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The potential of bioacoustics for surveying carrion insects

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ABSTRACT

Knowledge of the sequential cadaver colonization by carrion insects is fundamental for post-mortem interval (PMI) estimation. Creating local empirical data on succession by trapping insects is time consuming, dependent on accessibility/environmental conditions and can be biased by sampling practices including disturbance to decomposing remains and sampling interval. To overcome these limitations, audio identification of species using their wing beats is being evaluated as a potential tool to survey and build local databases of carrion species. The results could guide the focus of forensic entomologists for further developmental studies on the local dominant species, and ultimately to improve PMI estimations. However, there are challenges associated with this approach that must be addressed. Wing beat frequency is influenced by both abiotic and biotic factors including temperature, humidity, age, size, and sex. The audio recording and post-processing must be customized for different species and their influencing factors. Furthermore, detecting flight sounds amid background noise and a multitude of species in the field can pose an additional challenge. Nonetheless, previous studies have successfully identified several fly species based on wing beat sounds. Combined with advances in machine learning, the analysis of bioacoustics data is likely to offer a powerful diagnostic tool for use in species identification.

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Introduction

Estimation of the post-mortem interval (PMI) is a key factor in criminal investigations involving human remains, as it informs the scope and direction of inquiry around a reconstructed timeline of a crime. Inaccurate estimation of the PMI can misdirect the investigation, waste resources and hinder lines of inquiry. Ultimately, this may play out in court if not recognized and potentially lead to unreliable evidence and/or contribute to a miscarriage of justice. PMI can be estimated based on the physiological changes in the body in the first 96 hours after death or by examining the carrion insects present on and around the body at the time of discovery^{1,2}. Certain species of the order Diptera (true flies) associate with the body within minutes of death and remain in proximity until the late stages of decay. Adult, carrion breeding flies attend decomposing remains to lay their

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offspring and, where immature specimens are collected from remains, these specimens can provide a timeframe for the length of time a body has supported their growth. Their lifecycle consists of four stages: eggs, larvae (also called maggots), pupae and adult (fly), and is facilitated by the environmental conditions, primarily temperature^{3–5}. The time that it takes for their development under different temperature conditions has been documented for many commonly encountered species^{6–8}. Such temperature-dependent development data are used to model growth rates and allow prediction of specimen age based on the thermal conditions under which the specimen developed. Hence, their lifecycle and development on the body in accordance with the environmental conditions are used to estimate the period of insect activity and PMI⁹. Additionally, the arrival and colonization of a body by species, known as insect succession, is orderly and predictable for a given geographic region and climatic conditions^{5,10}. Knowledge of the expected insect succession patterns can provide a basis for estimating the time frame between death and colonization and combined with immature specimen age estimates can indicate a minimum PMI. In the late stages of decomposition, it is difficult to determine the oldest individuals of a single species, as often colonizing insects have completed their development. Thus, the community composition of all species present is instead used to predict the PMI based on knowledge of the predicted successional order^{11,12}.

Many successional studies have been undertaken throughout the world to establish successional data for use in entomological estimation of PMI. However, the arrival of the different insect species on the body and their abundance is dependent on climatic conditions, seasonality, geographical location, and habitat^{13–16}. For example, the community composition in agricultural land in Australia is significantly different to that of a bushland community¹⁷. This contrast is amplified by the season with some species acting as primary (early) colonizers in summer, while being secondary (subsequent colonizers) in other seasons or inactive altogether in the cooler seasons^{16,17}. Certain species, such as the 'hairy maggot blow fly' *Chrysomya rufifacies* Macquart 1842 (Diptera: Calliphoridae), are known as both a primary and secondary colonizer depending on the geographical location^{18,19}. The black soldier fly, *Hermetia illucens* Linnaeus 1758 (Diptera: Stratiomyidae), is universally known as a colonizer in the late stages of decay²⁰, yet a study of pig decomposition in South Georgia observed the species from the first week of cadaver placement²¹. Since succession can be different in different locations, baseline, reference data used for estimation of PMI in legal proceedings should ideally be from the same geographical area^{22,23}. Additionally, the individual conditions of the body, for example, presence of drugs²⁴ or inaccessibility,¹⁰ can also impact insect arrival timeframes and ongoing association with the body. Consequently, baseline data of insect succession, should be established under the various conditions and parameters known to influence the colonization of decomposing remains for improved accuracy when calculating the PMI.

