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AN OPTICAL SENSOR THAT DETECTS
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Major professor

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EVALUATION AND FIELD APPLICATION OF AN OPTICAL SENSOR THAT DETECTS FLYING INSECTS

By

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ABSTRACT

EVALUATION AND FIELD APPLICATION OF AN OPTICAL SENSOR THAT DETECTS FLYING INSECTS

By

Meghan Suzanne French

An optical sensor that was designed to remotely detect flying insects (OFIDIS; www.qubitsystems.com) was evaluated outdoors under full sunlight. Shadows cast by an artificial insect (an electric toothbrush oscillating at 53 Hz over a 120° angle), were detectable up to 35 m away from the sensor: they weakened with distance at a rate of $y=90^{2e-0.11x}$ ($r^2=0.9$ at $p < 0.001$). Shadowed signals were undetectable when sunlight intensity was reduced below 20,000 lux. Sunlight signals reflected from a mirror attached to the oscillating toothbrush remained detectable and strong ($y=-0.24x + 86$; $r^2 = 0.33$) beyond 25 m. The ability of this sensor to detect differences in abundance, as well as density in flying insect populations, was evaluated in three replicated site types: old-field, wetland, and parking lot. Insect signal abundance (based on differing fundamental wing-beat frequencies of each signal) at both old-field and wetland locations were higher than that of the parking lot and control (F=8.85; df=8; P>0.0001). Signal richness was greatest in the old-field (R=259) and least rich in the parking lot (R=88). However, diversity was greatest at the wetland ($H' = 2.26$; $H'_{max} = 2.7$), showing a high richness that was most evenly distributed from 100-1000 Hz. Combined with an appropriate data logger, this year 2000 sensor is a promising development for remote sensing of insects with wing-beat frequencies over 100 Hz, notably Diptera and Hymenoptera.

Dedicated with love to my mother and father whose encouragement and friendship
continuously guide me through life's greatest challenges.

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My project would not have been possible without the support of the sensor's inventor, Dr. Aubrey Moore and the folks at Qubit Systems, Ontario, Canada, who loaned the OFIDIS sensor and software. Thank you for the chance to evaluate your product as applied to my deep interest in animal conservation.

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Introduction to insect wing-beat frequencies, harmonics, and their application to insect detection

I. The Phenomenon to be explored

A female mosquito buzzes near your head on approach for a blood meal. The regular, high frequency beating of her tiny wings generates the necessary lift and thrust for this oriented flight, now near completion. However, these diminutive creatures are not always sufficiently stealthy to secure an uncontested meal. A by-product of even tiny beating wings is compression waves in air that, if generated in proximity to the human ear, are sufficiently energetic to set one's eardrum vibrating synchronously with her wing beats. The resultant high-pitched "whine-of-the-mosquito" is unmistakable to the experienced human host, causing us to cringe and swat in an attempt to avoid the imminent bite.

The core question to be explored in this thesis extends from the above commonly experienced phenomenon, in which, unintended cues emitted by the actions of one organism can sometimes be exploited as useful information by another. That question is: Do flying insects produce sufficient physical cues during flight to enable notable advances in rapid and remote detection, quantification, and possible identification by humans extending their natural sensory capabilities via available tools and techniques of the modern electronic age?

II. Mechanisms of insect wing beat

A. Flight powered by direct flight muscles. Insect wings are comprised of a short basal and longer distal shaft separated by a pivot point (Figure 1). "Primitive" insect orders like Odonata and Blattodea, contract elevator muscles attached directly to the basal shaft (Figure 1) to raise their wings. However, some authors (Brodsky 1995; Chapman 1998) suggest that indirect muscles are involved in wing elevation in some Odonata. There is full agreement that direct, depressor muscles, attached to the distal shaft just beyond the pivots, power the down-stroke. A series of smaller muscles (not shown in Figure 1) also attached directly to the wings govern lift, speed, and steering by regulating wing rotation, angle of attack, and exact stroke plane (Gullen and Cranston 1994; Dudley 2000). The up and down movement of a wing within the stroke plane is known as a wing beat.

Direct flight muscles are characterized as *synchronous* -- in the sense that delivery of at least one action potential to the neuro-muscular junction is necessary for every contraction (Dudley 2000). Since there is an upper limit to action potential frequencies (rarely sustained at > 100/s) and that only a few neurons innervate single muscle cells (Chapman 1998), wings powered entirely by direct muscles are restricted by this neural frequency limit.

The flight system of insects powered by direct flight muscles is often characterized as a primitive system (Brodsky 1995) associated with clumsy fliers like stoneflies, lacewings and roaches. However, under certain selective forces like predation, direct flight muscles can yield outstanding flight speeds and maneuverability, e.g., dragonflies.

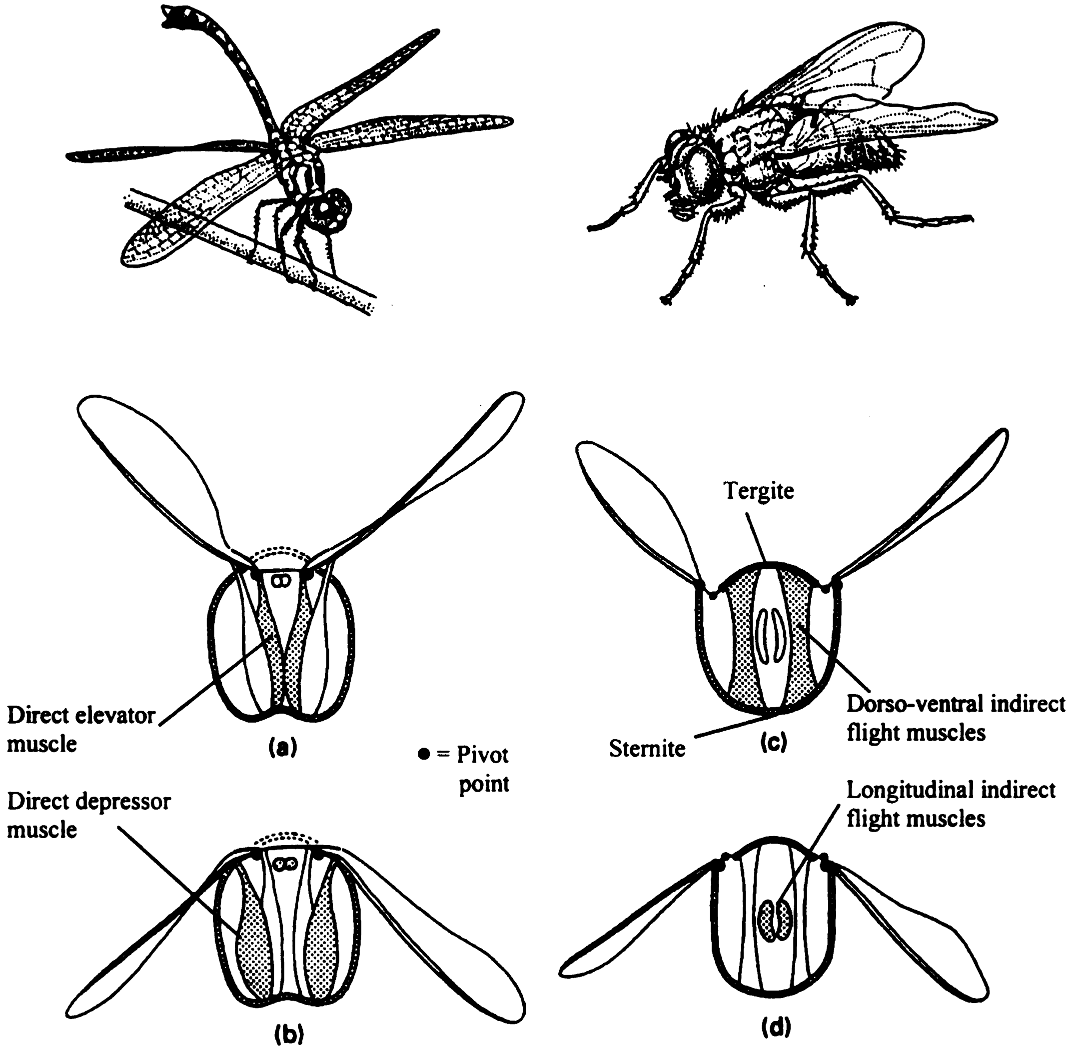


Figure 1. Direct ((a), (b)) and indirect ((c), (d)) flight mechanisms. Dragonfly thorax during (a) upstroke and (b) downstroke of the wings. House-fly thorax during (c) upstroke and (d) downstroke of wings. Stippled muscles are those contracting in each illustration. (Modified from Gullen and Cranston, 1994 with permission from Kluwer Publishing)

B. Flight powered by indirect flight muscles. Most flying insects power the strokes of their wings by massive indirect rather than direct muscles (Chapman 1998). Indirect flight muscles, attached to the walls of the thorax, are comprised of opposing muscle groups that distort the thorax in such a way as to raise and lower the wings of an insect by means of a pivot (Figure 1). Contractions of a dorso-ventral set of indirect flight muscles anchored to sternites and tergites of the thorax cause the thorax to compress dorso-ventrally and the wing to rise. Contractions of a set of longitudinal indirect flight muscles anchored to the anterior and posterior thorax causes the thorax to shorten longitudinally and the wings to move downward. Rather complex sets of non-massive direct flight muscles imbedded in the thorax and attaching directly near the wing base control such features as wing rotation, angle of attack, and realized stroke-plane. In essence, the indirect flight muscles generate power for flight, while the direct flight muscles govern how that power will get translated into lift, speed, and steering. In actuality, the wing-beat strokes of most insects are better represented as a *"figure 8"* rather than a simple up-down stroke.

The opposing indirect muscle sets are activated by action potentials delivered by motor neurons under control of a neural central pattern generator located in the thoracic ganglia (Chapman 1998). The action potentials are delivered to the respective neuro-muscular junctions in a coordinated pace that causes only alternating and never simultaneous contractions of opposing muscle groups. In fact, the complementary nature of these opposing muscle groups is so well refined that the stretch received upon contraction of either one of the pair is often a sufficient stimulus for immediate counter-contraction of the other. In this sense, this type of muscle system acts as a mutual oscillator that, once activated, can sustain its impetus for a burst of cycles before needing to be reinforced by another action potential.

Such flight muscles capable of multiple contractions per arriving action potential are termed *asynchronous* (Dudley 2000). A distinct advantage of this arrangement is that such insects can generate oscillation rates well in excess of the maximum possible rate for sustained delivery of action potentials (Brodsky 1995). Of course, this condition of having rates of muscle contraction exceed the limits for neural signal delivery can develop only concurrently with extraordinary designs for energy-efficient muscle contractions and oxygen-delivery systems. Both of these conditions are exemplified by the flight muscles of the higher insect Orders (Coleoptera, Hymenoptera, and Diptera) exhibiting highly ordered muscle fiber substructures (actin, myosin, and their attachments), an extraordinary number of mitochondria (Dudley 2000), and dense and regularized distribution of trachea throughout these muscles (Chapman 1998). While both the direct, synchronous and indirect, asynchronous flight mechanisms are found within fliers of extraordinary speeds and agility (e.g., dragonflies vs. bees and flies respectively), the indirect design is judged superior energetically (Dudley 2000).

III. Ranges in insect wing-beat frequencies

Insect wing-beat frequencies, usually expressed as cycles per second (Hz), vary depending on such factors as wing morphology, body size, body mass, and neuro-muscular mechanisms of power generation. Wing-beat frequencies for most insect Orders fall below 100 Hz (Figure 2). Non-Dipteran, aquatic insects have relatively small ranges, all falling below 100 Hz. Some Lepidoptera have the lowest wing beat frequencies ever recorded, with a total range typically between 4 Hz

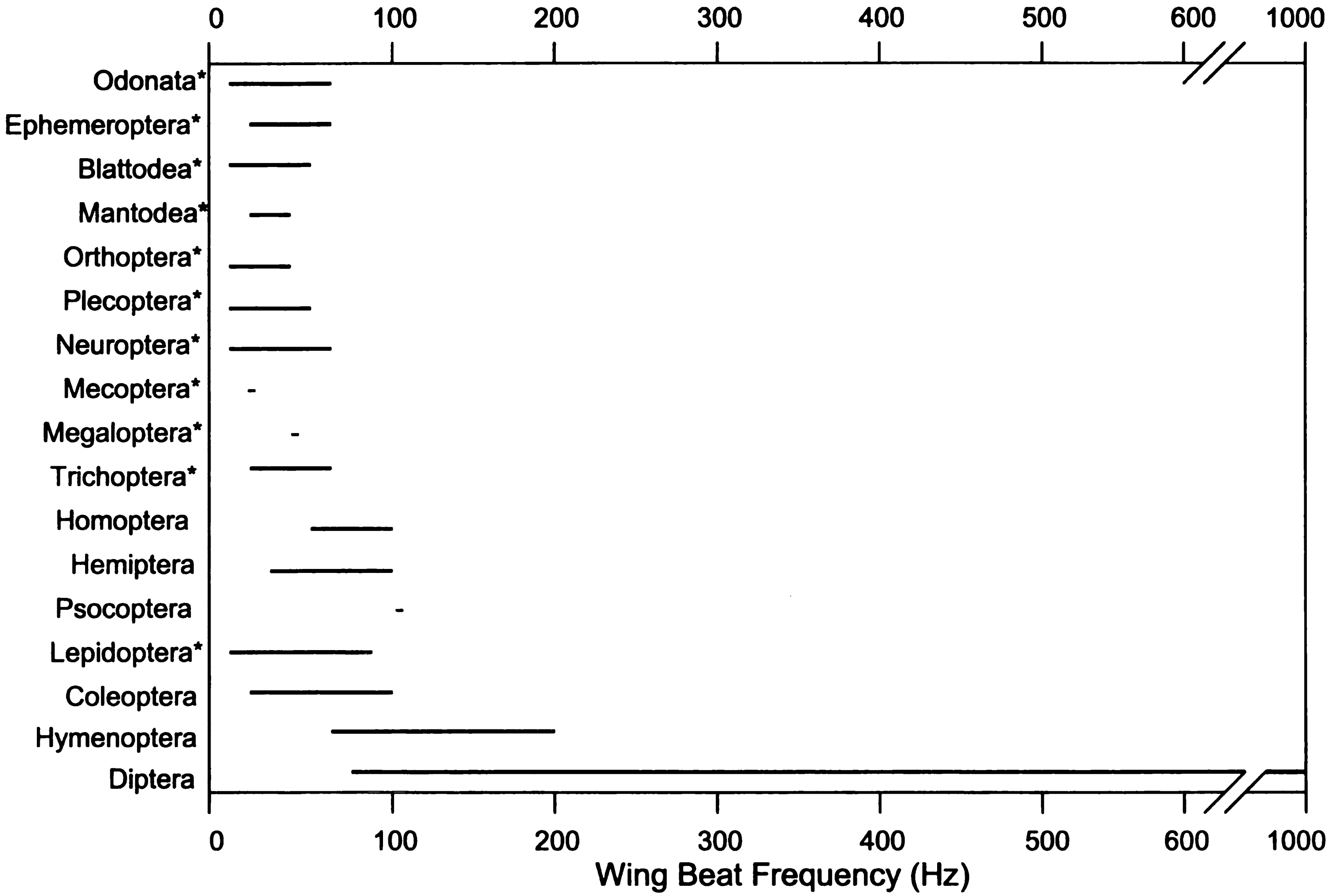


Figure 2. Wing beat frequency ranges for common insect orders. Information from Brodsky 1995, Chapman 1998, and Dudley 2000 was combined to make this figure. Orders marked with an * are synchronous, while others are asynchronous.

to 80 Hz. Only the Hymenoptera and Diptera markedly exceed the wing-beat frequencies of all other orders. No Hymenoptera beat their wings slower than ca. 70 Hz and most exceed 100 Hz, while midges can have wing beats as high as 900 per second. Diptera may be able to achieve such high wing-beat frequencies because they only need to energize two wings, while stabilizing themselves with halteres.

Wing-beat frequency does not necessarily determine the speed of insect flight. For instance, fast flying dragonflies are able to use an array of direct flight muscles to rotate their wings in such a way as to highly vary their flight speed and direction while defending their territory (Chapman 1998). But, they do this at low (well below 100 Hz) wing-beat frequencies. Butterflies also have a low wing-beat frequency and move at lower speeds than the dragonfly. However, butterflies are tremendous gliders, allowing them to conserve energy while in flight.

IV. Factors influencing wing-beat frequency

A. Body mass. Dudley (2000) established that body mass within various insect Orders is generally inversely correlated with wing-beat frequencies (Figure 3). Specifically, this pattern was observed by Casey et al. (1985) for contained, hovering euglossine bees (*Euglaema-Eufriesea* spp.). They exhibited a significant correlation ($-0.347 \pm 0.048x + 1.992$; $r^2 = 0.902$; $p < 0.0001$) with a body mass range of 0.1 g to 1.5 g and a respective, correlated wing-beat frequency range of 90 Hz to 240 Hz. Female mosquitoes whose mass increased after a blood meal were reported to decrease their wing-beat frequencies from 437 Hz to 433 Hz, or by approximately 2.4% (Ogawa and Kanda 1986).

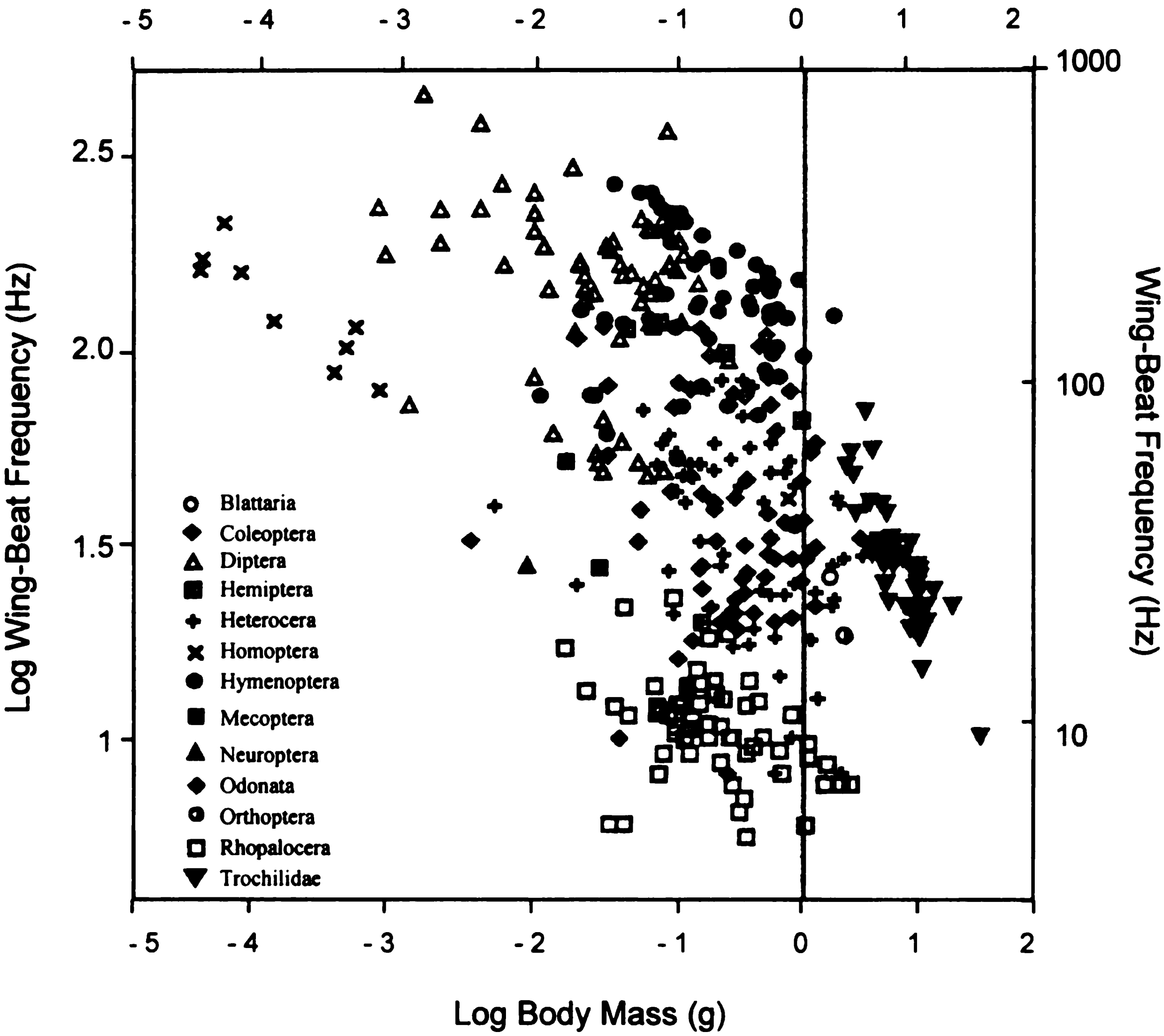


Figure 3. Wing-beat frequencies as they relate to respective log body mass (g) for some insect orders and one hummingbird family (Trochilidae). Data were compiled and plotted by Dudley 2000. Permission to reproduce this figure with some modification was granted by Princeton University Press.

