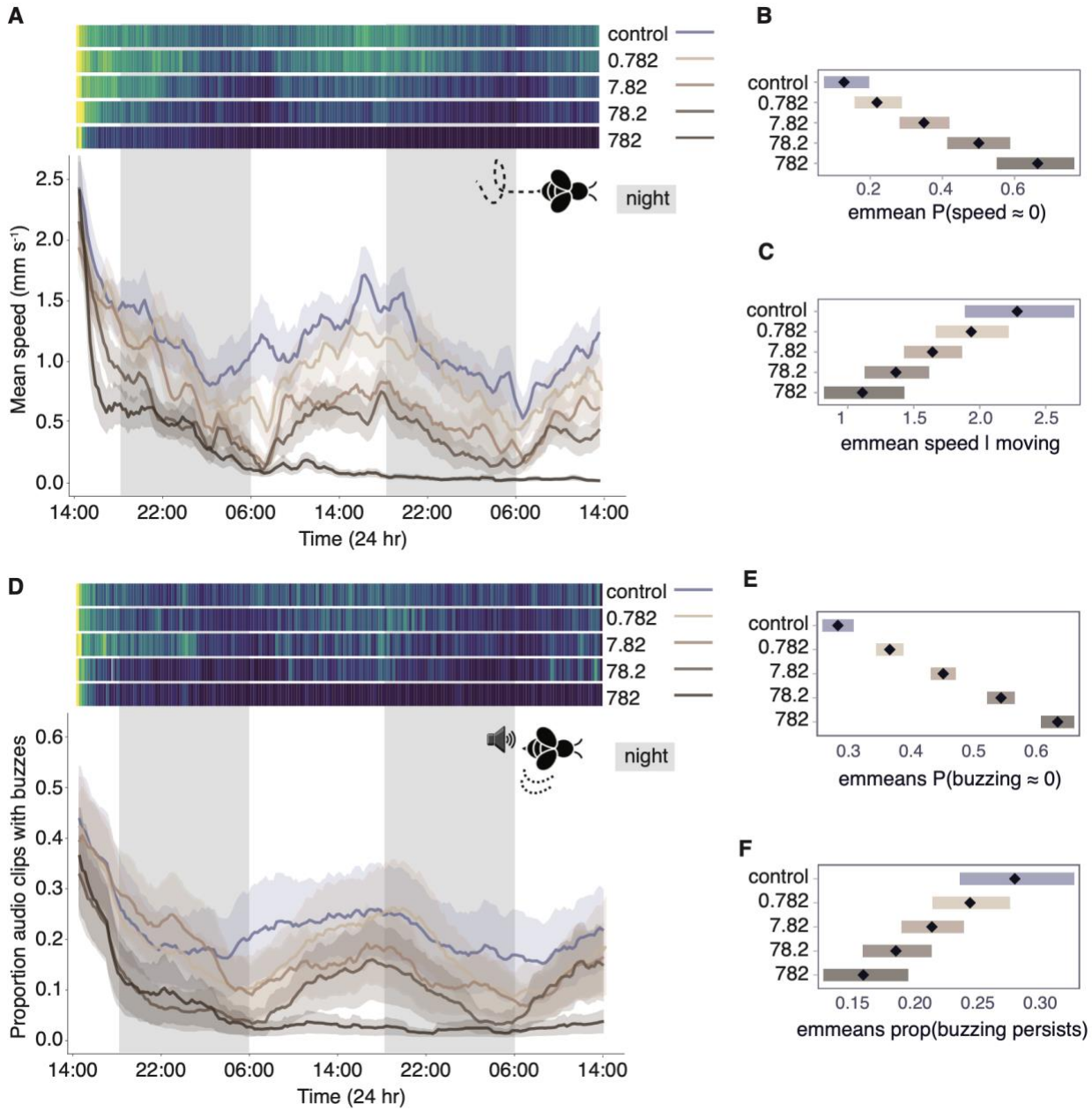

Figure 3: Moxidectin induces dose-dependent reductions in locomotion and buzzing.

(A) Mean locomotion speed over 48 h across moxidectin treatments; heatmaps are scaled to the maximum within each treatment group. (B) Estimated marginal means (EMMs) for the probability that speed falls below the movement threshold, showing dose-dependent increases in inactivity. (C) EMMs for locomotion speed conditional on movement, indicating more modest dose-dependent reductions among active bees. (D) Mean proportion of 1 s clips containing buzzing over 48 h across treatments; heatmaps scaled to treatment-specific maxima. (E) EMMs for the probability of no buzzing, showing dose-dependent increases in complete inactivity. (F) EMMs for buzzing persistence conditional on buzzing occurring, indicating smaller reductions once buzzing is initiated.