Sampling procedure and guidelines

Current procedures for building reference data of the carrion insect community includes invasive physical sampling of live and preserved larvae, fly capturing by sweeping, and subsequent laboratorial species identification²⁵. The preserved immature specimens are used to determine the life stage at the time of discovery for the purpose of calculation to

the day they were deposited, in accordance with previously obtained experimental data. The adult and/or reared live specimens are used for developmental data and identification using visual examination of key morphological features.

This physical and time-consuming approach is necessary and unavoidable at crime scenes but may introduce a component of error in data related timeframes due to investigator disturbance of the decomposition environment during collection. Using this method to conduct successional studies on a local and reproducible scale is also a challenge due to various concerns primarily associated with sampling design and time requirements. The sampling intervals can impact the data and community composition either by collecting an inadequate representative sample by under sampling, or even oversampling which can interfere with the population and consequently the related progression of decomposition/succession^{26,27}. Manual trapping can introduce bias as only the flies present in the trap will be counted. Sudden drops in temperature can cause temporary immobilization for certain species that are only active in narrow temperature ranges²⁸. The temporary sampling intrusion may also interfere with the colonizing insect's activity and mating behaviour. Visual surveying is also not always possible at isolated locations or where sampling access is restricted by replicating common death scenarios for example, where a body is wrapped, concealed, placed in cars, or buried. In such studies, the data rely on all the species, or their remnants collected at the end of a trial.

The sampling method and subsequent analyses require practitioners to commit to long-term studies covering seasons and annual replication. According to Wallman and Archer²⁹, there are 10 to 15 active practitioners in Australia and New Zealand. Limited researcher availability can result in inadequate replication and failure to sample all environmental, individual, and circumstantial impacts on insect lifecycles relevant to forensic cases²⁶. The low number of practitioners also limits the research on spatial variability. This implies that forensic entomologists may need to rely on previously published data from different regions, yet such data may not be accurate or accepted in a court of law. For example, most successional data publications in Australia originate from the Eastern states and Perth^{30,31}, while there are no published data from the Northern Territory; despite the significant variation in climate among these regions³². On a global scale, most research and publications originate from the USA³³, while other countries are still in the process of establishing standardized protocols for the collection and use of carrion insects in legal cases³⁴. The USA has been at the forefront of the field due to its experimental facilities that allow taphonomic research on human cadavers. Such facilities are now operational or launching in other part of the world, including Australia. Although, practitioners have raised the issue of interdependence between cadavers at the experimental sites^{26,35} as the ongoing access to nutrient resources promotes species richness and diversity, including a thriving population of opportunistic species³⁶.

To address for these concerns, more studies need to be performed on human cadavers with long and time-consuming insect surveying, invasive manual capturing of individuals, and identification along with interpretation of their presence by experienced entomologists³⁷. Recent advances in machine learning and audio processing offer an alternative methodology for obtaining such data that has the potential to minimize investigator disturbance of the body during surveying and reduce the heavy time

commitment of collection and identification. Here, we suggest the use of species-specific sound ('buzzing') and machine learning (ML) to identify species attending decomposing remains and thus, help forensic entomologists focus on the dominant species in the local area for further developmental studies, which could inform PMI estimations in local casework. In this paper, we review current knowledge on insect audio identification and discuss the potential for taphonomic insect database building.

History

The identification of insects using their sound is not a novel concept, particularly for pest control or prevention of fly-borne diseases. Its use was first mentioned in the publication of termite detection in 1909³⁸. Since then, an ever-increasing number of papers have been published with promising results for the detection of various insects' species^{39,40}. To assess if this method can be adapted to carrion flies for surveying, first there is a need to understand the mechanism of sound production and causes of species-specific sounds.