B. Wing size. An inverse relationship with wing-beat frequency holds for insects with large vs. those with smaller wings. Lepidoptera and Odonata have relatively large wings and lower wing-beat frequencies compared to other Orders. Even within a particular Genus, there can be considerable variation. Joos et al. (1991) studying free-flying bumblebees showed mean wing-beat frequencies of ca. 155 Hz and increased by up to 13 Hz per 2 mm change in wing length ($y=166.4x^{-0.80}$; $r^2 = 0.88$). Increased wing length was likewise correlated with slower wing beats in tethered, anopheline mosquitoes (Ogawa and Kanda 1986). Specifically, their mean wing-beat frequency ranged from 250 Hz at 5 mm wing length to 550 Hz at 2.0 mm wing length.

C. Maturation. Wing-beat frequency of a given species of hemi-metabolous insect is reported to change with maturity. For example, Australian locusts increased their wing-beat frequencies two-fold during the first few days as adults (Altman 1975). In addition, wing-beat frequencies of 2-day old male mosquitoes were observed to be up to 300% faster than those immediately eclosed, an increase from 200 Hz to 600 Hz (Ogawa and Kanda 1986). This trend was also observed by Moore et al. (1986), who showed that male *Aedes aegypti* (Say) increased their wing-beat frequencies by up to 150 Hz within their second day as adults.

D. Type of behavior. Insect behaviors affect wing-beat frequency. As insects take off, land, switch directions, hover and glide they modify the angle of their wings and in some cases, wing-beat frequencies. In experiments with tethered vs. free-flying Australian locusts, the free-fliers beat their wings 13% (3 Hz) faster (Baker et al. 1981). A study by Spangler (1993) compared four behavioral types of the honeybee (*Apis mellifora* L.) to show that tasks can affect mean wing-beat frequencies. Specifically, Arizona honeybee robbers had a mean wing-beat frequency of 235 Hz, while Arizona foragers had a mean of 220 Hz (2% difference). In a study that compared freely flying foraging and hovering bumblebees (Joos et al.1991), mean wing-beat frequencies for both groups differed by a mean of 4 Hz (3%).

These studies suggest that wing-beat frequency may vary more between castes in the social insects than between individuals behaving similarly. Dudley (2000) believes that wing beat frequencies of insects within an "unchanging behavioral context" remain consistent. He reasons that insects exhibiting a given behavior will settle on a common wing-beat frequency that represents an energy optimization.

E. Thoracic temperature. Physiological and environmental factors can influence insect wing-beat frequencies. Studies relating thoracic temperature to wing beat frequency show mixed effects. For instance, Ortelli (1989) reported that during take off flight, the beetle *Dineutus americanus* (Coleoptera: Gyrinidae) decreased its wing-beat frequency as its thoracic temperature increased ($y = -1.81 \pm 1.76x + 136.35 \pm 8.88$). But this was not the case in the other beetles he studied; either there were no effects of temperature or slight increases in wing-beat frequency were observed as thoracic temperatures increased. The greatest effects of temperature were observed in individuals of four Coleopteran families Scarabaeidae, Elateridae, Cantharidae, and Chrysomelidae, suggesting that variation is not taxon specific. Interestingly 3 of the 4 families had the highest mean wing-beat frequencies of all of the beetles tested and also showed high ambient temperature sensitivity in their wing beats. Japanese beetle (*Popillia japo*nica Newman) was most affected by an increase in thoracic temperature; as temperature increased from 33-43 °C wing beats increased from 113-140 Hz.

Studies with bumblebees (Joos et al. 1991) showed no correlation between wing-beat frequency and thoracic temperatures, while studies with dragonflies (*Tetrgonneuria cynosura*) (May 1981) showed that even after they warmed-up for flight, body temperatures continued to increase and so did their mean wing-beat frequencies by up to 20 Hz (50% increase). Even during free flight, dragonflies can have wing-beat frequency changes up to 10 Hz. These studies seem to suggest that consistent wing-beat frequencies are more readily found among insects, such as bees, that use asynchronous rather than synchronous muscles.

F. <u>Air temperature.</u> Ambient air temperatures also affect wing-beat frequencies of insects. They have had significant effects on the mean wing-beat frequencies of *Centris pallida* (Hymenoptera: Anthrophoridae); a 40 Hz decrease occurred down to 210 Hz between 25° and 35° (Roberts et al. 1998). The authors suggested that this decrease might have been due to water loss from the insect in response to the higher temperatures, and /or the decrease in metabolic rate.

Foraging, stingless bees, (*Trigona jaty* F. Smith), increased their mean wing-beat frequencies from 180-300 Hz between 22° and 32° C (Unwin and Corbet 1984). However, only slight changes in mean wing-beat frequency occurred among three groups of flies studied: *Calliphora vicina* (5.4± 0.04 Hz), *Drosophila melanogaster* (2.9± 0.04 Hz), and *Musca domestica* (2.9± 0.04 Hz) (Unwin and Corbet 1984). The results agreed well with the original measurements made of these flies by Soltavalta (1947).

In other studies with honeybees, little or no effect of air temperature was seen among individuals (Spangler 1992), but mean wing-beat frequencies of free flyers did comprise a range of 152-278 Hz. Spangler believes that the differences in wing-beat frequencies of freely flying honeybees resulted from fluctuations in thoracic temperatures occuring when insects are thermo-regulating before and during flight. This information again suggests that constancy in wing-beat frequency can be influenced by behavioral state, particularly warm-up.

While it is important to understand the factors that may be influencing the wing-beat frequencies of insects, the context of the studies should be noted. Insects that are confined (Moore 1996) or tethered (Baker et al. 1981) or hand-held (May 1981) may be behaviorally constrained, unable to reach and maintain the maximum wing-beat frequency they typically can in nature. These studies tell us something about fluctuations in wing-beat frequencies, but they all have constrained the insects in some way, making the possibility of fluctuations in wing beat greater.

The greatest constancy is likely to be found among insects with the indirect flight muscles system equilibrated by sustained flight. However, species identifications purely on the basis of fundamental wing-beat frequency are judged unlikely.

V. Wave properties of insect wing beats: Harmonic content

The flexible body parts of a flying insect represent a medium that can express complex wave properties under the influence of the strongly oscillating flight muscles in the thorax. In this context, membranous wings can be thought of as flexible wands anchored at the pivot (Figure 1), and the muscles powering wing beat can be considered on-going generators of highly regular and energetic waves imparted to the wing shaft, as well as surrounding body parts. The physics of this system can be approximated by more familiar models like a human hand regularly imparting waves into a stretched rope whose other end is fixed, or to the plucked string of a familiar musical instrument. Let us now consider some of the most important properties of such resonating systems in preparation for more complex analysis of insect wing beat that goes beyond simple fundamental wing-beat frequency.

Different musical instruments (e.g., guitar vs. banjo) sounding the identical note (e.g., middle C) vary in tonal quality as influenced by each instrument's size, shape, mass, and material composition. Each emission begins with the pluck of a stretched string (Figure 4 A). Initially, the guitar and banjo string can be expected to vibrate with a common fundamental frequency assignable to the condition where the full string alternates between being either bowed up or bowed down (Figure 4 B). However, this condition quickly becomes more complicated (enriched) as the wave propagating along the string reaches an end and is reflected back into the string repeatedly. Following well-established physical laws governing all types of waves (Berg and Stork 1995), a series of standing waves arises exhibiting nodes precisely at 1/2, 1/3, 1/4, etc. the total length of the vibrating string (Figure 4 C – E). The loops of string between these respective nodes vibrate at even integer multiples of the fundamental frequency as set by the total number of loops of one given length. Thus, a length of string between nodes of short spacing vibrates at a much higher frequency than that between distantly spaced nodes. The term *harmonic* and a number reflecting the fold increases over the fundamental are assigned to these discrete ascending steps in vibration frequency (Figure 4).

The collection of harmonics associated with a given fundamental vibration is also referred to as its overtone series (Berg and Stork 1995). It turns out that the number and relative intensity of harmonics varies for different instruments sounding the identical

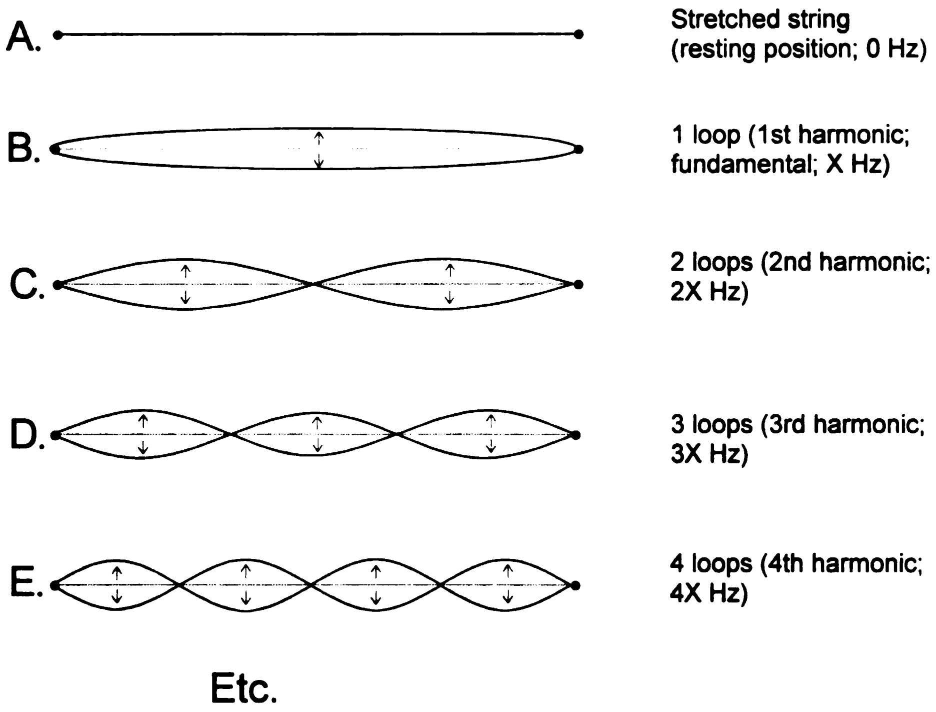


Figure 4. Representation of the lower harmonic series of possible standing waves in a stretched string.

note. However, it is important to recognize that the fundamental vibration need not always be the most intense, and that certain harmonics in an ascending series may appear minor or absent. It is the relative patterns in harmonics that impart richness in tonal quality to the human auditory system. Indeed, the overtone series for a guitar vs. banjo sounding middle C are distinct. We listeners learn to associate unique patterns in overtones to particular instruments or particular voices.

In actuality, the vibrations shown in Figure 4 B – E occur simultaneously. Thus, the condition of the pure standing waves shown in Figure 4 would not be visible to the careful observer of the plucked guitar or banjo string. The actual pattern in standing waves along the length of string would be a summation even more complicated than the simple summation shown in Figure 5 for a fundamental vibration (1st harmonic) summed with its 2nd harmonic. Fortunately, software for analyzing complex vibrations and visualizing patterns in harmonics is now readily available (Canary® 1.2.4 at http://birds.cornell.edu/BRP/SoundSoftware.html and TRex® 2.0 at www.qubitsystems.com).

Harmonic theory can be applied to the waves generated by insect wings. Knowing that insects differ in size, shape, mass and flight behaviors, we can expect insects to differ somewhat in harmonic patterns of wing beat. The extent to which this is true is partially revealed by the data of this thesis and of high importance to the future of this type of research.

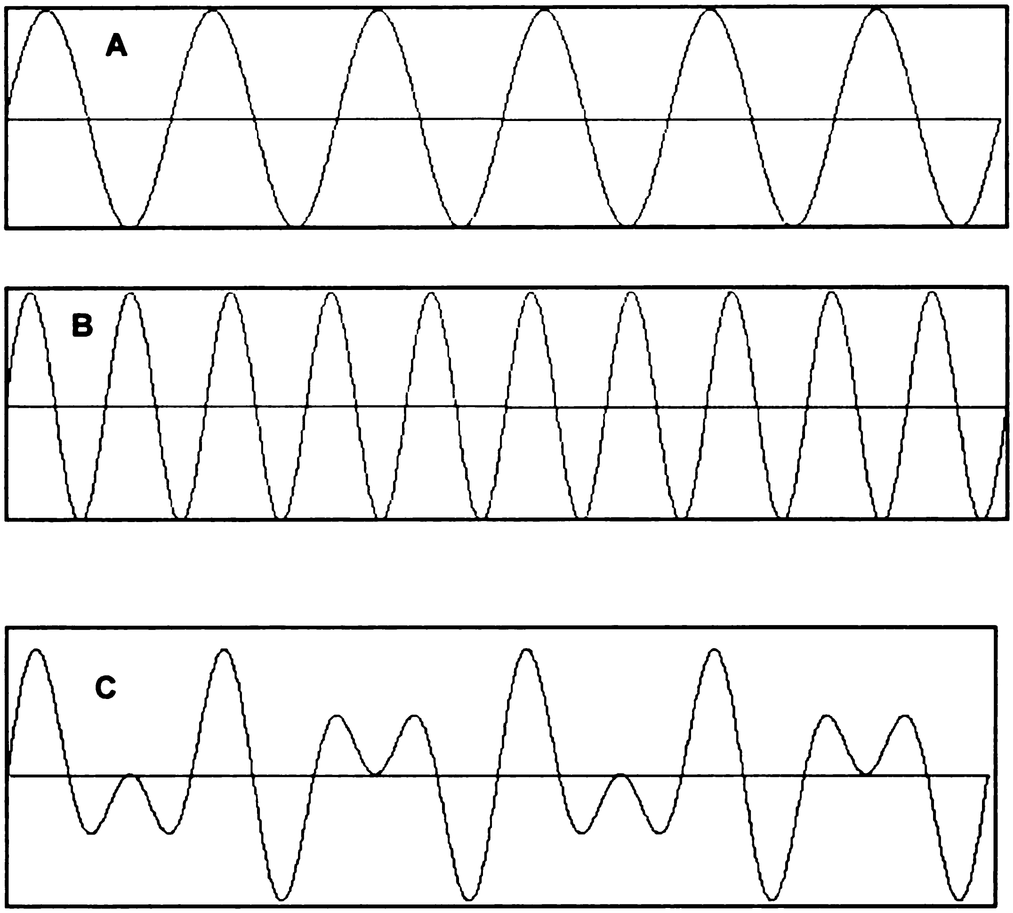


Figure 5. A complex wave (C) results from the summation of the standing waves (A) (Harmonic 1) and (B) (Harmonic 2) that comprise it.

VII. Developments in remote sensing of insects

Remote sensing may be defined as the collection of information about an object without being in physical contact with the object (Sabins 1987). Sabins further stated that the term remote sensing is restricted to methods that employ electromagnetic energy as the means of detecting and measuring target characteristics. Based on this definition, it seems plausible that anything from cameras to radar detectors used in the history of entomology are indeed remote sensing instruments.

Entomologists have historically relied on aerial photographs and satellite imagery to assess insect damage to crops and forests (Riley 1989). Distance photographs allow researchers to observe changes over vast geographic areas that may not be as easily summated by local measurement of damage. However, there may be disadvantages to this more sophisticated methodology, including the financial and time costs of gathering the data. Up-to-date satellite images of specific areas on earth are not always easily available and usually come with a cost to the researcher.

Videography is commonly used to capture insect behavior as it applies to insect movement. Aphid flight patterns have been monitored using camera techniques (Hardie and Young 1997) and three-dimensional moth flight trajectories have been mapped for fliers too low to the ground for accurate radar detection (Riley et al. 1990). The response of drones to queen bee pheromone has also been captured by videography (Loper et al. 1993).

Application of radar systems to the monitoring and detection of insect biota has met with considerable success. Radar techniques have included Doppler, harmonic, tracking, and millimetric-wavelength radar (Riley 1989). Radar systems have been used at a macro-level to track the movement of migrating locusts (Riley 1980 and Schafer 1976 cited by Riley 1989) and to ascertain insect flight migration trajectories (Hobbs 1989; Rui-Lu et al. 1989). Used in localized experiments, radar systems have detected and documented moths following a pheromone plume (Riley et al. 1998) when these behaviors could not be captured on video because of the long flight distances involved. Radar has also been used to remotely sense ground beetle movement (Mascanzoni and Wallin 1986). Radar offers many benefits for monitoring insects, such as the ability to collect and record real-time data. In addition, radar is thought not to harm the insect or modify its behavior during a study. Unfortunately, the high cost of using radar reduces the practicality of this tool for daily or seasonal insect monitoring.

VIII. Measuring wing-beat frequencies

Insect wing beats were first catalogued and published by Sotavalta (1947). Amazingly, this researcher measured wing-beat frequencies over 100 insects by simply listening to them and assigning frequencies based on his perfect pitch (Sotalvalta 1947). This is an uncommon ability. More typically, quantifying wing-beat frequency has more recently relied on microphones placed closely to the insects of interest (e.g., Ogawa and Kanda 1986; Roberts et al. 1998). The recorded sounds are analyzed through an oscilloscope or sound-analysis computer software. Microphones coupled to a system for amplifying and recording signal are cheap and portable tools for researchers. However, microphones can detect sounds from insect wings only when positioned within a short distance (often less than 1 m). Intensity of the weak acoustical signal diminishes with the square of distance. Therefore, microphone use has been limited to typically experiments where the target insects are contained within small cages. Other interesting techniques for recording insect wing beats included the use of high-speed film photography (Baker 1981) and electromyographic (EMG) recordings (Foster and Robertson 1992). Both of which require researchers to be present during the study.

Detection of insect activity (including flight, but not wing-beat frequency) was also made easier with the advent of electro-static actographs (Backlind and Ekeroot 1950 as cited by Berry 1972; Edwards 1960; Berry 1973) and later versions using infrared (Eaton 1980) and radar (Buchan and Moreton 1981; Schaefer and Bent 1994). These technologies are beneficial in that they can run in the researcher's absence. More esoterically, Macauley (1974) was able to use thermal gradients disturbed by flying moths to detect insect flight. While these types of sensors were used only to measure activity and not wing-beat frequencies, their automated data collection and circuitry design became foundational in the development of newer, optical sensors.

Unwin and Ellington invented an optical tachometer in 1979. This device detected and amplified minute changes in light intensity across the surface of a photo-diode as modulated by the movement of the body parts of flying insects even decimeters away from the sensor. From the optical tachometer signal, fundamental wing-beat frequencies of insects could be extracted by oscilloscopic analysis.

More specifically, the device was comprised of a lens and a photo-diode integrated into an amplified circuit board. The unit was battery powered and had a simple electrical analogue output recorded over time on devices such as a tape recorder. The system was used to detect wing-beat frequencies of bees and flies in Costa Rica (Unwin and Corbet 1984) as well as various beetles during Oertli's (1989) thermoregulation work.

Dr. Aubrey Moore of Ottawa, Ontario, Canada has, over the last 15 years, substantially improved optical tachometer technology. This research yielded the Optical Flying Insect Detection and Identification System (OFIDIS) now manufactured and distributed by Qubit Systems® (www.qubitsystems.com), of Kingston, Ontario, Canada for use as a remote sensing, pest-monitoring tool for field use. The system has two main components: 1) An optical sensor (Figure 6) with imbedded photocell inside, and 2) wave analysis software called TRex 2.0. The sensor's photocell is able to detect very rapid changes in light intensity (beyond 1000 Hz) caused by insect wing beats in the presence of sunlight or an artificial light source. Insects can either diminish light falling on the sensor by shadowing it, or increase the light on the sensor's surface by reflecting light onto it. The millivolt electrical changes in the sensor circuitry can be amplified and recorded as raw electrical signal using a digital recording device, such as a mini-disc player. The individual signals can then be analyzed for their fundamental frequencies as well as harmonic content. Dr. Moore postulates that harmonic patterns may be taxon-specific, in which case a neural network database could be trained to recognize insects of interest.

Moore's initial studies using the precursor to the OFIDIS sensor were conducted on mosquitoes (Moore et al. 1986) and aphids (Moore and Miller unpublished data). The mosquito studies showed that the sensor and its analysis software were capable of distinguishing between *Aedes aegypti* (L.) and *A. triseriatus* Say 84% of the time when analysis was conducted only on the fundamental frequencies (also called primary frequency or 1st harmonic). When analysis was done on the subsequent 4 harmonics of each mosquito's signal, 82% accuracy was achieved.

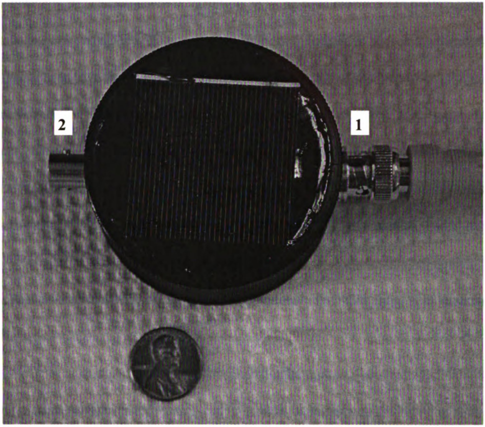


Figure 6. The OFIDIS sensor is 3 cm high, 6 cm in diam, and weighs 126 g. It is waterproof and very durable; electronics are contained within a protective epoxy. Connector 1 delivers the sensor's output relative to a resting voltage, while connector 2 delivers absolute voltage.