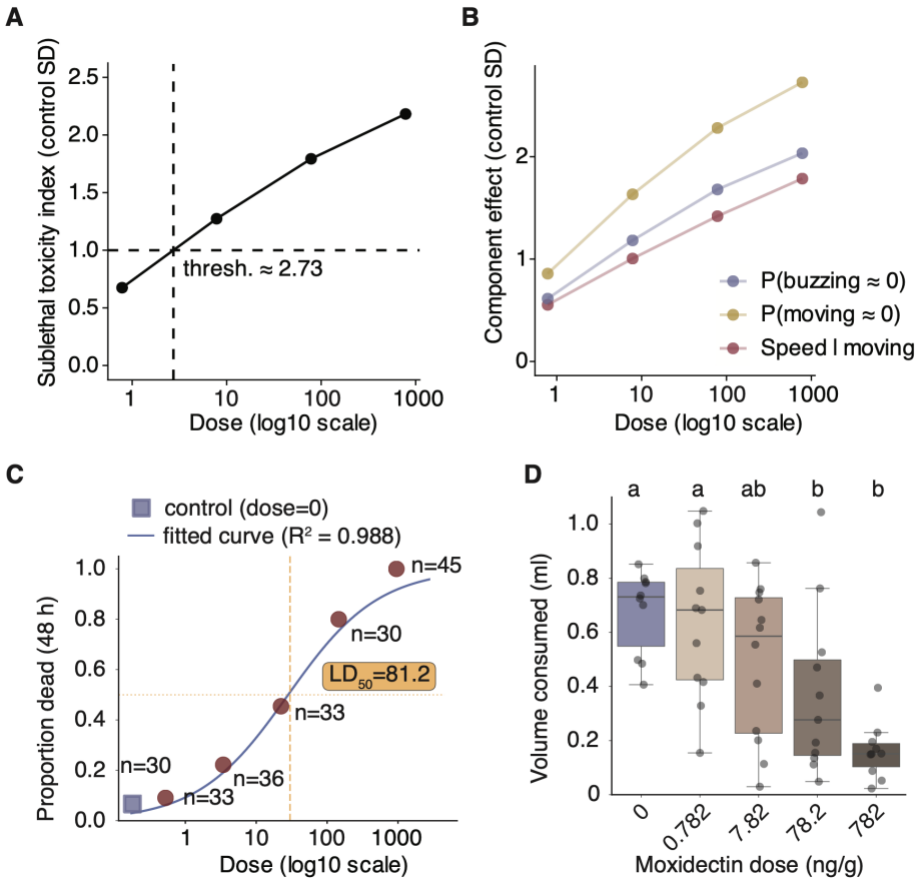

Figure 4. A multimodal behavioural toxicity index detects sublethal effects below lethal thresholds.

A: Sublethal toxicity index integrating deviations in locomotion and buzzing relative to control variability. The dashed line indicates a threshold of functional abnormality at one control standard deviation, crossed at 2.73 ng bee^{-1} moxidectin. B: Relative contributions of individual behavioural components to the composite toxicity index, showing dominance of changes in behavioural engagement over graded performance effects. C: 48 h contact mortality curve with an estimated LD_{50} of 81.2 ng bee^{-1} moxidectin. D: Total sugar syrup consumption per cage (three bees per cage) across control and moxidectin treatments. Letters denote significant differences between doses based on estimated marginal means.

Table 1: YOLO model benchmarking across bee species.

Model	Species	N validation images	Precision	Recall	F1-Score	mAP50	mAP50-95
model 1	bumble bee	50	0.98	0.97	0.98	0.98	0.77
	ivy bee	38	0.50	0.45	0.47	0.47	0.37
	honey bee	40	0.76	0.67	0.74	0.74	0.39
	hoverfly	38	0.83	0.67	0.75	0.75	0.36
model 2	bumble bee	50	0.98	0.98	0.98	0.98	0.85
	ivy bee	38	0.99	0.99	0.99	0.99	0.85
	honey bee	40	1.00	1.00	1.00	1.00	0.84
	hoverfly	38	1.00	0.99	0.99	0.99	0.64