Sound production

Insect sound is defined by Potamitis et al.⁴¹, as the '*muscle power contraction leading to mechanical vibration of the sound-producing structure and finally to acoustic loading of this source and sound radiation*'. Dipterous flies, for instance, produce vibrational signals by beating the wings in the air^{42,43} using a coordinated system involving a set of power and steering muscles and the nervous system⁴⁴. Dipteran wings are made of translucent, micro-thin chitin with interconnected veins^{45,46}. Most fly wings are covered in small hairs on the surface, called microtrichia⁴⁷. They also have hairs running longitudinally on the top of the wings and the bottom of the wings, increasing the wing surface area, and facilitating flight. The wings are connected to the flight power muscles either directly or indirectly. Synchronous power muscles activate as a response to neural input to generate upstroke and downstroke movement of the wings around their base⁴⁸. These muscles require electrochemical flow of ions and therefore take time for activation. Similarly, structures are found in insects with larger wings, such as moths and butterflies.

Notably, smaller winged, flying insects, relative to body size (e.g. blow flies and bees), require faster wing beats to stay airborne and therefore use asynchronous flight muscles that operate on oscillatory stretching and contracting, after the initial neural input^{48,49}. These highly coordinated and repetitive wing movements create a resonant system and therefore a predictable sound output⁴⁹. The faster wing beating of such smaller winged taxa creates a higher frequency output. To the human ear, the difference of the wing beat frequency (WBF) is translated as the loudness and pitch of the source; for example, between the high-pitched buzzing of mosquitoes and the more resonant buzzing of the large blow fly. The swift rotation of the wings creates vorticity at the base of the wing, which travels along the surface and expels from the tip^{50,51}. This vortex is known as the leading-edge vortex and enables both the lift and drag motion of the fly^{50,51}. The larger the wings are, relative to the body, the greater the displacement of particles in the air which creates more powerful vortices, thus requiring less wing beats to sustain the insect in the air^{52,53}.

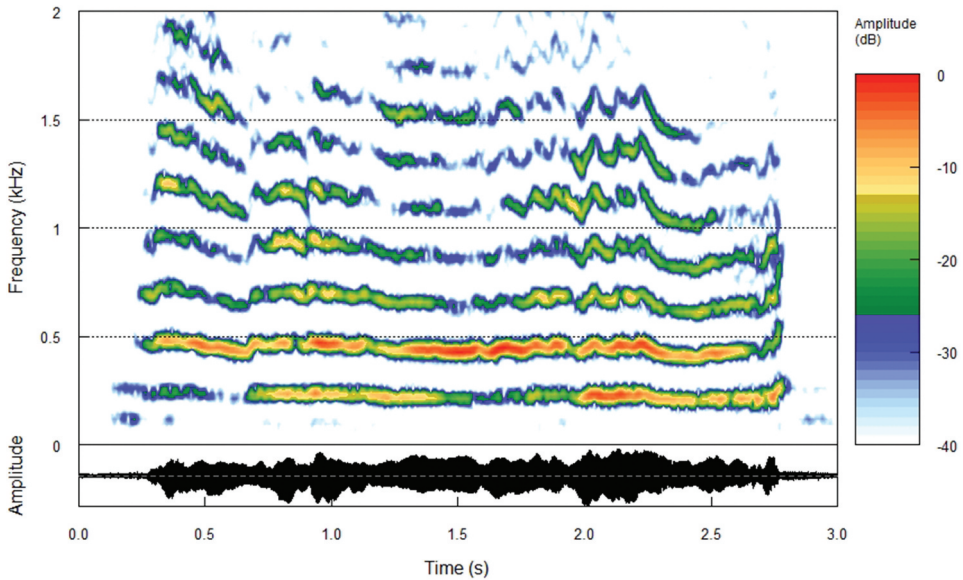


Figure 1. An example of a fly's WBF output, illustrating the fundamental frequency and overtones. Spectrogram (top) and oscillogram (bottom) of a wingbeat of *Lucilia cuprina* wiedemann 1930 (Diptera: Calliphoridae), generated in RStudio using seewave⁵⁶ package.

In addition to the influence of wing size relative to body size on WBF, an insect's response to environmental stimuli can also alter WBF. The steering muscles are essential for dynamic stability flight and the insect's response to environmental stimuli. They act indirectly on the wing hinges through mechanoreceptors to generate time-varying deformation in flight, and in turn changing the frequency output⁵⁴. A comprehensive review of fly aerodynamics is available in a series of papers by Ellington⁵⁵ and Dickinson^{44,50}.