During aphid studies in the laboratory, individual frequencies of 4 species overlapped, but their means were significantly different ($p = 0.05$) from each other. This outcome suggested that there is variation of wing beat frequencies within a species and that using only wing-beat frequency to distinguish individual insects will not suffice for identification. Moore suggested that harmonic content of the wing-beat frequency might provide more individualized characterization for more accurate identification of that insect. However, considerable work remained to be done on the OFIDIS system. For example, the active space of the sensor was not yet characterized nor was the system broadly tested in the field for recording a wide range of insects. Beginning in year 2000, Dr. Moore and Qubit Systems sought collaborators to assist in this research.

IX. The potential application of OFIDIS relative to other insect monitoring techniques

Insect detection and monitoring are fundamental to pest management and conservation programs addressing habitat quality. Insect populations can rise and fall dynamically within and between seasons due to many biotic (e.g., predator density) and abiotic (e.g., degree day accumulation) factors (Southwood 1978; Samways 1994). This reality can make insect detection and monitoring a challenge, especially since the patterns associated with population fluctuations are not known for all species in need of control or protection. An insect detection device that features real-time data collection, over a large area, and that is portable and cheaper than radar or more detailed than aerial photographs would benefit management programs.

A. Pest management. Flying insects can be detected using sticky traps with or without lures, light traps, sweep netting, suction devices and through indirect means, such as parasitoid emergence (Pedigo 1996). Unfortunately, initial detection and/or reaching the action potential of a pest population usually warrant insecticide application and the long-term monitoring necessary to delay spraying is halted (Dent 2000). This is often the case even if natural control of the pest were possible. Stinner (1983) asserted that using light, pheromone, and suction traps for early detection of pests is problematic because of insufficient studies converting trap catch into accurate density estimates.

More recently, studies have been conducted to measure the reliability and accuracy of trapping systems deployed in greenhouse and cropping systems. Heinz et al. (1992) studied the time-efficiency use of sticky traps in greenhouses. They saw that a major problem with trap deployment was not only the cost of the traps themselves, but also the labor needed to sort and identify the arthropods caught. This unique study established a specific number of traps, that when deployed, would give a reliable estimate of pest density that could be used for pest management decisions.

Trap design must be conducive for collecting the highest number of insects of interest within specific crops. Knodel and Agnello (1990) determined that non-sticky pheromone traps were better than the sticky variety within New York orchards. Leafhoppers, which are very quick, erratic flyers, are better collected through suction trapping than through a beating tray (Herms et al. 1990). Yet for slower moving insects, like lady beetles, visual inspection/ scouting was judged the most efficient sampling technique so long as time was not a factor (Michels and Behle 1992). In addition, abiotic factors such as temperature and light intensity will affect catch density. For instance, pea aphids and their predators are most efficiently caught by sweep netting at late morning hours (Schotzko and O'Keefe 1989).

I now join Dr. Moore in suggesting that several OFIDIS sensors placed within a crop or orchard could serve a grower well by gathering real-time data about number and possible type of flying insects. This optical sensor could be used in conjunction with traps and scouting protocols to enhance monitoring activities. With an added light source, like a laser beam, night monitoring could be achieved.

B. Insects as indicators of habitat quality. Insects are regularly used as indicators of habitat quality. Tiger and ground beetles, as well as butterflies, ants and flies play important indicator roles in terrestrial habitat quality studies, while mayfly larvae may be sampled and compared during water quality studies (New 1995). For the terrestrial studies of insect indicators, or even for those conservationists trying to monitor for protection, the OFIDIS sensor could prove useful. The OFIDIS sensor could be applied to insect density and abundance studies within many land and aquatic environments.

Some conservationists have described and ranked the insects that would be good indicators in certain regions of the world. Brown (1997) and Brazilian colleagues created a list of valuable insect indicators of environmental changes in the Brazilian Atlantic Forests. This list includes flying insect families and subfamilies from Hymenoptera (*Meliponinae, Euglossinae*), Lepidoptera (*Nymphalidae, Lycaenidae, Saturniidae, Arctiidae*), Diptera (*Muscidae, Tephritidae, Phlebotominiae, Bibionidae*), Coleoptera (*Elateridae, Cerambycidae, Cassidinae*), Hemiptera (*Pentatomidae*), Homoptera (*Menbracidae*), and many odonates. He further mentions that using an array of insect indicators, like those mentioned above would better indicate the extent of disturbance. Monitoring a collective group of insects within one region would avoid drawing conclusions about habitat quality from monitoring one taxon. But, this can be viewed as so time-consuming as to be judged impractical. The OFIDS sensor could contribute valuable multi-species data when deployed in fields and waterways throughout an ecological region being evaluated for animal diversity and habitat quality. Moreover, this electromagnetically based system has considerable potential to operate remotely.

X. Objectives of thesis research. Study objectives were to: 1) Characterize the OFIDIS sensor's active space when operating under sunlight, and 2) Determine whether this system capturing optical signals from insect wing beats could yield useful measures of insect abundance and diversity in a few types of natural and disturbed areas in central Michigan.

Chapter 2

Evaluation of an optical sensor and waveform analysis software system for detection and taxonomic characterization of flying insects using wing-beat frequency and harmonics: Response to controlled, artificial stimulation

Introduction

Insect detection and identification is fundamental to any conservation monitoring program or pest management plan involving insects (Samways 1994; Pedigo 1996). For example, the Optical Flying Insect Detection and Identification System (OFIDIS), designed by A. Moore (Moore 1998) and marketed by Qubit Systems (www.qubitsystems.com) of Kingston, Ontario, Canada, was created to detect and, in-so-far-as possible, identify flying insects quickly and remotely by using their wing-beat frequencies and wing-beat harmonic content as identification characters.

The OFIDIS sensor's design builds on the optical tachometer invented by Unwin and Ellington (1979), in which rapid, minute changes in light intensity across a photo-diode increase or depress electrical signal output. This tachometer was used in studies measuring how wing-beat frequency of bees and flies increased with ambient air temperatures (Unwin and Corbet 1984) and how increased thoracic temperature in some beetles caused an increase in wing-beat frequencies (Oertli 1989).

The OFIDIS sensor (Figure 6, chapter 1) includes a photocell that detects rapid light changes across its surface caused by beating insect wings and moving body parts. The photocell and additional circuitry are contained within in a 3 cm thick, 6 cm diam, weatherproof case that collectively weighs 126 g. Electrical signals, created by an insect's shadow or reflection of light onto the photocell of the sensor, are detected as a change in voltage. These electrical data can be stored on a recording device, such as a tape recorder or mini-disc player. OFIDIS has accompanying waveform analysis software (TRex 2.0) that displays waveforms and harmonic spectra from captured signals. However, other waveform analysis programs, such as Canary 1.2.4, a well-established program developed by the Bioacoustics Research Program at the Cornell Laboratory of Ornithology, also display waveforms and harmonics of captured signals in alternative forms.

Initial studies using an OFIDIS predecessor, designed by Moore, were conducted on mosquitoes (Moore et al. 1986) and aphids (Moore, unpublished data). The sensor and its analysis software were capable of distinguishing between laboratory-held *Aedes aegypti* (L.) and *A. triseriatus* (Say) 82% of the time. Analysis was conducted on the fundamental frequency (also called primary frequency or 1st harmonic) and the 4 consecutive harmonics from each mosquito's record. In studies of aphids in the laboratory, individual frequencies of 5 species overlapped, but their means were different from each other ($P<0.05$). These studies suggest that there may be sufficient variation of wing-beat frequency and harmonics to distinguish among some taxa. Moore concluded that using only the primary wing beat frequency (1st harmonic) of an insect is a less discriminatory characteristic compared to using both the insect's primary wing beat frequency as well as additional harmonics.

Both the mosquitoes and the aphids used in the above studies were held within flight arenas under artificial lighting. These conditions may have modified their flight behavior by forcing more take-offs and landings and wing buzzing rather than equilibrated free flight. Insects that are warmed-up and flying in their natural environment might have less variation in wing-beat frequency and harmonics.

If particular insect taxa generate unique harmonic patterns with their wing beats, it is possible that some could be identified and counted remotely, using the OFIDIS system (Moore, per. comm.). Recently, OFIDIS was offered to collaborators worldwide for testing. Objectives of this research were to: 1) characterize active space for shadowed and reflective stimuli detected by the OFIDIS sensor operating under sunlight, 2) determine how light level affected signal intensity, and 3) evaluate the utility of TRex 2.0 and other wave analysis software in displaying the signals recorded from the sensor.

Material and Methods

Recording techniques. All data were recorded on a mini-disc player (Sony® MZ-R37, Japan), using 74 min, Memorex® mini-discs. The sensor was connected to the mini-disc player by an RCA cable (Radio Shack® 278-962). The mini-disc player's lineout was directly connected to the input of a Gateway Performance PC or Power Mac with a 16 bits or higher soundcard. Light intensity readings were made with a Lutron® Lux 100 photometer (Lutron Electronics, Taipei, Taiwan).

Various wave-analysis programs are available to researchers evaluating biological sounds and electrical data. The OFIDIS wave-analysis program, TRex 2.0, has been evolving concurrently with this research. Therefore, it was important to use additional software to provide contrasting measurements of the data collected. Canary 1.2.4 was chosen to accompany this research and provide a comparison to TRex.

Shadowed signal intensity over distance. A 1 x 1 m mirror was used to redirect sunlight horizontally along a 50 m sidewalk before it struck the shaded wall of a building.

The OFIDIS sensor was placed in the sun-lit spot on the wall with its face perpendicular to the redirected sunbeam. A helper maneuvered an oscillating (53 Hz at a 120° angle) Braun® electric toothbrush (Type 4726, Kronberg, Germany) to which was affixed a blackened 1 cm square mirror chip between the sensor and the mirror, breaking the beam of light at 1 m intervals progressing either toward or away from the sensor. Mean intensity for each signal (10 reps) at each distance was measured by Canary 1.2.4 software.

Reflective signal intensity over distance. In preliminary use of the OFIDIS sensor in the field, I discovered that insect signals could still be detected even when the sensor was facing away from the sun on a cloudless day. Although the inventor and distributor have emphasized shadowing as the predominant mechanism of signal generation, it became apparent that insects are capable of reflecting enough light onto the sensor for their detection. This remained true even when the sensor was already fully illuminated by sunlight.

All tests characterizing reflective signal were conducted after 2 pm when the sun was not directly overhead. A 25 m rope was first stretched along the ground. The OFIDIS sensor was placed at one end of this transect with its face perpendicular to the ground and facing away from the sun. A hand-held, oscillating, electric toothbrush with an attached 1 cm^2 mirror was held 0.5 m above the ground and maneuvered to reflect sunlight until it struck the sensor as sensed by the investigators via auditory output from a small, amplified speaker (Radio Shack 277-1008C). This manipulation was done at every 1 m interval while moving along the 25 m transect. Undoubtedly, insect body parts are not perfect mirrors. However, measuring the response of the OFIDIS system under this controlled and reproducible activation offered insights into how the system is likely detecting insect signals when shadowing is impossible. Mean intensity of signal (10 reps) over distance was quantified using Canary 1.2.4 software. Data were expressed on a relative scale (0-100%) relative to maximum signal.

Effects of light intensity on signal strength. The investigator stood on the ground holding the OFIDIS sensor directly toward the sun in late morning on a cloudless day. A helper stood on top of a ladder and placed embroidery hoops (25 cm in diameter) holding one to six layers of polyester, shear cloth (20 intersections per cm) between the sun and the oscillating, electric toothbrush held approximately 2 m from the sensor. Light intensities adjacent to the sensor were recorded for each cloth density along with signal intensity of the toothbrush operating only in the shadowed mode.

Harmonic patterns as influenced by the variation of stimulus approach to the sensor. Tuning forks of 430, 440 and 523 Hz were struck and placed over the sensor in full sunlight. Each fork was first displayed with both vibrating prongs shadowing the sensor and then twisted so one prong was eclipsing the other over the sensor. Five repetitions per position were recorded and each tuning fork signal's harmonic spectrum was analyzed and displayed using Canary 1.2.4.

Testing TRex harmonic measurement capabilities using standard tuning forks. The sensor was placed face-up on the floor in a darkened room. An illuminated laser pointer was securely placed 1 m above the face of the sensor. The laser pointer was used because of lack of clear skies for several days in a row at the time of this test. Tuning forks of frequencies 430, 440 and 523.25 Hz were individually struck and placed in the laser beam. Five repetitions per tuning fork were recorded. TRex 2.0 analyzed each recorded signal, displaying harmonics for each tuning-fork signal that were then visually compared.

Effects of signal duration on TRex detection. The music synthesizer within Creative Sound Studio® software was set to piano mode. Middle C (523 Hz) was played at both 0.5 and 1 s intervals and recorded as wave files on the computer using Creative® recording options. This note was used because it matched the frequency of one of the previously used tuning forks. Resultant wave files were played through TRex 2.0 to generate waveforms and harmonic spectra for each middle C piece of varying duration. The TRex waveforms were visually compared and their subsequent harmonic spectra were compared through cluster analysis of Teach Me Data Analysis® (Lohniger 1999) to determine if differences in harmonic patterns resulting from both the 0.5 and 1 s signals would be separated into two distinct clusters.

Results and Discussion

Measuring shadow signal intensity over distance. Shadowed signals were always most intense when the stimulus was immediately in front of the sensor. The negative exponential decrease in signal intensity with distance from the sensor (Figure 7) was to be expected for a small object casting a shadow on a target surface lit by sunlight. The explanation is well known (Minnaert 1993) and involves the non-linear narrowing of the umbra (darker part of the shadow) and the swelling of the penumbra, (lighter parts of the shadow) as the eclipsing object is moved from the target toward the sun.

While shadowed signals from the toothbrush were sometimes detectable up to 45 m away both by the analysis software and the human ear upon acoustical playback, 30 m

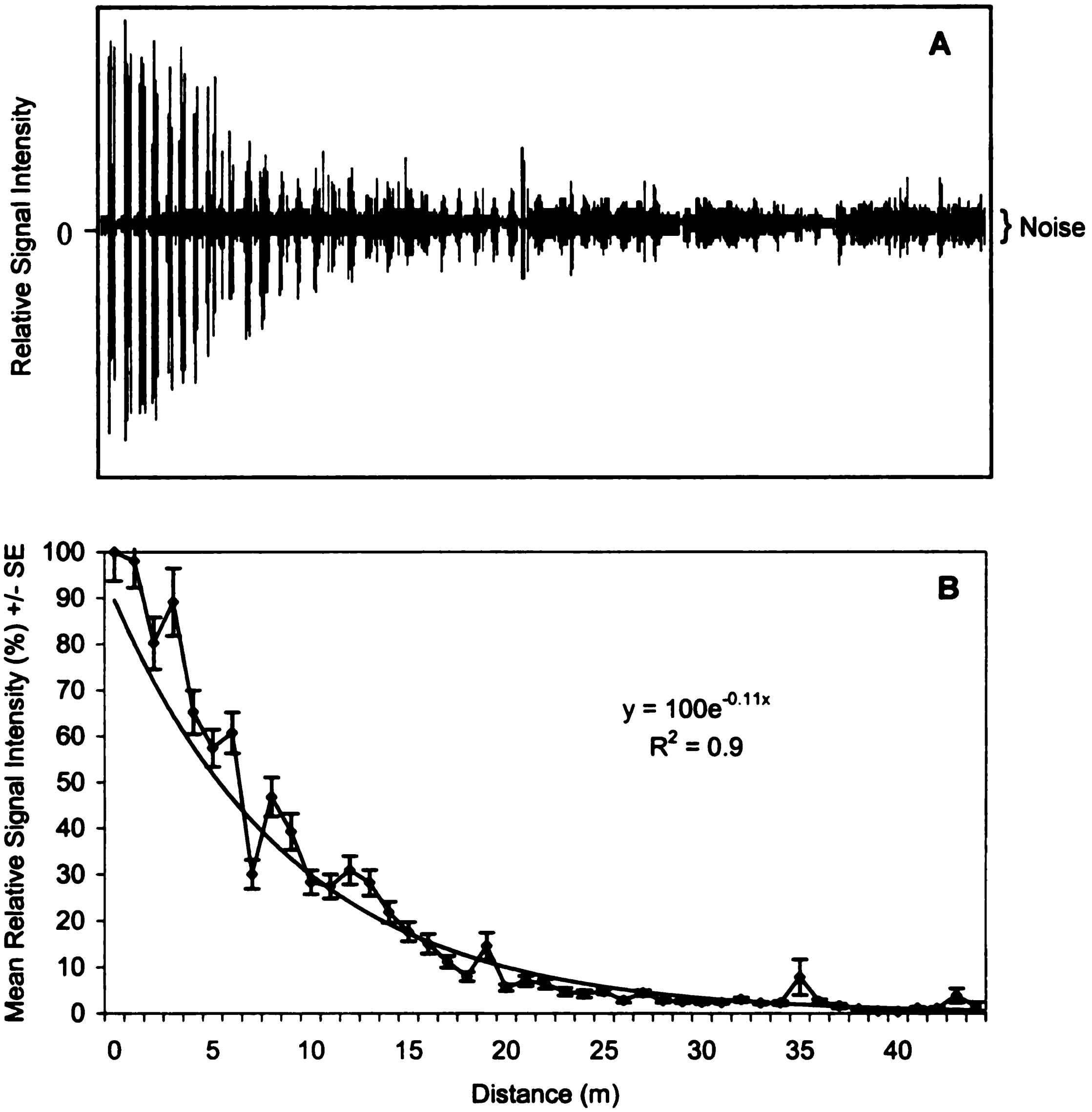


Figure 7. Shadowing toothbrush signal as a function of distance. Panel A shows an individual record with a typical signal-to-noise ratio. Panel B shows mean signal intensity for 10 replications.

or greater signal was most often amidst equal or greater amplitude of noise caused by atmospheric haze and wind. Without a filter option to eliminate this noise, shadowed signals beyond 30 m cannot be reliably analyzed by TRex 2.0 or Canary 1.2.4. Nevertheless, the OFIDIS sensor impressively has a much greater distance of detection than microphones used in previous insect flight studies (Ogawa and Kanda 1986; Roberts et al. 1998).

Additionally, I noticed that weaker, distant signals produced fewer harmonics. More testing of how strong a detected signal must be in order to allow a wave analysis program to produce usable harmonic patterns is needed. As of this writing, Dr. Moore has established a minimum threshold of signal intensity of 500 bits for TRex to analyze a signal and display harmonics (Moore per. com.)

Measuring reflective signal intensity over distance. Reflective signal from the mirrored toothbrush diminished considerably less per unit distance than did shadowed signal (Figures 7 and 8). A shallow and negatively linear slope was documented when mean signal intensity was regressed on distance. The decrease over the first 2 m did appear to be sharper than that at greater distances.