The wing beats of a fly cause a disturbance of the air particles which move in a back-and-forth motion in response to the compression and retraction of the medium (air) through which the insect flies. The pressure from each displacement causes the consecutive particles to be displaced, creating a wave. The wave is composed of a 'fundamental' frequency and overtones or harmonics (Figure 1). Harmonics are used to describe frequencies that are an integral multiple of the fundamental, while overtones are used to describe any frequency above the fundamental. The different overtone pattern is the result of the different physical properties of the sound producing source, which create a distinctive sound⁵⁷. In the case of flies, the overtone pattern is influenced by the wing morphology, size, and structural properties, such as venation, hairs, and flexibility, and therefore contain species-specific information.

The amount of particle displacement in the air caused by the wings is determined by the force of the fly, which in turn is influenced by the size, sex, and age. However, the effect of some of those variables on WBF has not been systematically studied and remains conflicting between studies.

Sex

Sexual dimorphism is present among fly species, and this consequently creates differences in the WBF. The abdomen of the female fly is larger due to the biological and physiological factors related to reproduction, and to compensate for the larger body mass the wings are also larger. For example, the wing area of the female *Lucilia sericata* Meigen 1826 (Diptera: Calliphoridae) has been recorded as 16 mm², while the male only at 13 mm²⁵³. Sueur et al.⁵³, recorded the fundamental frequency and second harmonic for the males and females *L. sericata* and observed similar fundamental frequency but a higher second harmonic in the male. The fundamental frequency and second harmonic of the males was on average 195 Hz and 390 Hz respectively, while the female average at 191 Hz and 377 Hz⁵³. This suggests that the fundamental frequency could be used as an indication of the species and the harmonics as an indicator of sex, traits, or other communication cues. Other studies of the same species have reported a larger difference of 13 Hz⁵⁸ and 23 Hz⁵⁹ in the fundamental frequency of males and females. But for different species, such as the mosquito *Aedes aegypti* Linnaeus 1762 (Diptera: Culicidae), the reported difference between males and females is noticeable at more than 200 Hz difference^{60–62}. The female produces a fundamental frequency of 664 Hz in free flight in the field, while the male at 982 Hz⁶¹. Laboratory recordings of the same species show a smaller difference and smaller WBF of 511 Hz for the female and 711 Hz for the male⁶⁰. This intraspecies difference in fundamental frequency across studies may be the result of different environmental conditions. The opposite trend, where the female produces a higher fundamental frequency than the male, has been observed in *Musca vetustissima* Walker 1849 (Diptera: Muscidae)⁵⁹.

Communication

Certain species of flies can also actively control their wings to convey a signal intended for a specific receiver⁴³. However, most research to date has been focused on mosquitoes, which are not representative of the whole order Diptera. Examples of acoustic communication cues have been recorded in the form of harmonic convergence^{63–67}, wing fanning^{58,68–71}, wing buzzing⁷², wing flicking⁷³ and wing vibrating with whole body waggle⁷⁴. These acoustic communication cues last less than a minute⁷⁴. Thus, the brief WBF change is insignificant for the purpose of fly identification. If other long-lasting communication cues are observed, similar to those found in bird communication, they should be accounted for in any classification model.

Age

The physiology of the fly changes from emergence until sexual maturation. The flight muscles and reproductive organs continue developing gradually the first few days^{75–77}. For instance, the WBF of the mosquito *Ae. aegypti* increases by around 100 Hz in the first few days, starting just below 400 Hz and increasing close to 500 Hz on day four⁷⁶. The WBF then stabilizes until day 10 and afterwards decreases again, caused by the consumption of finite energy⁷⁸. In a more recent study⁷⁹, the WBF increase

was observed to subtly increase until week 3. Similar increases in WBF with age have been noted in other flying insects, such as the rice leafroller moth⁸⁰. The increase in WBF can also be associated with improved capability of flight and sexual maturation⁵⁸.

Size

Body size and wingspan determine the number of wing beats needed to produce lift and maintain in the air. Byrne et al.⁸¹, and Burkart et al.⁸², recorded the sound of flies and bees, respectively, and found a negative correlation between the body size and WBF. Larger flies produced a lower WBF, and smaller flies produced a higher WBF. This was also observed in bumble bee species⁸³, as well as bee colonies where the large queen produced a lower WBF than the smaller workers⁸⁴. However, flies weighing below 30 mg did not fit in this general model as they seem to have adapted a different flying style to account for their exceptionally small size⁸¹. Other studies found no difference in WBF between mosquitoes of different sizes^{62,85} within a species, however these studies explored a relatively narrow size range not representative of all sizes found in nature. Conversely, studies of different mosquito species found that the larger flies generated higher wing beat frequency than the smaller flies^{79,86}. While the size of the mosquitoes is not reported in these studies, the study of Byrne et al.⁸¹, counted the mosquito, *Ae. aegypti* in the category below 30 mg.

Ultimately, the size of the wings and the amount of pressure they can tolerate from the body of the fly will determine the wing loading ratio^{81,87}. It is calculated by the division of the fly's body mass (m), measured in grams over the total wing area (S), measured in square centimetres, shown in Equation 1.

$$WL = \frac{m}{S} \quad (1)$$

This means that flies with a larger body relative to wing area will compensate by beating the wings more frequently in comparison to flies with smaller body and same wing area. This has been experimentally supported by loading a fly with a nylon thread and observing an increase in WBF to account for the extra weight⁷⁶.

Sound propagation

Sound attenuates as a result of atmospheric absorption or reflection on obstacles in the environment, and distance from the sound source⁸⁸⁻⁹³. The largest acoustic obstacle in the environment for flies is leaves, dispersing the energy of the sound wave^{88,94}. In addition, changes in temperature, humidity, wind speed and the density of vegetation, can further attenuate the acoustic signal. The amplitude of the sound wave decreases with the increase in distance from the source by a factor of 6 dB per metre⁹⁴. As the amplitude of the fly sound is already small, such an amount of attenuation will limit the efficiency of acoustic communication in nature. It has been suggested that this could result in fly adaptations and therefore acoustical geographic separation⁹⁵. The degree of attenuation can also impact the quality and quantity of acoustic data collected in nature.

The orientation of a receiver around the sound source is also important for the perception of sound⁹⁶. The sound radiation of the wing beats in the air changes around the fly as a result of the vortices created during upstroke and downstroke movements. The fundamental frequency is known to have the highest energy output and that is true when perceiving the sound from the front of the fly⁵³. Sueur et al.⁵³, found that when the microphone is positioned at the sides of a fly species, *L. sericata*, the air movement results in the second harmonic displaying the highest amplitude. They argue that the lack of knowledge of how sound radiates relative to position has led earlier research to disagree upon a fundamental frequency of certain species⁵³. In hovering flight, the fundamental frequency emits a dipole radiation pattern, while the second harmonic emits a monopole radiation pattern, thus displaying higher amplitude on the sides⁹⁷. At the rear of the fly, however, the frequency output is a combination of the flight tone and unstructured frequency output. This is a result of the complicated wing motions and air vortices that shed from the tip of the fly's wing^{44,52}. Klopsch et al.⁹⁷, observed the same sound field of dipole fundamental and monopole second harmonic for *Calliphora erythrocephala* Macquart 1834 (Diptera: Calliphoridae), but in their study the fundamental frequency preserved the highest amplitude in all directions. Similarly, the small mosquito *Ae. aegypti* shows no radiating changes and a dominant fundamental in all directions⁶⁰. The size of the experimental species may play a significant role in the sound radiation patterns as the shedding of the vortices has been observed to be different among insects of different sizes⁹⁸. This should be further explored for the forensically important species as flies as in the wild are rarely flying in a straight trajectory; they manoeuvre to avoid obstacles and accelerate and decelerate for take-off and landing, particularly around a cadaver.