This modest decrease in signal intensity with distance is undoubtedly related to modest decrease in light intensity reflected from a perfect mirror over distance. This potential benefit in the insect sampling context needs to be contrasted against a lower probability of hitting the sensor with light with increasing mirror distances. At distances 10 m and greater it often took the mirrored-toothbrush handler up to 30-60 s to hit the sensor and sustain a signal, whereas it only took a few seconds when the handler was

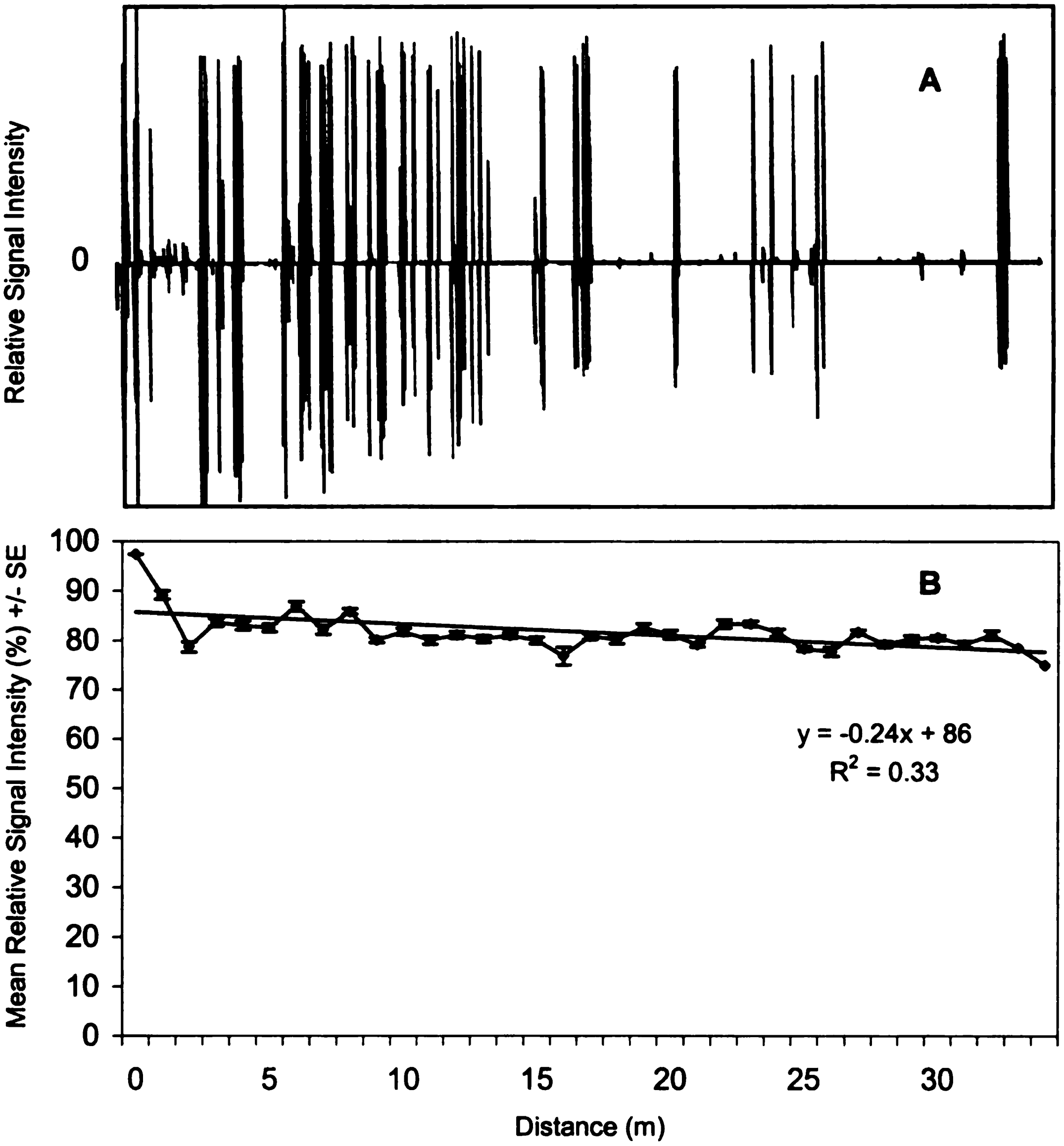


Figure 8. Reflective toothbrush signal as a function of distance. Panel A shows an individual record with a typical signal-to-noise ratio. Panel B shows mean signal intensity for 10 replications.

only 1 m away. These experiences suggested that although the signal strength of sunlight may remain very strong over considerable distances, the probability of reflected light striking the sensor would decrease appreciably with distance. Preliminary trials in which the mirror was positioned off the transect line showed that the active space for reflective signals is a broad cone rather than a cylinder. Although incomplete, this research has established that combined active space for reflected plus shadowed signals is large enough to make insect detection by the OFIDIS sensor a common rather than uncommon occurrence.

Further research is needed to determine the exact distance limits for insect detection by this sensor and the probability of detecting different-sized insects within this limit. Such details will allow a user of the sensor to make more informed decisions about which insects to measure (e.g., migratory aphids perhaps 100 m above the sensor or pollinating bees moving between plants in a crop).

Effects of light intensity on signal strength. Shadowed signal intensity decreased considerably once two cloth layers were placed between the sensor and the toothbrush stimulus (Figure 9). As shown by information combined in panels (B) and (C), a minimum sunlight intensity of 20,000 lux was necessary for the OFIDIS sensor to detect a shadow-produced toothbrush signal from ca. 3 m. Cloud cover or other conditions dimming light intensity will decidedly reduce the OFIDIS active space. Full sunlight is a requirement for maximal detection of shadow-generated signals. Although not formally tested here, decreasing light intensity will likely similarly diminish reflected insect signals.

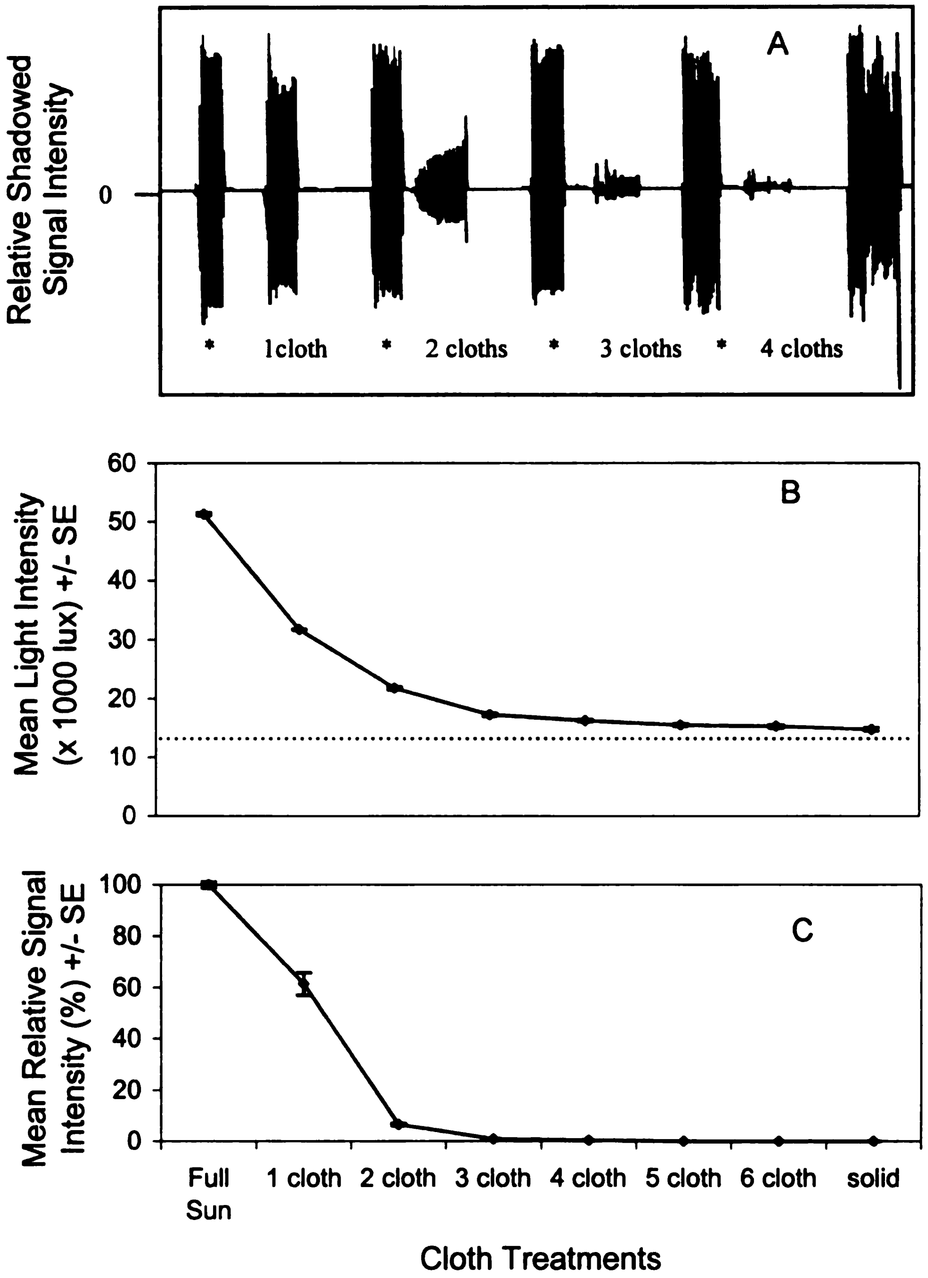
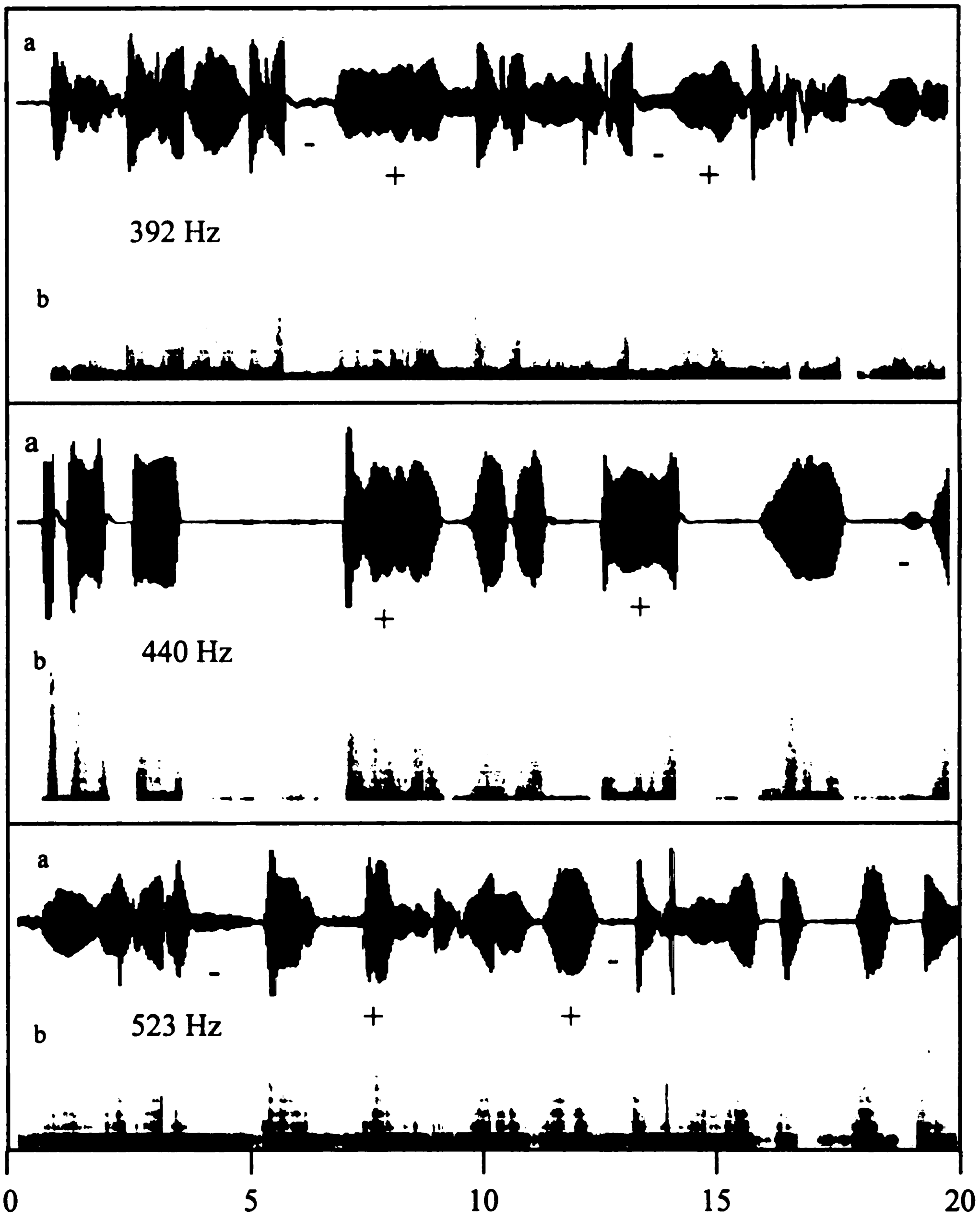


Figure 9. Shadowed signal intensity (A), mean light intensity at the sensor face as a function of density of cloth screens (B), and signal intensity relative to the maximum detected for each cloth treatments (C). Dashed line in (B) shows light intensity due to diffuse sunlight indirectly striking the light meter from sources below the cloth.

Harmonic patterns as influenced by variation of stimulus approach to the sensor. Each tuning fork signal became markedly less intense when the prongs were shifted from horizontal to vertical with respect to the sensor face (Figure 10). The harmonics produced by each tuning fork varied in their intensity and clarity in direct correspondence with overall signal intensity (Figure 10). However, whenever there was sufficient signal strength (e.g. 523 Hz) to produce harmonic spectra, the harmonic pattern was conserved, i.e. in no cases were there reversals in intensity of harmonics. These results must be taken with caution, but nevertheless, they support the idea that signals with identical fundamental frequency can produce the same harmonic pattern.

Testing TRex harmonic measurement capabilities using standard tuning forks. TRex displayed very similar harmonic patterns for each tuning fork that produced strong enough signal (Figure 11). However, TRex declined analyzing up to 50% of the signals produced due to weak or clipped signal (those exceeding an amplitude limit). At times, I could hear a clear signal upon acoustical playback, but TRex did not detect it for unexplained reasons.

It appears that there is a trade-off inherent in TRex: fewer signals are worthy of analysis, but those that are sufficiently intense, produce measurable harmonics. This information is important for future training of a neural network capable of identifying a particular insect signal within multiple signals (Moore 1998). Identifying specific insects of interest will be the strength of OFIDIS, especially if the same consistencies of harmonic patterns for a given taxon are observed and a reliable database is developed in which to compare field-acquired signals.



Time (s) after tuning fork was struck

Figure 10. Signal intensities shown as bandwidth (a) and harmonic spectra (b) of three tuning forks vibrating at identical fundamental frequencies. (+) = examples of when the arms of a fork were horizontal vs. (-) = arms vertical with respect to the face of the sensor. The intervals between maximum intensity of bands in the harmonic spectra are equivalent to the fundamental frequency of each tuning fork.

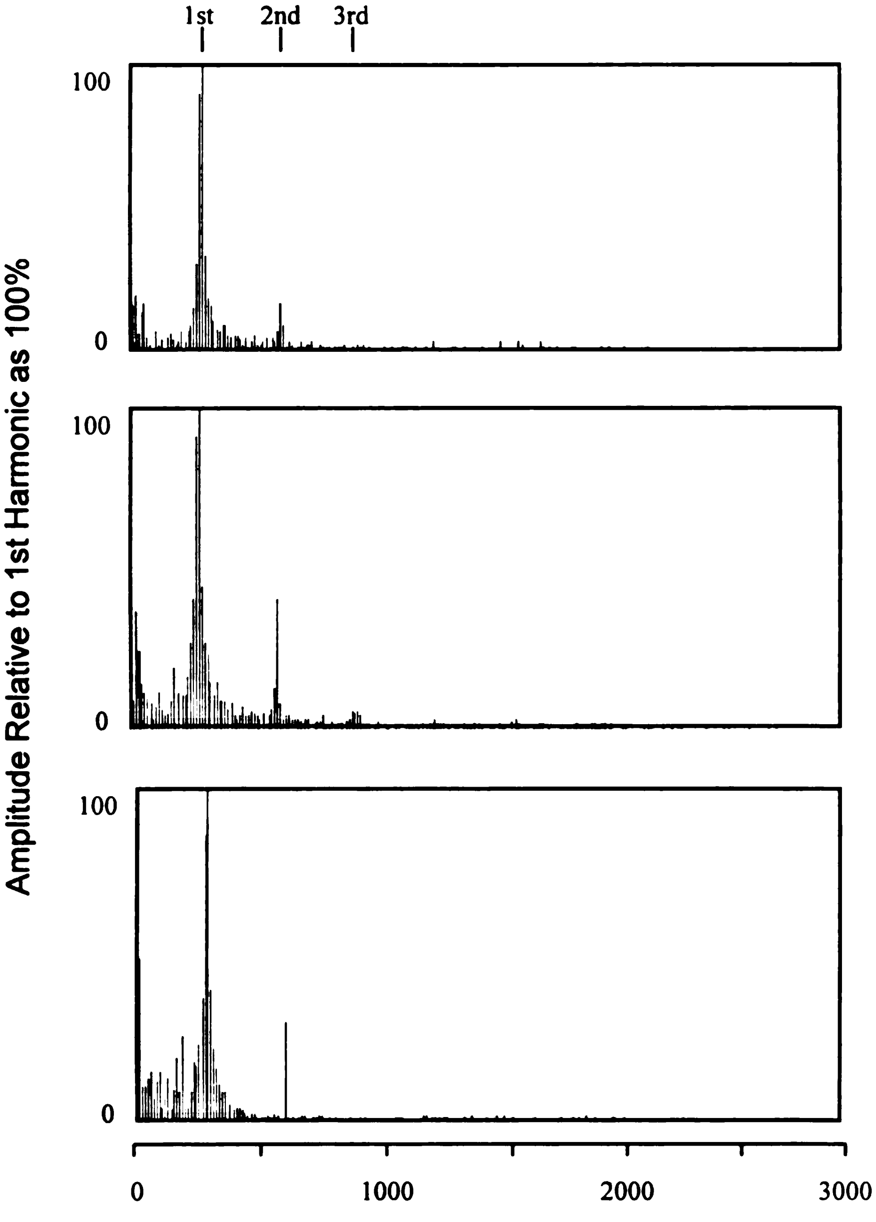


Figure 11. Harmonic patterns for 3 separate signals made by a 430 Hz tuning fork as analyzed and displayed by TRex software.

Effects of signal duration on TRex detection. Signal duration influenced the harmonic spectra produced by TRex. When comparing harmonic spectra produced by 0.5 s signals versus 1.0 s signals (Figure 12), slight differences in the amplitude of each harmonic distribution are apparent. These slight differences in harmonic amplitude caused an almost perfect division of the short and long signals into two major classification groups (Figure 13) when analyzed via cluster analysis using full frequency distributions of harmonics (Figure 12). This outcome demonstrates that signals generated by the same source can be over analyzed so as to detect differences due to subtleties in behavior. Developers and users of OFIDIS need to guard against attributing all variations in signal to authentic differences in signal generators.

Relevance of these results for OFIDIS use in the field. As reported herein, the active space of the OFIDIS sensor is sufficiently large to detect many different flying insects in varied environments under full sunlight conditions. Shadowing insects can be detected up to 30 m away. However, the active space for capturing reflective insect signals has not been officially delimited. As a safety margin against missing flying insect signals, the OFIDIS sensor should be treated as having an unbounded active space for reflective signals. For accurate comparative measures of insect abundance in differing sites, the sensor should be pointed at open sky under full sunlight.

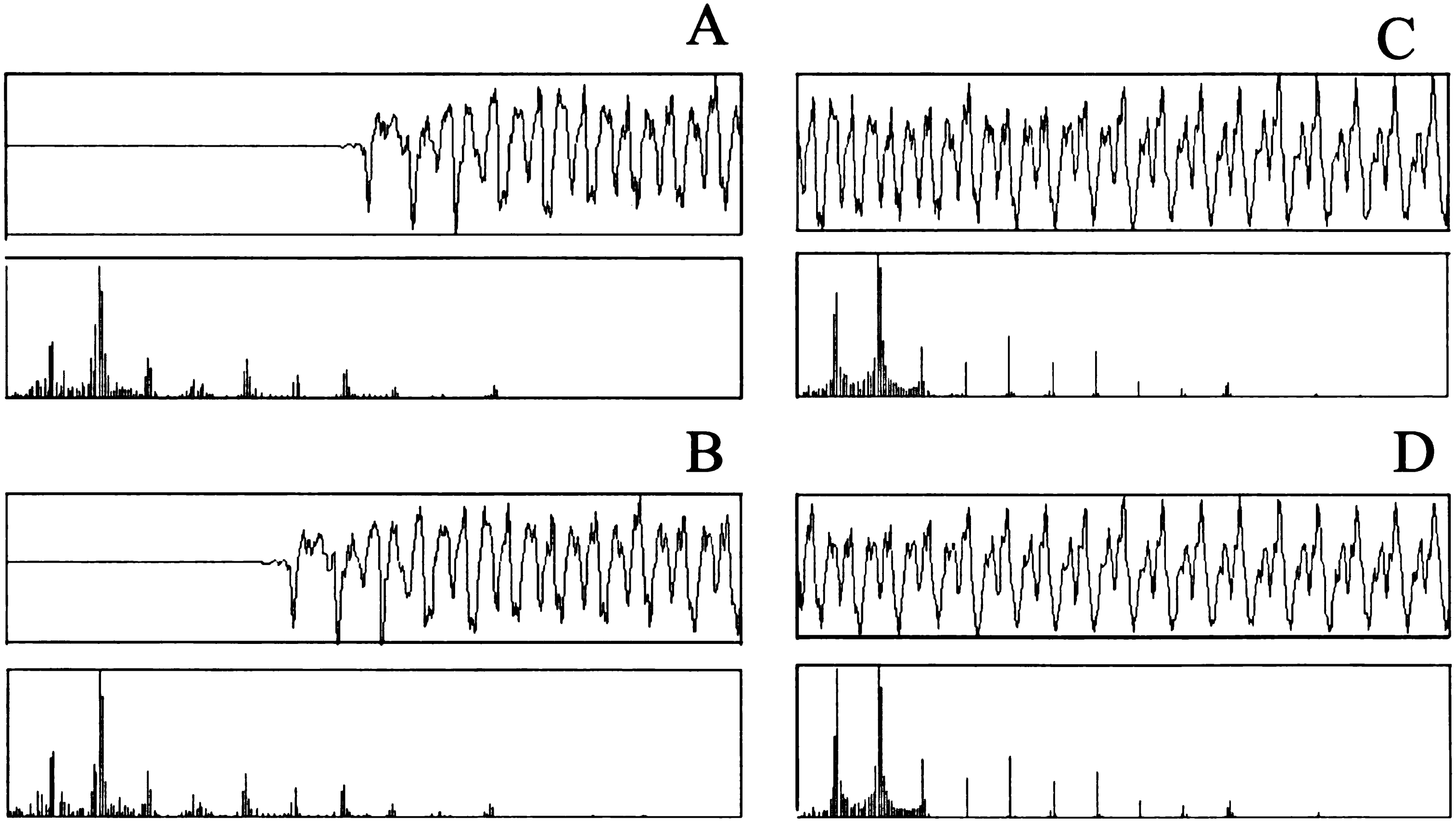


Figure 12. Middle C (523 Hz) piano signals displayed over time (top panel of each pair) and harmonic spectra measured by TRex. (A) and (B) = ca. 0.5 s (C) and (D) = 1 s, respectively.