Temperature

Temperature changes the air density and consequently the aerodynamic support. At higher temperatures, the air particles are more dispersed, reducing the flight performance of the fly. It is expected that the flies will compensate by increasing their WBF linearly with the increase in temperature^{99,100}. Although this general statement does not consider the various mechanisms of thermoregulation adapted by insects. The increase in WBF will have a cooling effect on the large surface of the smaller flies but warming effect on the small surface of the larger flies as the thoracic heat gained from the muscles will exceed the amount of cooling on the surface¹⁰¹. Therefore, the smaller flies are expected to increase their WBF to cool at high temperature, while the larger flies are expected to minimally increase the WBF or slowdown their activity all together^{102,103}.

The relationship between WBF and temperature has been acknowledged in many research papers^{76,101,102,104,105}. Unwin and Corbet¹⁰¹ observed a linear increase of the WBF and temperature for *Calliphora vicina* Robineau-Desvoidy 1830 and *Musca spp.* (Diptera: Calliphoridae and Muscidae respectively). The change is not reported as a rate per °C; although it is evident from the graphs in their article that the change is steeper for the smaller *Musca spp.*, than the larger *C. vicina*. For the even smaller fly, the mosquito *Ae. aegypti*, the change reported is up to 13 Hz/°C^{79,85}.

Moisture

Moisture affects the air density and again the flight performance. Depending on the micro-structure and material of the wings, small air bubbles can be trapped to provide a surface for water repulsion to a certain degree and direct the water drops away from the fly^{47,106,107}. The degree of wettability is the measurement of the contact angle between water droplets and the surface of the wings¹⁰⁸. A recent study of three dipteran species, among which are the forensically important, *L. sericata* and *Chrysomya marginalis* Wiedemann 1830 (Diptera: Calliphoridae), determined unequal water permeability¹⁰⁹. *L. sericata* wings showed hydrophobic properties, while *C. marginalis* exhibited strongly hydrophilic behaviour¹⁰⁹. Water permeability of the wings can increase the mass of the wing and the wing load of the fly, and subsequently it can shift the resonant system. Besides the direct impact of the raindrops, the increase in humidity following rainfall may affect the wing indirectly by demanding an increase in frequency and power to maintain in the air. Some studies of mosquitoes reported an increase in WBF with the increase in humidity, albeit minor^{104,105}. It is worth noting that there are studies that have investigated the effect of the relative humidity on flight activity^{110,111}, but only a few studies are available on the effect of humidity on WBF.

Insect audio classification

Despite the WBF variations caused by the internal and external variables, practitioners have managed to successfully classify and identify different species of insects. Most studied are the singing insects: cicadas (Hemiptera)^{112–117}, katydids (Orthoptera)^{116,118,119}, and crickets (Orthoptera)^{115,120}, and the agriculturally and health important: bees (Hymenoptera)^{102,121,122}, and mosquitoes (Diptera)^{100,116}. The singing insects produce distinctive sounds based on dedicated sound producing structures¹²³, so they will not be discussed here. Bees and mosquitoes produce WBFs like that of forensically important fly species. In the absence of published literature that investigated the forensically important fly species, bees and mosquitoes are the most suitable analogues for developing a classification system.

The general workflow for developing a classifier includes data collection and processing, feature extraction and selection, classifier training, and evaluation. For audio classification is it the extraction of suitable audio features that will determine the merit of the classifier¹²⁴. The audio features, meaningful acoustic properties of the sound source, can be as simple as the fundamental frequency, or more complex transformations, such as those used in human speech recognition systems, for example in Siri and Alexa. Transformations convert the sound waveform to a compact and meaningful representation that shows the potential discriminative properties of the different sound sources¹²⁵. The extraction and representation of the audio features can be completed in several domains, most used are the temporal, spectral, and cepstral domain¹²⁶. Temporal features are the easiest and fastest features to compute as they are extracted directly from the time domain and consequently do not require any transformation¹²⁶. They are a useful indicator of a signal within noise-free recordings and thus can be used to reduce data storage. Spectral features are the largest group of features that characterize the frequency content of the signal. Cepstral features are

more complex and require more computation as they are extracted from the log magnitude of the frequency spectrum. Arbitrary use of all the different features can lead to misclassification based on overlapping or irrelevant features to the sound of interest¹²⁷ and since there is no set of features that can be applied to different types of sound with equal success rate, finding the right audio feature/s is solely dependent on the target sound. As the wing beat of small flies preoccupies the low-frequency range where many other background noises are present, some features, particularly temporal features, can be ineffective, unless the audio undergoes filtering and processing. To overcome this issue, practitioners have developed different types of opto-acoustic sensors that capture the wing beats as they move through a receptor as they do not suffer from background noise^{100,128}. Besides dipteran species, optical sensors are well suited for aphid species as the sound they produce has even lower frequency and amplitude. Attributing to the success, these sensors are now available for commercial use, an example is the Wingbeat Recorder® (Insectronics, Greece). This sensor was used to record six aphid species and later classify to species with accuracy of 80%, based on multiple audio features¹²⁹.

Extracted audio features are then fed into an appropriate machine learning (ML) algorithm, which is responsible for generating identification predictions. The approach of the ML algorithm can be based on supervised or unsupervised learning. Supervised learning requires prior training with labelled data to make a prediction output on new data. Different models are available and have been successfully used in the literature, including decision trees, random forest (RF)⁸⁴, support vector machine (SVM)^{130,131}, artificial neural networks (ANN), including deep learning models^{57,132,133}, and others. Unsupervised learning does not require labelled data; instead, it explores the entire dataset and looks for patterns or relationships between the data points. This approach has failed to produce an effective identification method of four Dipteran flies using the fundamental frequency in our earlier work¹³⁴. Although, it outperformed the supervised models in identification of mosquito species using multiple audio features¹³⁵. Some studies have compared multiple machine learning algorithms on the same dataset, yet no single algorithm consistently outperformed the others^{84,136–138}. Regardless, the goal of the model should be aligned with the capabilities of the algorithm. Some algorithms, such as the SVM, can perform well with small data quantity, while others, such as ANN, require large datasets. There is also a trade-off between interpretability and accuracy. Regression based models are generally simpler and the relationship between the sample points can be interpreted, while other more complex models, such as the neural network, are harder to interpret and may be used with a black-box approach, but generally offer better accuracy. Therefore, the choice of algorithm is an iterative process and should be based on the desired outcome, complexity of the problem, data amount, and computing time.

Moreover, commercially available algorithms and software are available and promote the building of a self-classifier, however, often revolving around the detection of clear and repetitive sound patterns or vocalizations. For instance, Kaleidoscope Pro (Wildlife Acoustics Inc., Concord, MA, USA) can cluster datasets based on predefined frequency range, duration, and inter-syllable gap of the signal. While this can be effectively applied for birds, bats, cicadas, and crickets, it is ineffective for the wing beats of flies. Flies do not produce syllables or precisely timed bursts of signal, and the duration of their wing beats is dependent on the distance travelled.

Research in the field

Bees have been the target of acoustic monitoring research as a non-destructive method for observing the health of the colony and early prevention of population decline¹³⁹. One study successfully classified three bee species with precision rates between 73% and 88%, and one hornet species, the Japanese yellow hornet, *Vespa simillima xanthoptera* Smith 1868 (Hymenoptera: Vespidae), with a precision rate of 100%¹²¹. The authors used SVM and considered the fundamental frequency and Mel Frequency Cepstral Coefficients (MFCC), which is a psycho-acoustic feature, regularly used in human speech processing. The average fundamental frequency of the hornet, around 100 Hz, is lower than that of the bees allowing for more accurate recognition within its own frequency range. The WFBs of the bees in their study overlap between 200 and 300 Hz, and therefore there are some misclassifications between species. Another study¹⁴⁰ also considered the fundamental frequency and MFCC, and tested multiple algorithms, namely logistic regression, SVM, RF, and decision trees. They successfully classified the flight and sonification sounds of 15 bee species with highest accuracy of 69% when multiple ML algorithms are combined¹⁴⁰. Moreover, Gradisek and colleagues⁸⁴ developed an internet and mobile application for recognition of bumble bee species with a reported accuracy of 86%. Public use of such applications allows the algorithm to strengthen its recognition capability and accuracy rate by reinforced learning. However, the quality of the recordings can be an issue. Generally, the fundamental frequency of all flies' ranges from 100 to 1000 Hz¹⁴¹ and in this range there are many background sounds. This is why some researchers choose to record the training data in noise-controlled environments or in specifically designed and acoustically treated recording studios. In such instances, the accuracy can reach up to 99%¹⁴². However, during field studies, the noise levels are more challenging and often beyond the researchers' control. Using optical sensors, the bumble bee species were recorded and classified with an accuracy of 88%, based only on the fundamental frequency⁸³.