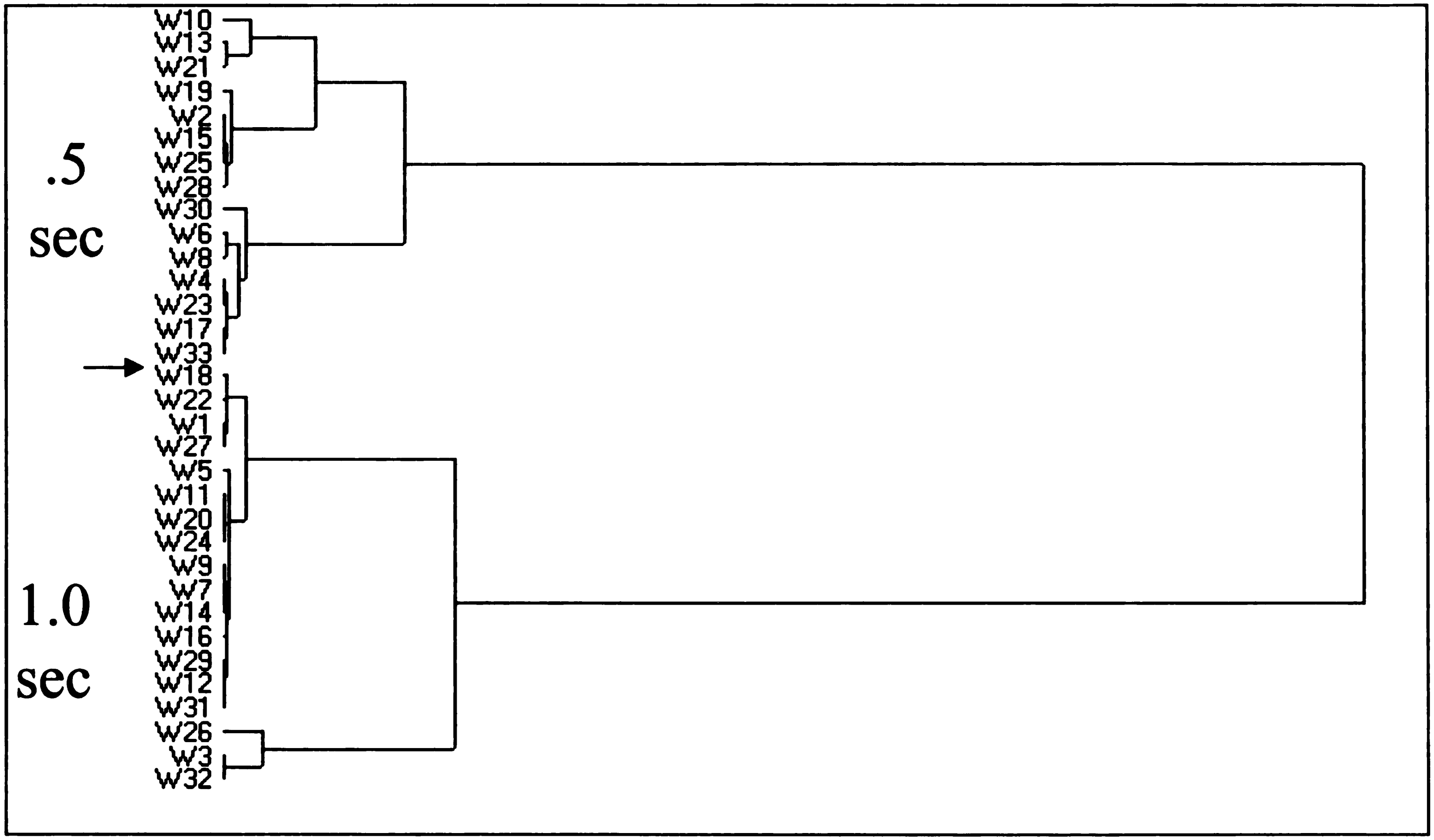


Figure 13. Cluster analysis of the TRex measured harmonics for Middle C signals (n=33)of two durations. The arrow indicates the major split between clusters.

Chapter 3

Field application of the OFIDIS sensor: Measuring flying insect signal abundance and diversity in three field-site types

Introduction

Insect conservation is an important and on-going part of conservation biology and endangered species protection. According to Pyle et al. (1981), the greatest cause of insect extinction is the destruction of necessary habitat. The United States Endangered Species Act (ESA) currently legally protects over twenty endangered and threatened insect species (USFWS 2000). Most of these insects are found in the Western USA states of California and Oregon, which have undergone tremendous human population growth and land use changes since ESA inception in 1973. In addition, worldwide invertebrate protection is broadly supported through the activities of the World Conservation Union (IUCN), World Wildlife Fund, and various other conservation groups.

Critical to the process of protecting ESA-listed species and adding new species to the threatened or endangered status list is acquisition of obtainable and reliable population data. Without proof that a species is declining, the U.S. Department of the Interior is unlikely to endorse protection (Houck 1993). Among the animal species needing protection are some insects.

Insect populations fluctuate from year to year, ranging from heavy declines to outbreaks (Samways 1994). For instance, the threatened California Bay checkerspot butterfly *(Euphydryas editha bayensis)* has many isolated populations, all of which fluctuate dramatically from year to year (Ehrlich and Murphy 1987). In these cases, it is often unclear to researchers and wildlife managers as to why the insect population is fluctuating and whether or not a decline is temporary or indicative of a potential loss of the species. Unlike mammal studies, where data regarding gender ratios and dispersal patterns are often well defined for managers devising an appropriate conservation plan, insect studies often lack such resolution.

Insects serve as important environmental indicators of habitat quality. Steytler and Samways (1995) examined the larval and adult Odonata colonization of a man-made, urban lake in South Africa as a means of monitoring the lake's water quality. After the lake was constructed, Odonata species richness in the area doubled from 12 species to 26 species. These same authors (1996) used Odonata as indicators of habitat disturbance by examining species richness along a river flowing through a forest, residential area, park and city. Species richness was highest in the park and lowest in the forest, which had been disturbed by commercial harvesting.

Collecting and identifying insect indicators is time consuming. Pearson and Cassola's (1992) use of tiger beetle species as indicators of regions high in biodiversity relied heavily on accurate human observation. At their site in Peru, it took 50 observational hours to find 93% of the tiger beetles known to live there. In addition, Integrated Pest Management practices are often not implemented in place of traditional pesticide control because of the pest scouting costs (both time and labor) to growers (Wearing 1988). Hence, there is a need for more rapid assessment of insect richness and population. Oliver and Beattie (1993) and others are testing the methodology of rapid assessment of biodiversity (estimates of species richness of insects using recognizable taxonomic units (RTUs) or a morpho-species approach rather than actual identified insect species) to help conservation agencies survey habitats faster. It is currently thought that the inclusion of invertebrates in habitat assessment is excessively costly in both time and labor. New technologies capable of remote detection, identification, and counting of insects might enhance such assessments.

In the current research, I deployed the OFIDIS sensor into three field-site types (wetland, old-field, and parking lot) in an attempt to measure relative abundance and diversity of flying insects. This research represents the first steps in determining: 1) whether or not the OFIDIS sensor is a reliable tool for measuring flying insect activity under a set of standard weather conditions, and 2) the extent to which captured insect signals vary in fundamental frequency.

Materials and Methods

Study design. Research was conducted between July 7 and September 6 of Summer 2000 at three field-site types: wetland, old-field, and parking lot. Each site type was replicated three times for a total of 9 sites. Because insect activity was expected to vary with temperature, humidity, and perhaps wind conditions, data collection and analysis were blocked by day in the sense that each site was sampled on a given day and replicates were accumulated over days. Order of visitations was randomized; but certain sites were paired to consolidate driving time. Another restriction in this randomized complete block experiment was that full sunlight was required for at least 9 of the 10 sampling min at each site. Past work on the OFIDIS sensor (Chapter 2) established that its active space was proportional to light intensity. This limitation was costly, as Michigan skies rarely proved cloud-free for the full 6 hours needed to complete one full block of sampling. Nevertheless, seven days of insect signal data were collected and analyzed.

Research sites. Locations were selected for their site type and were all in the state of Michigan, within 25 mi of Michigan State University (See Appendix 2 for the maps of the sites and their approximate areas).

The Rose Lake flooding site (42° 48.775 N, 84° 21.501 W; Shiawassee Co., MI) is a wetland surrounded by forest. It is within the protected Rose Lake Wildlife Research Area. Swimming, fishing, or boating is not commonly practiced in this body of water, making it the most pristine of the wetland sites used in this study. Hewes Lake (42° 31.445 N, 84° 20.272 W; Ingham Co., MI) is located within the Dansville State Game Area, which is surrounded by forest. Boating, fishing and swimming are permitted here. Central Park pond (42° 43.795 N, 84° 25.310 W; Ingham Co., MI) is situated within a park in a suburban setting and highly maintained by mowing regimes. People do fish here, but the pond is too small for boating; swimming is not allowed.

The Rose Lake old-field site (42° 48.775 N, 84° 21.501 W; Clinton Co., MI) is also within the Rose Lake Wildlife Research Area. This once-cultivated land is undergoing secondary succession and is populated by a large variety of plants. It is the largest old-field in this study. The Dansville old-field site (42° 48.770 N, 84° 23.450 W) within the Dansville State Game Area is surrounded by forest. Legg Park old-field (42° 41.665 N, 84° 22.949 W; Ingham Co., MI) is surrounded by forest, railroads, and a gravel road leading visitors to a grassy picnic area and forest trails.

The Kmart parking lot (42° 31.445 N, 84° 20.272 W; Ingham Co., MI) was the largest of all the parking lots used in this study. Residential areas and businesses surround it. Hannah Plaza parking lot (42° 43.165 N, 84° 27.646 W; Ingham Co., MI) had the most car traffic activity of the lots. Manly Miles parking lot (42° 43.007 N, 84°

27.519 W; Ingham Co., MI) was the least busy of the lots, but did contain the most trees and most abundant vegetative border.

Equipment set-up. The OFIFIS sensor (Figure 6) was fastened to the top of a 1 m high green garden stake that could be held vertical by insertion into the ground or a supportive stand at parking lots. The sensor always faced directly upwards toward the sunlit sky. Signals at each site were recorded on a mini-disc player (Sony® MZ-R37, Japan) for 10 min. During recordings, I stood several meters away from the sensor so that my possible attraction of deer flies and other insects did not affect the measurements. Cloud cover for a total of more than 1 of the 10 min disqualified a sample. Wind speed and local temperature at the initiation of each recording were measured with a combined anemometer/temperature probe (Omega; Manchester, UK), while a Lutron® Lux 100 photometer (Lutron Electronics, Taipei, Taiwan) was used to measure light intensity.

Counts of signals from the three site types. I listened to each 10-min recording played through speakers (Boston® Digital BA735) connected to a Gateway® Performance PC and counted the number of audible insect signals. Count means ± SE were calculated and graphed in Excel; significance between means was tested by Proc glm and means were separated by Least Means Square (SAS Institute, 2000). A Multiple Linear Regression (R-Square Selection Method, SAS Institute 2000) was run to establish the extent to which abiotic factors (date, time-of-day, wind, site type, location, temperature, wind speed, and light intensity) affected count data.

Wing-beat analysis of signals from three site types. Signals collected from the Rose Lake flooding, Dansville old-field, and the Kmart parking lot on the dates of July 20, August 16, and September 6, 2000 were used for this analysis. Dates were chosen to achieve representative coverage of the 3-month field season. Each 10-min recording was analyzed by Canary 1.2.4 sound analysis software (Cornell Bioacoustics Lab, Ithaca, NY)) for fundamental frequency and harmonic content. The harmonic spectra for each signal were printed and the fundamental frequencies of the wing-beat signals were calculated by hand because no software was found that could perform these calculations with desired precision (Figure 14). Calculations of fundamental frequencies consisted of using a high-precision metric ruler to measure the mean interval between harmonics and equating this distance to Hz in accordance with the y-axis scaling provided by the software. In some records where the scatter plot of harmonics were too compressed or smudged for measurements, the waveform (Figure 14B) was further expanded and the fundamental frequency assigned by measurement scaled to the calibrated x-axis. Precision of these fundamental wing-beat frequency measurements was quantified by having the two researchers taking measurements each re-measure 20 signals randomly selected within the range of 100-1000 Hz.

Signal diversity analysis. Frequency distributions of the wing-beat frequencies from each site location were made so that signal richness, diversity (using the Shannon-Weaver diversity index), and relative diversity could be compared between sites (Zar 1999).

Insect wing-beat signal capture for 33 collected insect specimens. Insects from diverse flying taxa were collected from site locations and the Michigan State University campus by sweep net. Individuals were placed in either a 1 x 1 m screened cage with the sensor positioned pointing downward from the center of the top of or in a

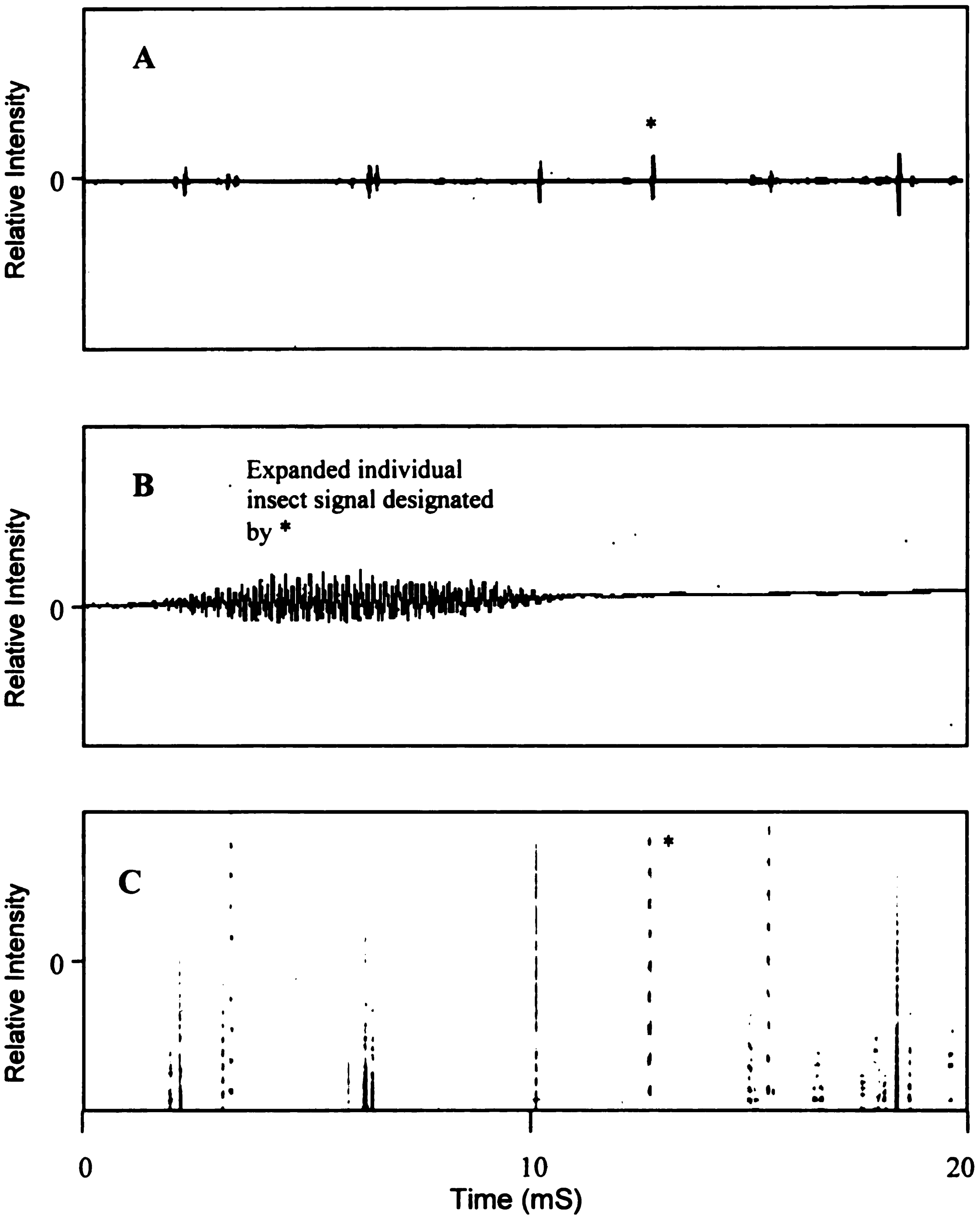


Figure 14. Types of data displayed by Canary 1.2.4 sound analysis software, used to analyze insect signals in this research. An insect signal in (A) can be expanded to show its entire wave form (B) and subjected to spectral analysis to make a spectrogram (C) of the harmonics. The intervals between each harmonic band are equal to the fundamental frequency of the analyzed signal.

10 cm diam, clear container placed on top of the sensor. Insect signals were recorded in full sunlight and the precise time they interacted with the sensor was noted for accurate linking of signal to given specimen. Individual signals were processed by the Canary 1.2.4 sound analysis program to generate a printed waveform used to measure the fundamental wing-beat frequencies, and, where possible, a spectrum of the harmonics. The 33 insects were frozen and then preserved according to the Michigan State University entomology museum procedures (Gary Parsons personal communication) and submitted to the museum as voucher specimens.

Results and Discussion

Wing-beat signals from the field. A sample of the waveforms for each of the 33 collected insects is displayed in Figure 15, arranged in ascending wing-beat frequency. Diptera and Hymenoptera were the most prevalent insect Orders collected, and their wing-beat frequencies (Figure 15) fell well within the expected ranges for these Orders (Figure 1). Harmonic spectra are not included here, as many of the signals did not yield distinguishable harmonics under captive conditions. The reason(s) for this absence are unknown and a bit disappointing, since I was able to see very rich harmonic spectra for signals recorded during the abundance/diversity study and in the tuning fork studies (Figure 10, Chapter 2). So far, insects with lower fundamental frequencies produce harmonics that can be undetectable. Therefore to obtain a fundamental wing-beat frequency, calculations must be based on expanded oscilloscopic waveform. Nevertheless, data begin to establish a database for wing-beat waveform and