Mosquitoes have also been the focus of bioacoustics research to aid in the reduction of mosquito-borne diseases and to develop a targeted pesticide spraying method that would not be destructive to other favourable insects. Generally, the fundamental frequency of mosquitoes starts around 500 Hz⁶⁰, noticeably higher than that of bees and most other flies starting around 150 Hz⁵⁹, therefore the accuracy rate of mosquito classification is relatively higher. Several studies have explored acoustic classification of mosquitoes. One study successfully classified eight mosquito species of similar morphology with an accuracy of 79%¹⁴³. Similarly, other studies successfully classified mosquito species with overlapping fundamental frequencies¹³⁸ and accuracy of 94% and 93%¹⁰⁰. González-Pérez et al.¹³⁸, considered multiple audio features including the fundamental frequency, spectral power density, and MFCC, and using multiple algorithms, of which deep neural network yield the best results. Batista et al.¹⁰⁰, used the fundamental frequency and circadian rhythm to adjust the probability of species appearance. Some studies on mosquitoes have also made their audio databases publicly available, promoting collaborative research^{62,143,144}. Using optical sensors, Ouyang et al.¹³⁵, recorded three mosquito species, extracted 12 features from the cepstrum and tested supervised and unsupervised machine model with 80% accuracy of genus identification. An earlier study, which also used optical sensors to record five mosquito species, achieved an accuracy of

73% when used the fundamental frequency and harmonics from the spectrum to train an artificial neural network¹⁴⁵.

Despite considerable interest throughout the years, insect bioacoustics research did not progress until the post-2000 due to the absence of recording equipment that could capture the low amplitude insect sound while neglecting the high amplitude background noise. The advancement of recording technology meant that the issue of background noise and the other previously reported concerns in bioacoustics research are no longer a hindrance. Background noise can now be easily reduced with directional microphones, frequency filters, amplification adjustment and the use of optical sensors. Large data storage is also available, providing researchers the means to generate, store and share large datasets, as well as crowd source data, albeit subject to quality¹⁴⁶. Still, further research is required to understand the effect of environmental conditions on WBF and its impact on the recognition capabilities, as well as the value of different audio features for species identification. Such research will greatly benefit from collaboration between practitioners from various disciplines, namely forensic entomologists, and computer scientists.

Outlook

There are numerous potential benefits to using an audio identification system in carrion insect surveying. Primarily, offering a non-invasive method of data collection that may deliver uninterrupted and continuous monitoring regardless of the external conditions. Data processing is considerably faster than manual identification, further reducing the need of long-term species preservation. Once established, it would require minimal human supervision, enabling more widespread studies in less accessible locations as often encountered in homicides. It can be deployed seasonally or annually to provide a timeline of the species assemblage and preference of weather conditions. Lastly, it may offer more unified approach to surveying the flies whilst reducing the bias in selective capturing of anticipated species. While this tool is not designed to replace practitioners, it can assist them in identifying locally significant species and prioritize further studies on those species.

In summary, there is strong evidence in the literature for the potential for audio recordings of insect flight to be used as a diagnostic tool to identify species, based on analysis of WBF. Bioacoustics research involving insects has been used in numerous applications; however, no studies are currently published that implement this in the forensic entomological workflow. Factors impacting WBF, such as environmental variables, sound radiation patterns, and wing-related communication cues, present challenges in field applications relating to forensic succession data. However, further development of the underlying data and processing/analysis techniques (e.g. machine learning) are likely to address these challenges, establishing classification parameters and greatly refine classification accuracy for field application. Upon review, the potential for bioacoustics monitoring in the field is substantial and the approach offers considerable advantages over traditional field monitoring in a forensic context. Further research is warranted to advance the development and implementation of the approach as a viable diagnostic tool for use in forensic studies of carrion surveying.

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