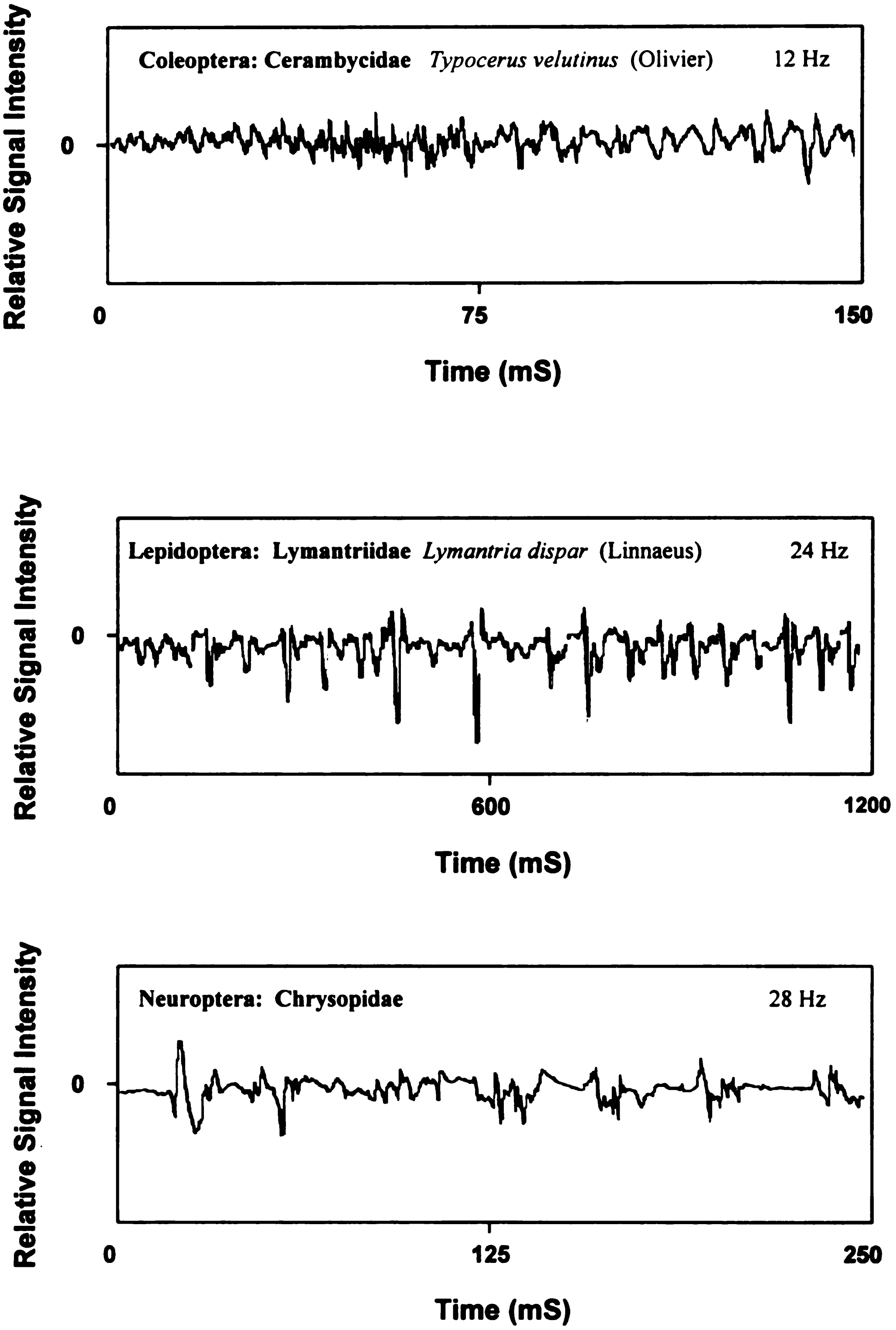
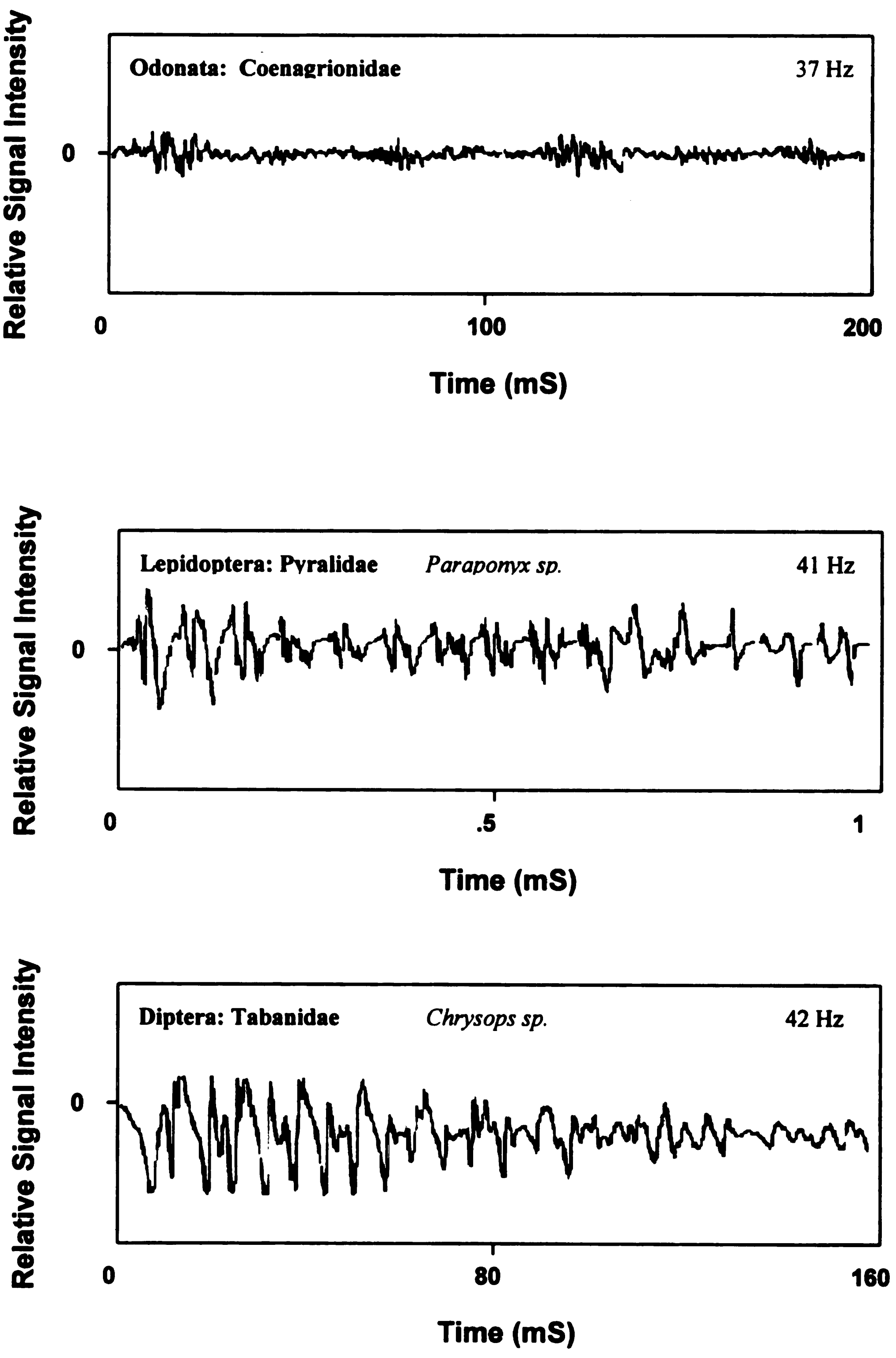


Figure 15. The individual waveforms for each of the 33 insects caught and identified. Identification of each insect was taken to the most specific taxonomic name with the possible keys available.



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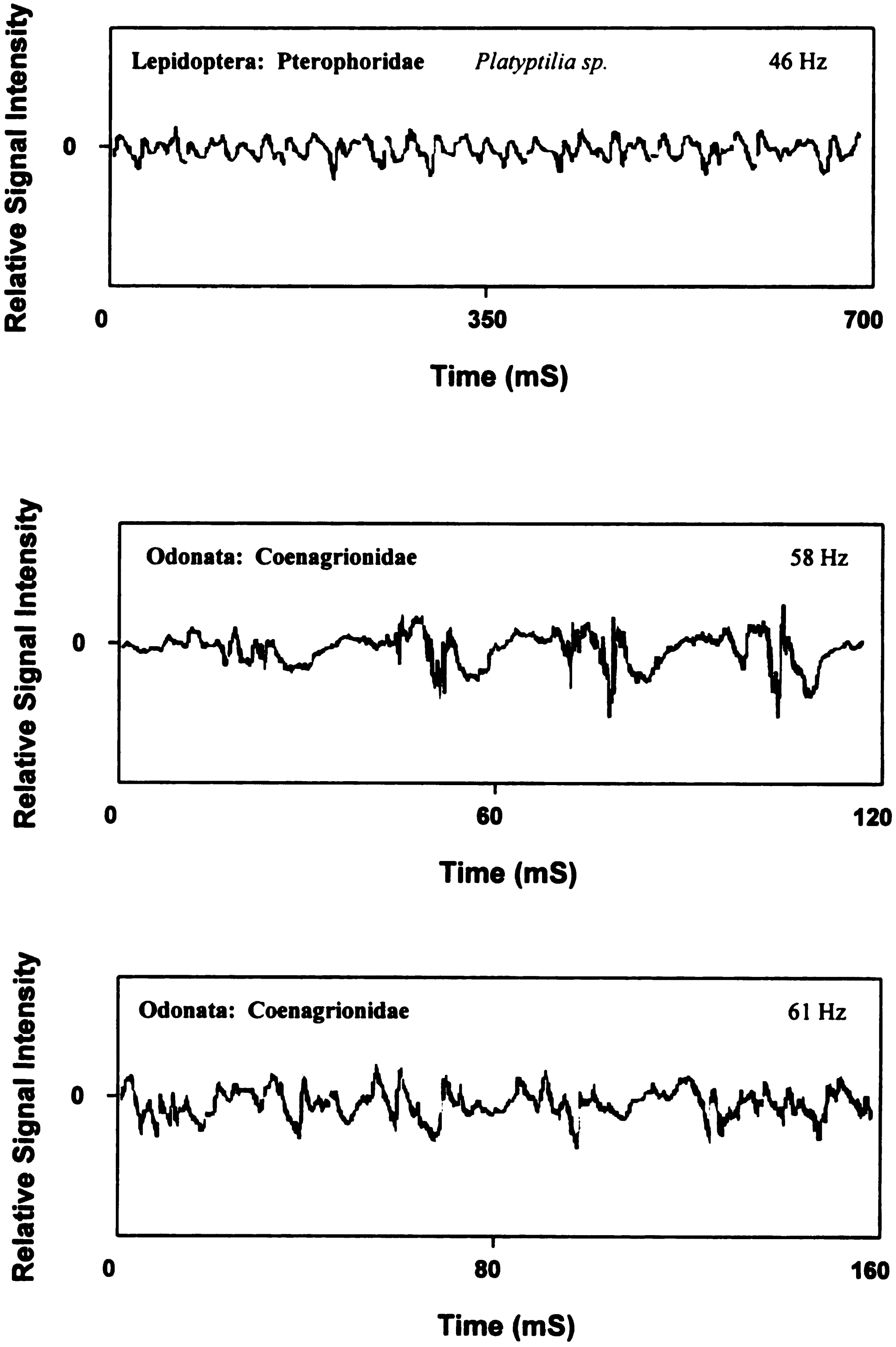

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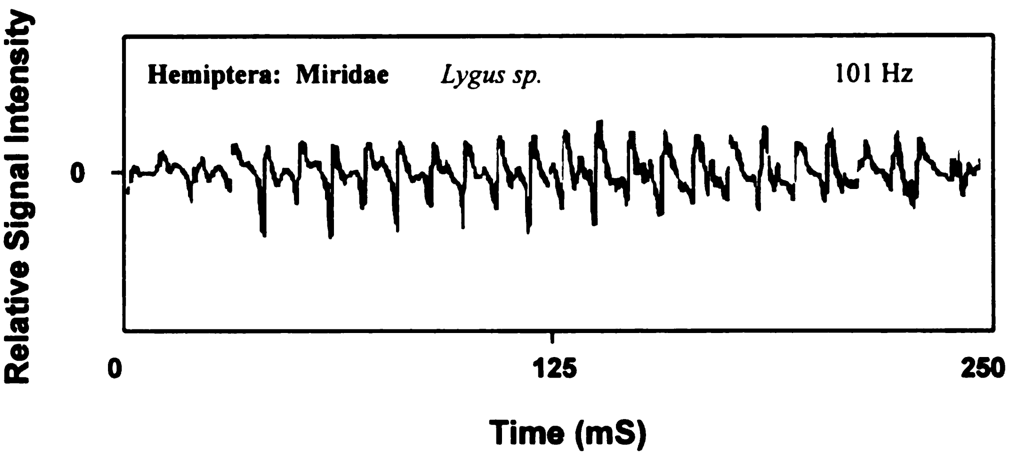

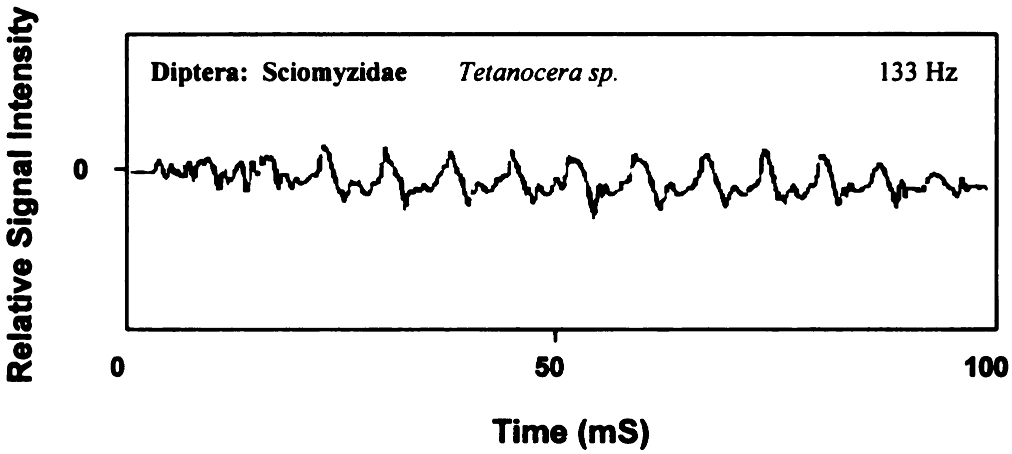

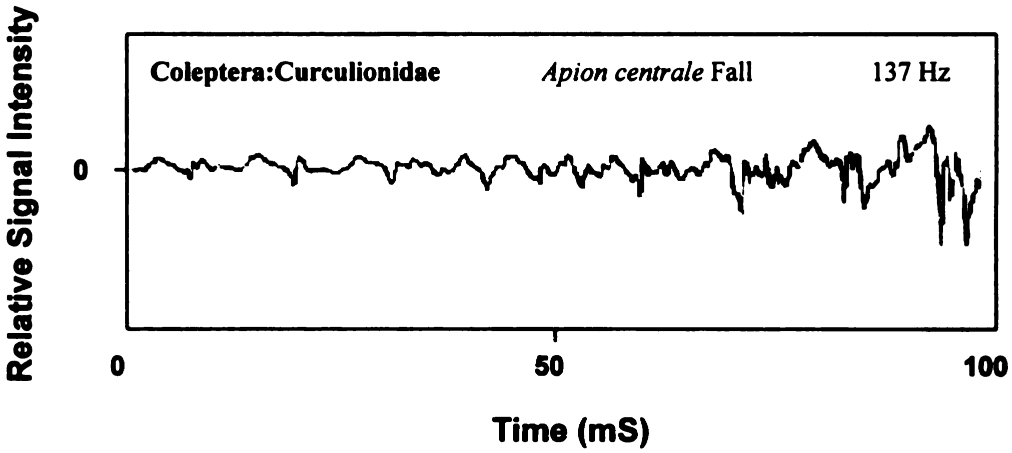

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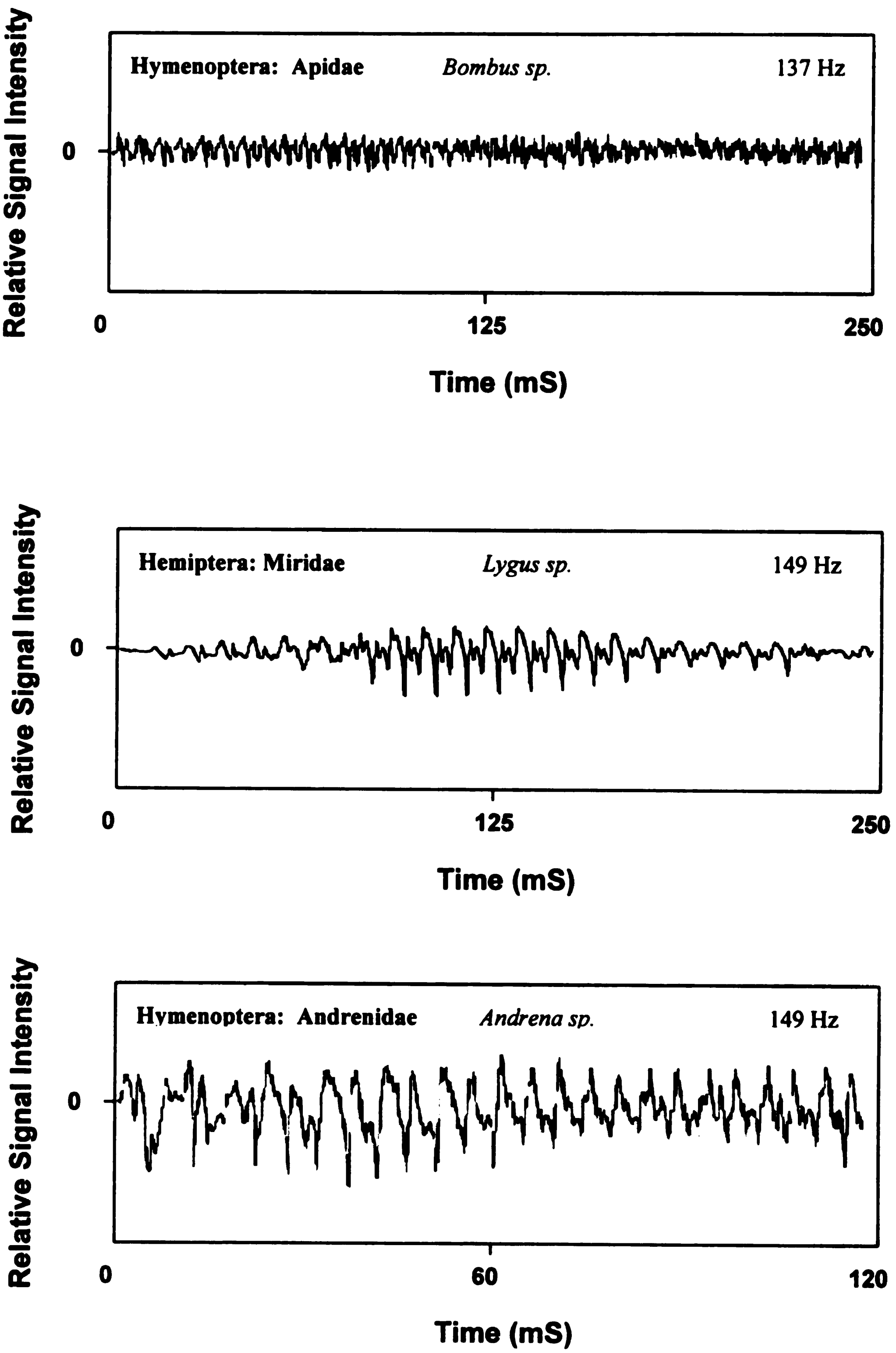

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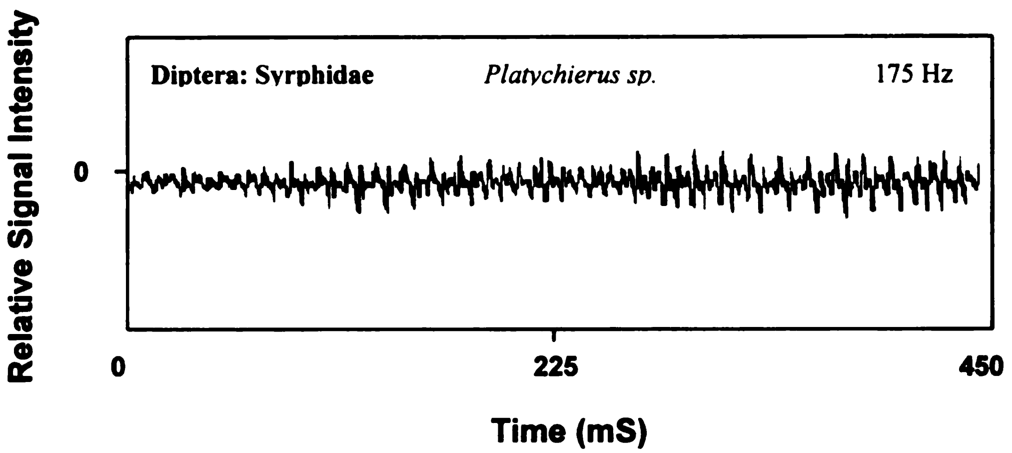

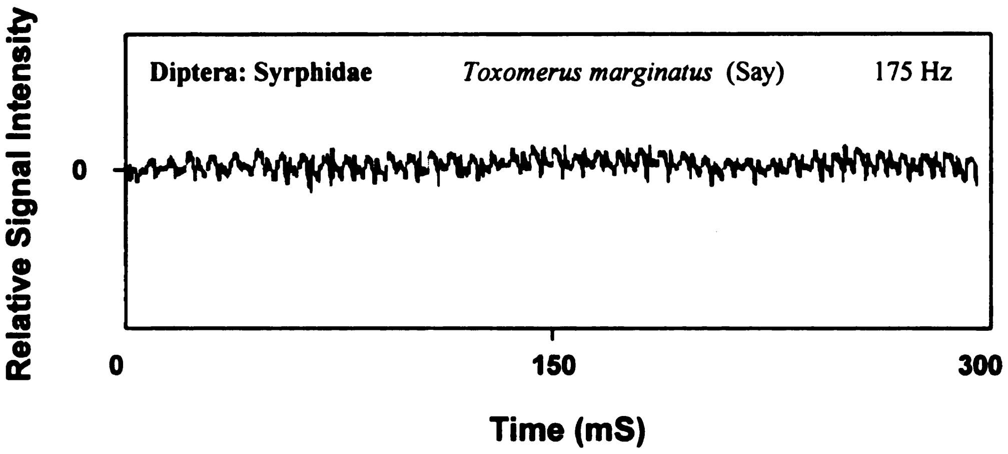

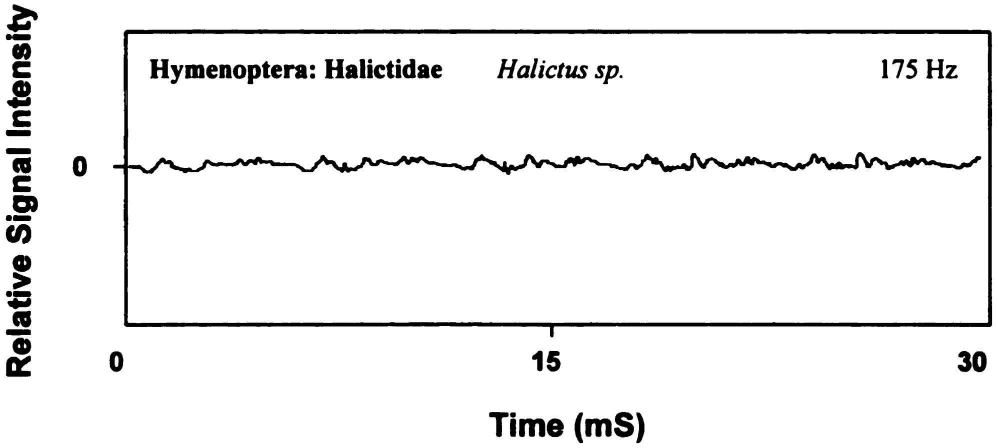

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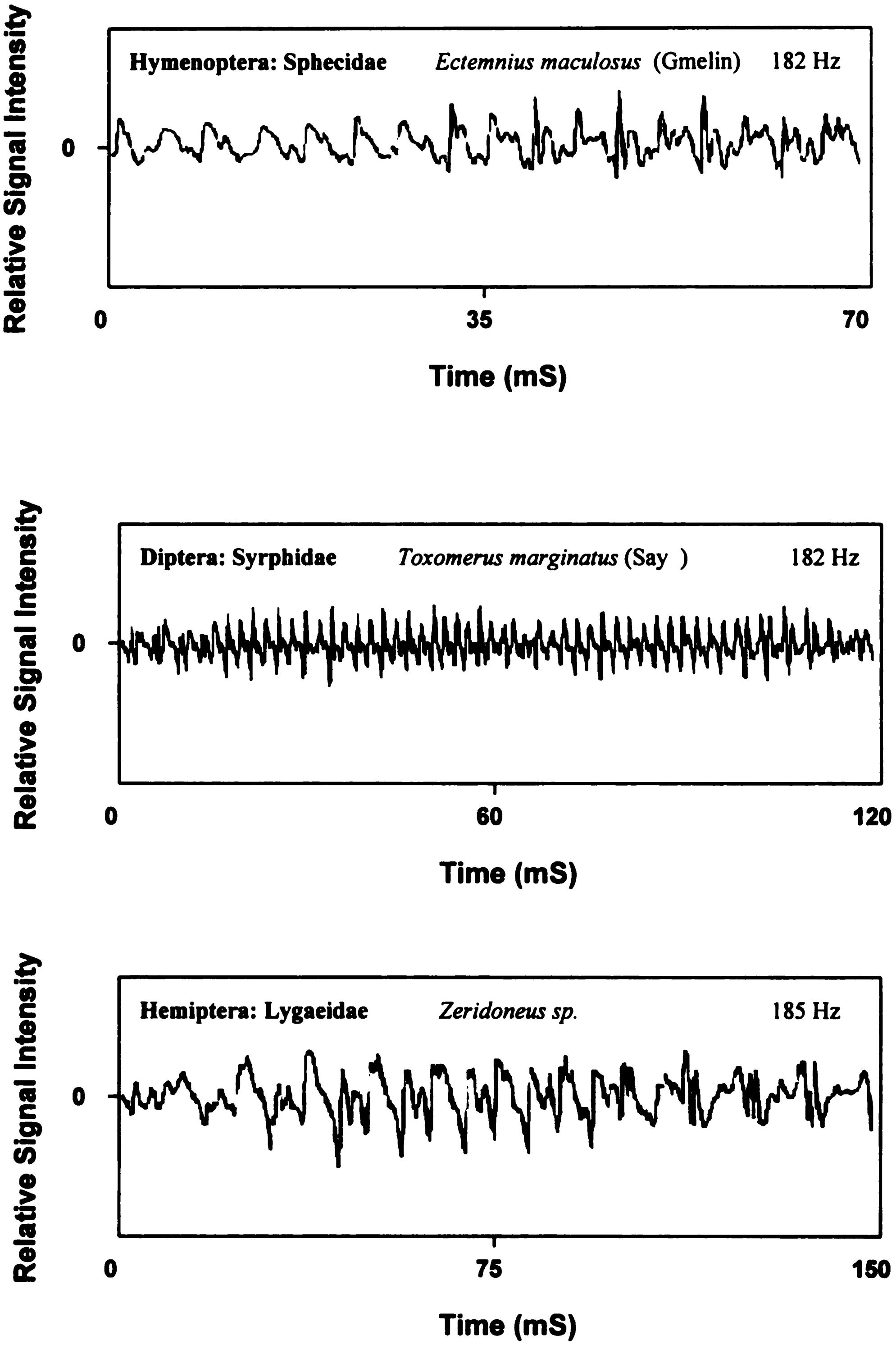

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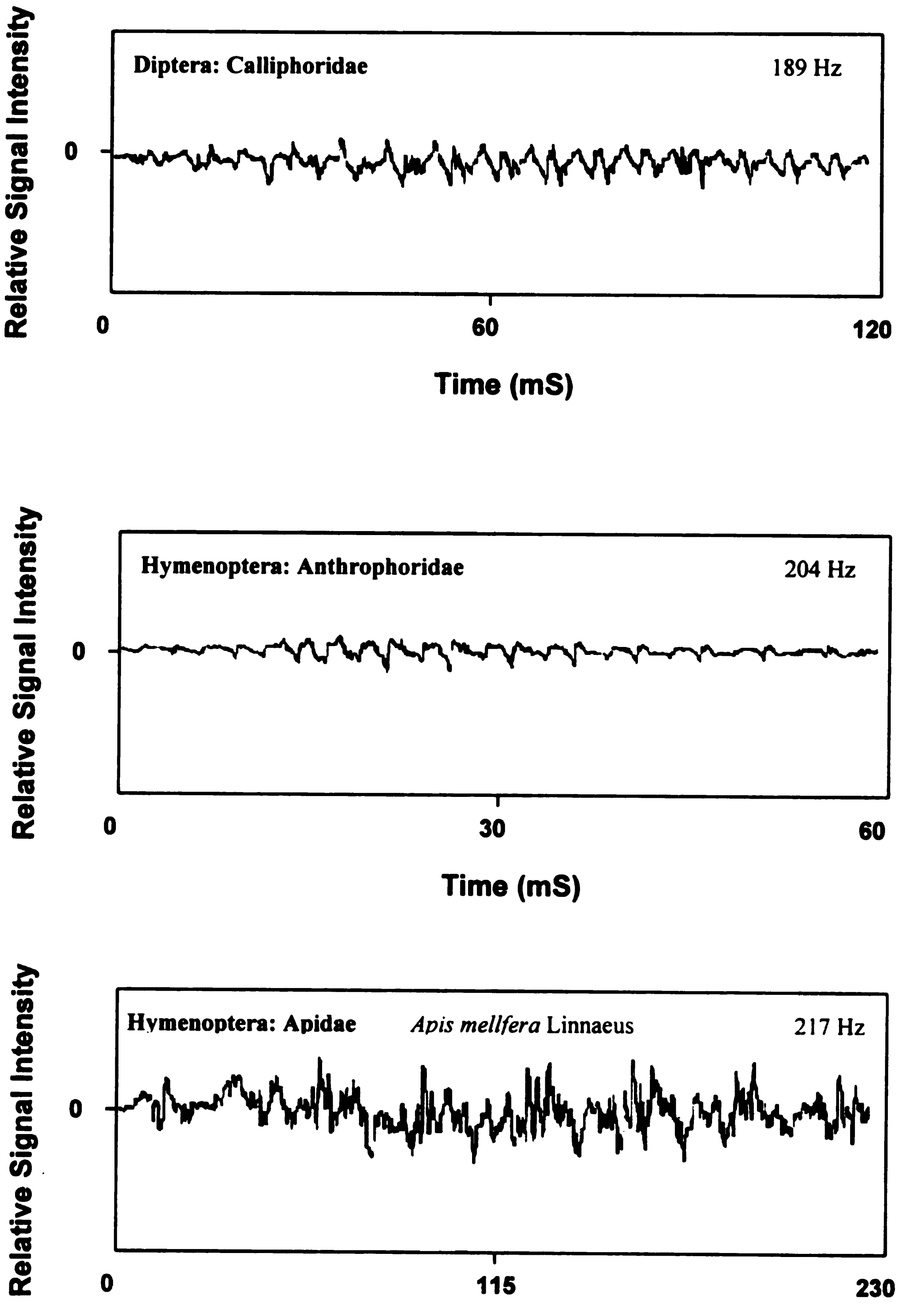

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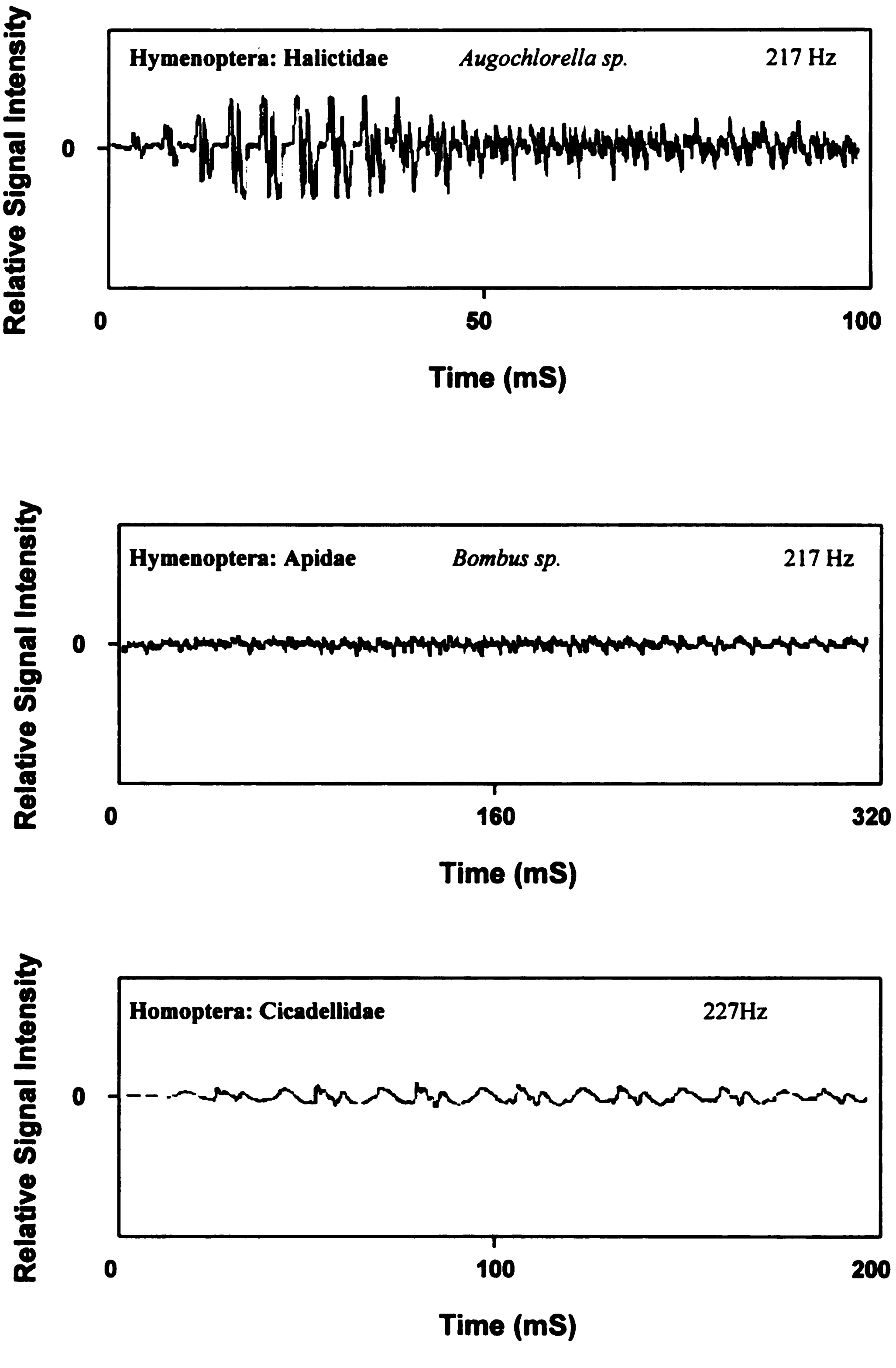

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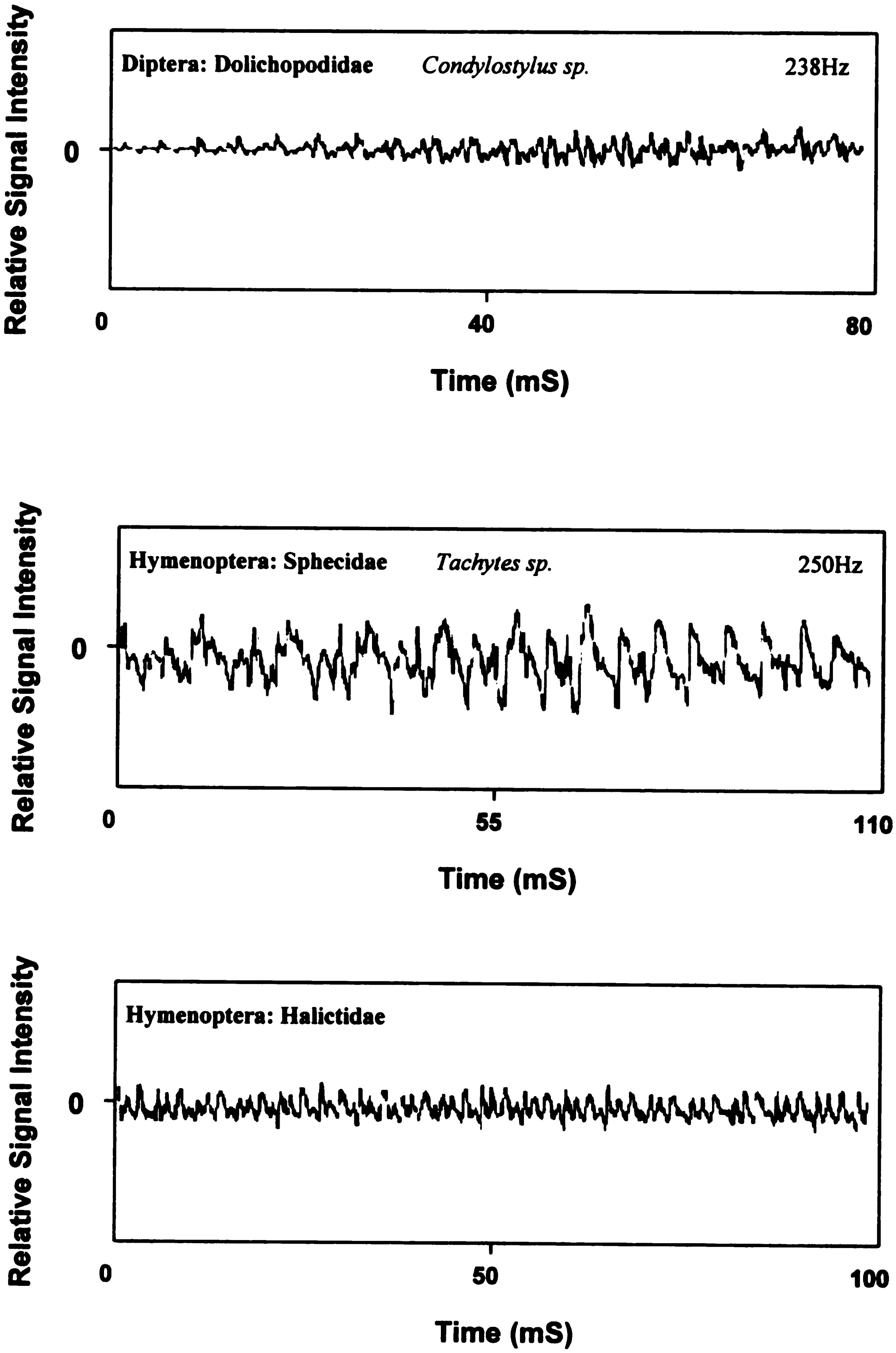

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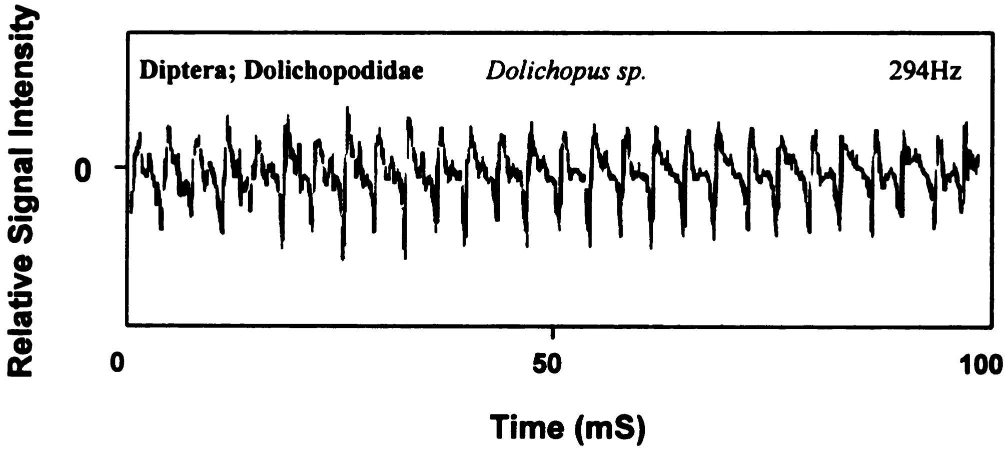

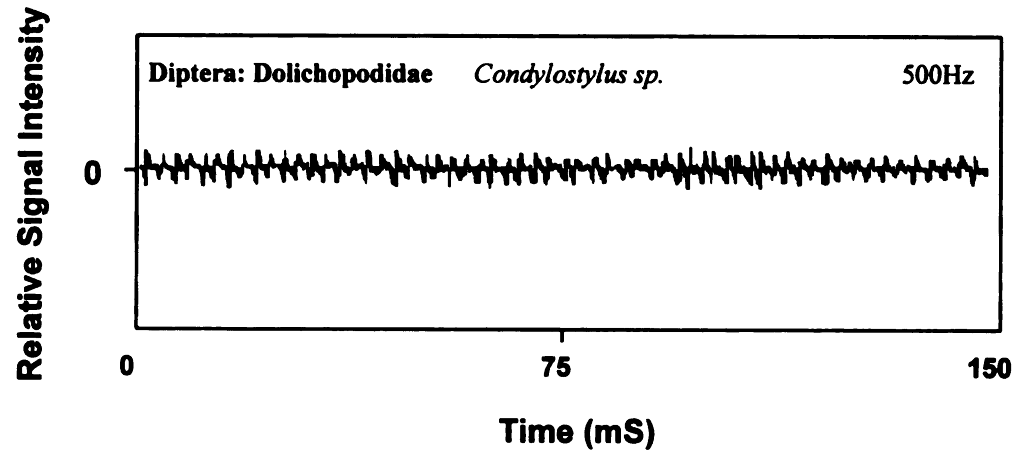

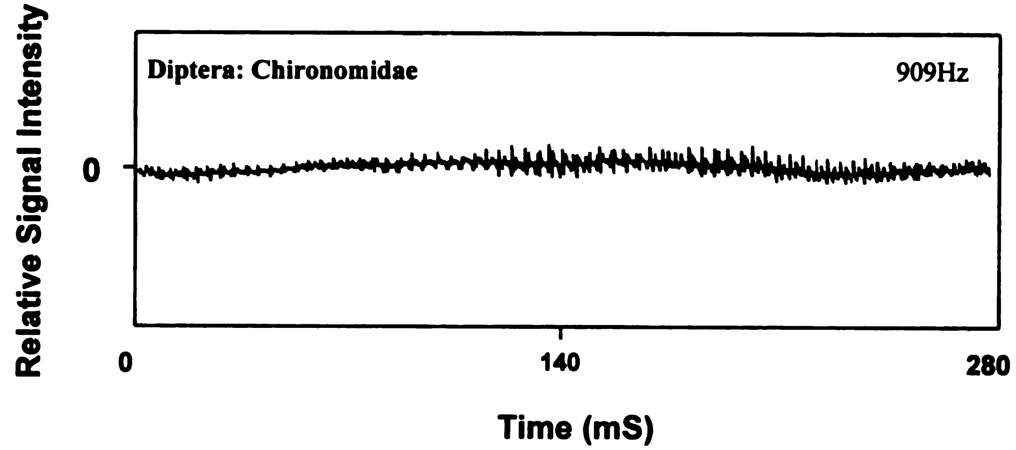

Figure 15. Cont'd.

frequencies as sorted by insect taxon (Figure 15). It is reassuring that all wing-beat frequencies recorded here for a given taxa agree with the values compiled in Figure 2 (Chapter 1) for insects generally.

Counts of signals from three field sites. The count data were normal as determined by the Shapiro-Wilk (P=0.0836, W= 0.9557) test and blocking was effective (F= 6.00, P = 0.0001 when H>1) (SAS Institute, 2000). Remarkably high numbers of insect signals per 10 min sample were detected for all site types (Figure 16). This was true even for parking lots, originally included as a type of negative control. Moreover, there was a difference among the sites (F = 8.85, df = 8, p < 0.0001). The equivalent field and wetland means proved different from the parking lots and the control (Figure 16). It is encouraging to see that a hard number can be obtained to verify the extent to which large areas of pavement and buildings lower the numbers of flying insects. Notably, these results establish for the first time that optical tachometer devices such as the OFIDIS sensor are capable of collecting data useful in making statistical comparisons between habitats.

Interesting count fluctuations occurred at some of the study sites across the season (Figure 17). Among the old-field sites (Figure 17B), Dansville regularly had the highest count of the three fields. One 10-min recording contained an amazing 1,215 insect signals (Figure 17A). Perhaps this occurred because at Dansville old-field, the sensor was close to forest edge. Also, the Dansville site had dense populations of flowering plants, which may have lured many visitors to the site that subsequently crossed the sensor.

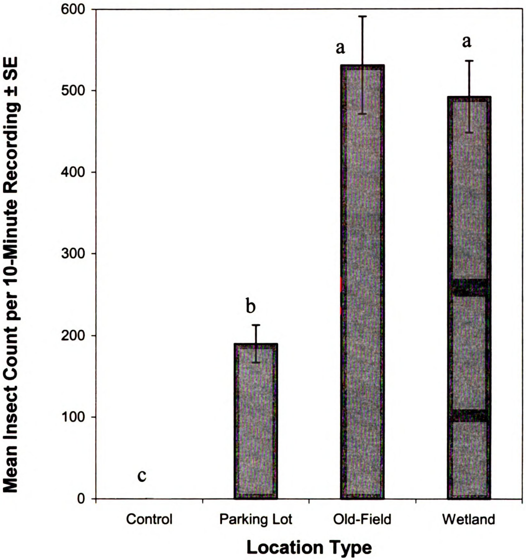

Figure 16. Mean insect signal counts (± SE) per 10 min sampling period across all sampling dates. No signals were detected when the sensor was shielded by and opaque cover (control). Treatments bearing a common letter are not different at p = 0.05.

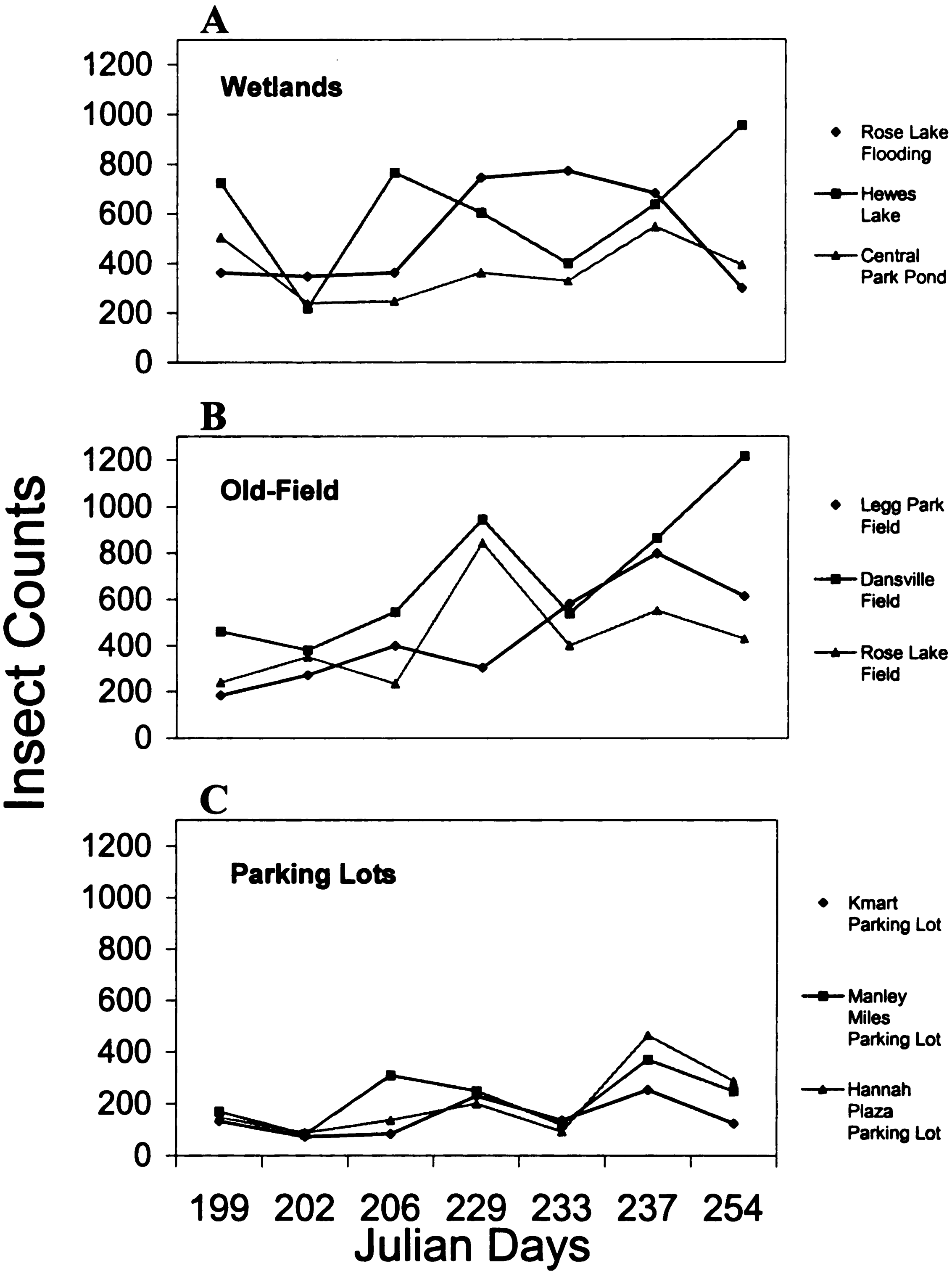

Figure 17. The number of insects detected per 10 min recording as a function of habitat type and sampling date during summer 2000.

In the multiple linear regression analysis, location (irrespective of site type) explained the most variation (r^2=0.36) compared to the other variables: time-of day (r^2=0.24), site types (r^2=0.22), temperature (r^2=0.22), date (r^2= 0.14), light intensity (r^2= 0.03), and wind speed (r^2 <0.001).

Some interactions are evident between variables of the MLR model. For example, wind and location together (r^2=0.38) account for more variation than either factor did alone. These data show, not surprisingly, that certain location types are prone to more wind, (e.g., the Kmart parking lot). However, wind alone explained very little variation (r^2=0.07) in the entire model, suggesting that this optical sensor was equally effective at detecting insects under windy and calm conditions.

Diversity in wing-beat signals from three site types. The standard deviation for repeated by-hand measures of fundamental frequency was 2.5 Hz. This precision was considerably better than measurements made by Canary 1.2.4, which showed 10-20 Hz differences within one signal's estimation. Figures 18-20 present (at a 2 Hz resolution) frequency distributions of all fundamental wing-beat frequencies detected at each field location for 3 dates. It is important to recognize that with a measurement SD of 2.5 Hz adjacent bars do not represent statistically different measures of wing-beat frequency, but that those separated by 5 Hz would do so.

If there were no differences in signal richness or diversity between site types, one would expect similar frequency distributions across sites (Figures 18-20). However, signal richness was the greatest at the Dansville old-field site (Table 1). The Rose Lake flooding site was 83% as rich as the Dansville old-field site, while the Kmart parking lot was only 41% as rich as Rose Lake flooding and 34% as rich as Dansville old-field.

Table 1. Signal richness (R), diversity (H') and relative diversity (J') compared for all three sites used in diversity study analysis

Site	R	H'	J'
Dansville Old- Field	259	2.15	.80
Rose Lake Flooding	215	2.26	.84
Kmart Parking Lot	88	1.87	.69

$H'_{max} = 2.7$ for all sites

H_0: Equal abundance among k (500) categories for all sites when the maximum diversity is 2.7

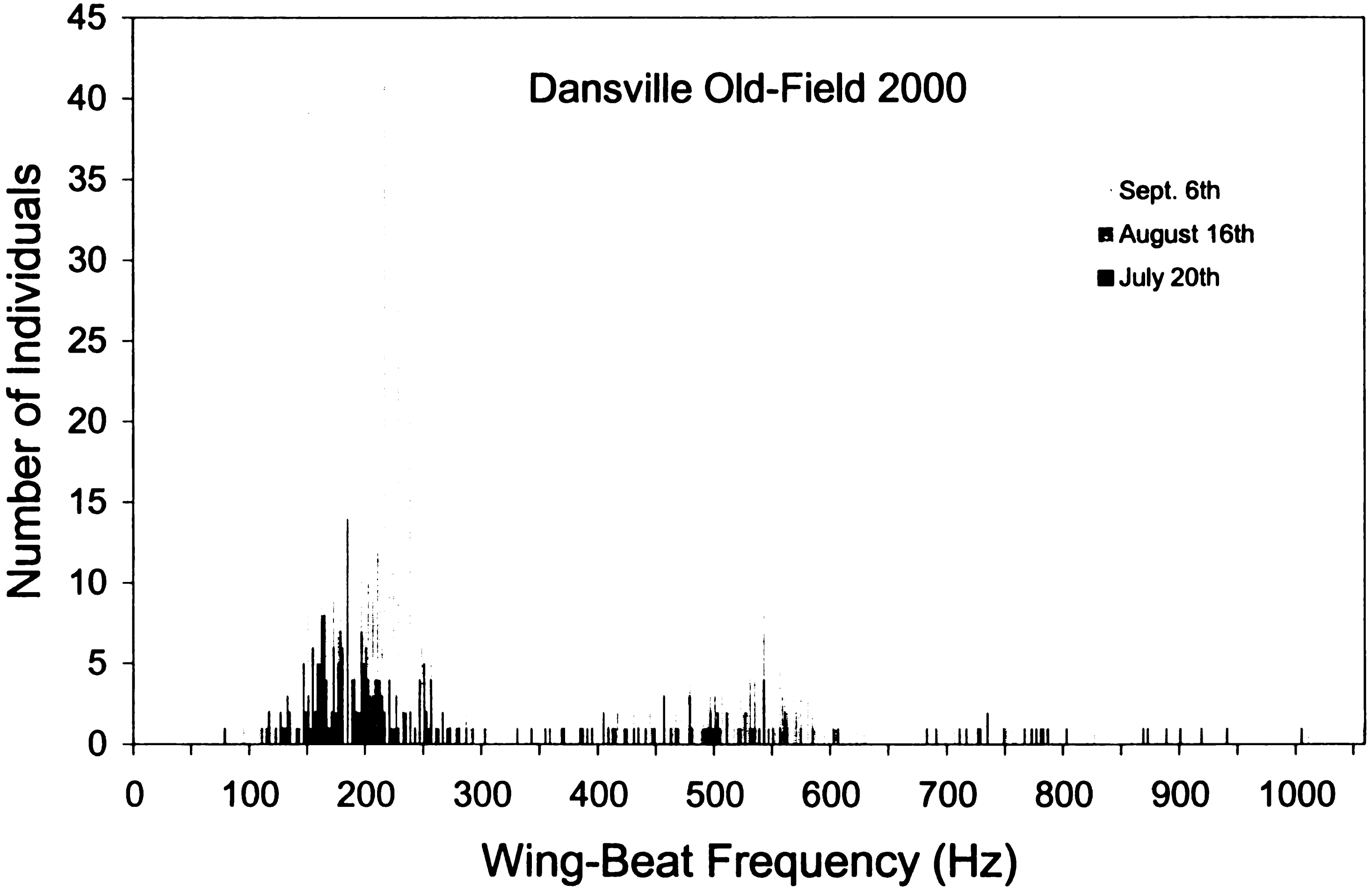

Figure 18. Frequency distribution of all wing-beat frequencies measured on 3 dates at Dansville old-field.

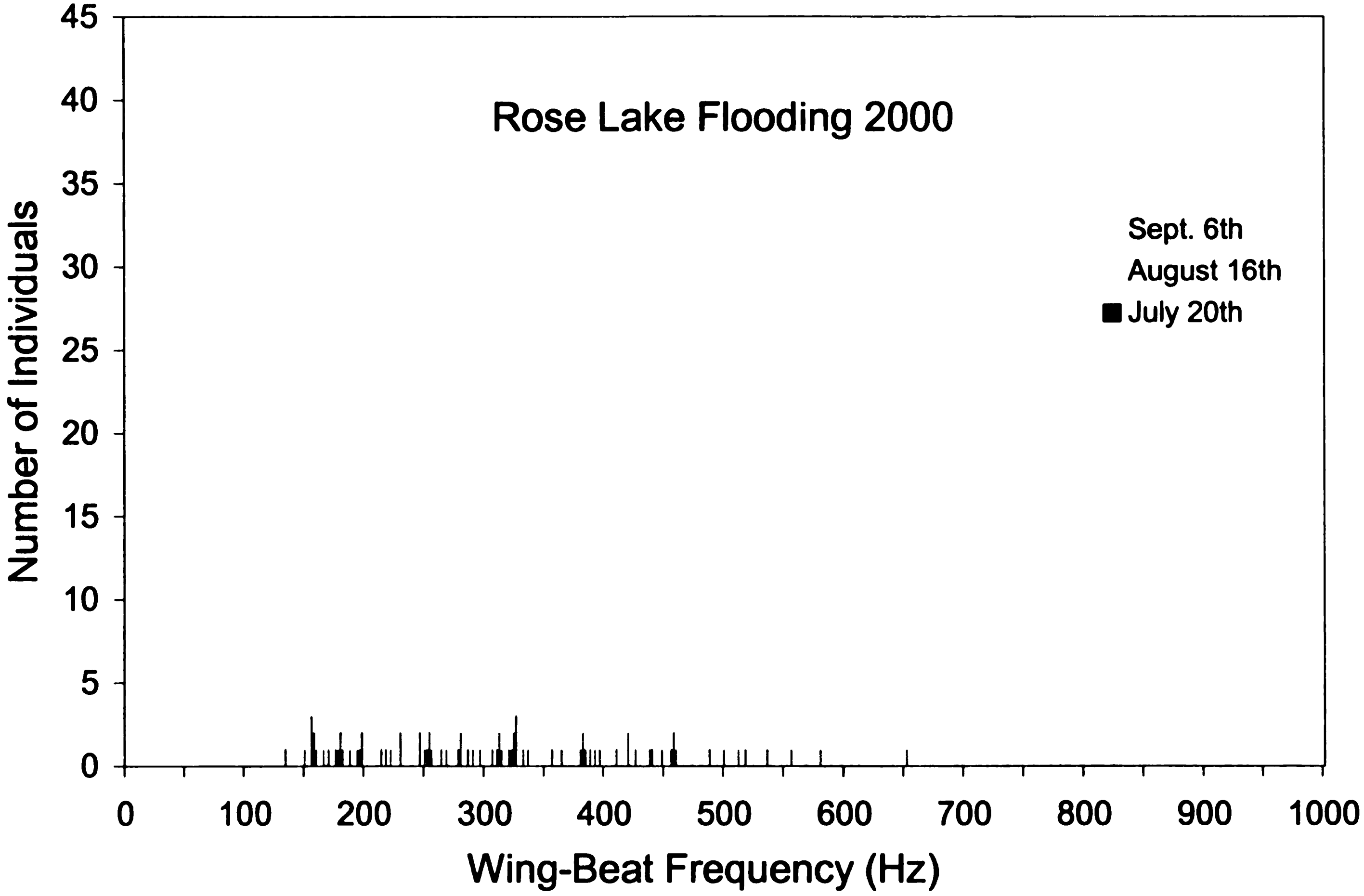

Figure 19. Frequency distribution of all wing-beat frequencies measured on 3 dates at Rose Lake flooding.

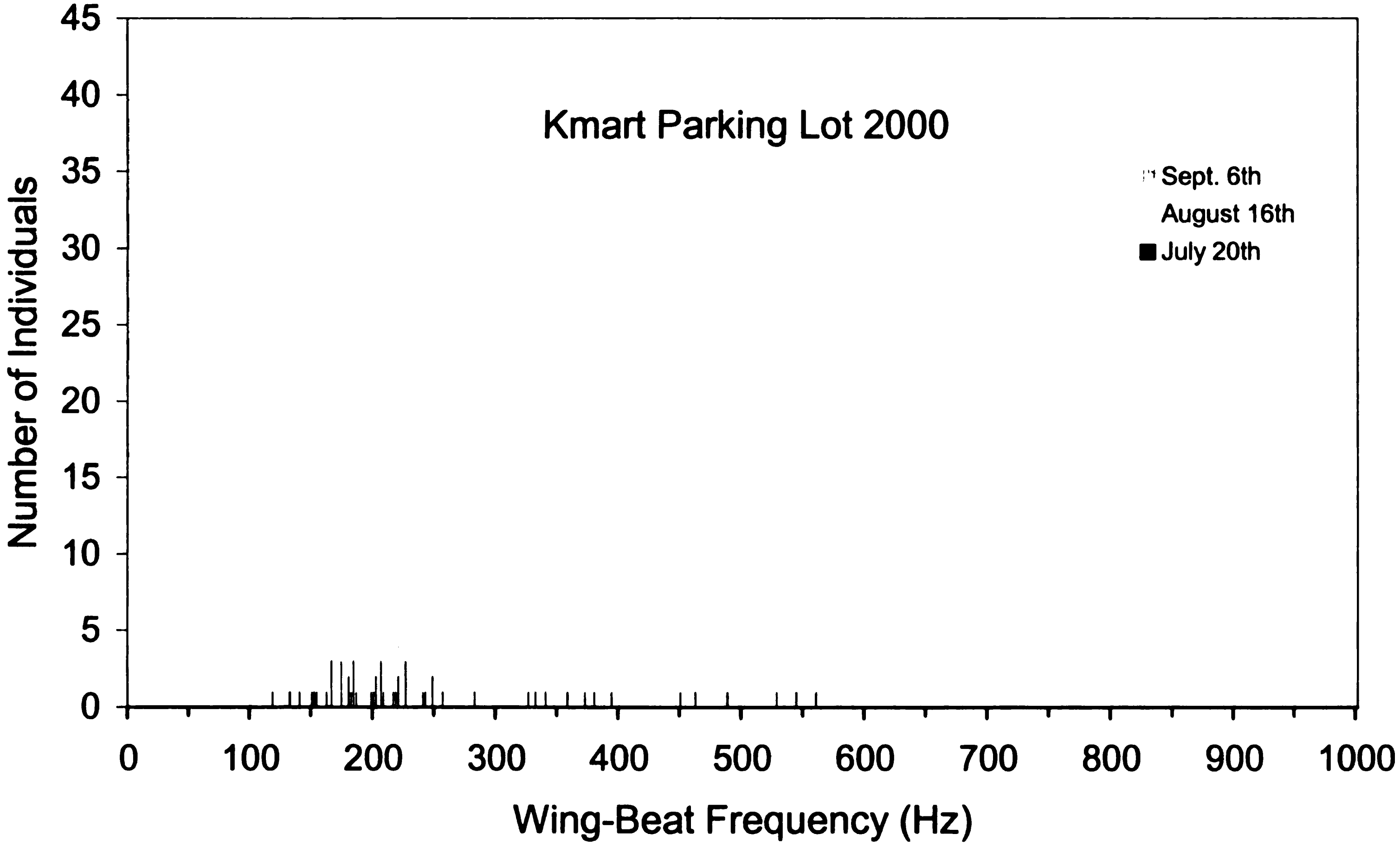

Figure 20. Frequency distribution of all wing beat frequencies measured on 3 dates at the Kmart® parking lot.

Signal richness alone might indicate that Dansville was the most diverse of the three sites. However, a better measurement of diversity is an index of the distribution of data compared to the total number of categories. To do so, I applied the Shannon-Weaver index to the frequency distribution data. A higher H' value (Table 1) indicates a more diverse data set based on the number of categories filled and the evenness of the data. Rose Lake flooding had the highest H' value of 2.26 (H'_{max} = 2.27), ranking it the most diverse site (Table 1). Values of H' are much easier to compare when they are converted to a proportion of maximum diversity of the entire data set (J'). The Rose Lake flooding site was 84% as diverse as the entire data set (Table 1), while Dansville old-field was 80% and Kmart parking lot 69% as diverse as H', respectively.

The composite distribution of individual signals (n=1,088) and their frequencies at the Dansville old-field site (Figure 18) showed that insects with wing-beat frequencies between 100 Hz and 300 Hz (peak = 216 Hz) were most prevalent. Based on (Figure 2, Chapter 2), I suspect that these came from Diptera and Hymenoptera along with perhaps some Coleoptera and Homoptera. These signal frequencies were also common to the old-fields (Figure 19) and the parking lots (Figure 20), where there was something even more peculiar- no insects were detected having a wing-beat frequency above 600 Hz. To be sure that this was not an affect of the time-of -day at which parking lots were sampled (Table 2), I analyzed a separate Kmart 10-min recording made at 4:35 pm on July 17th 2000. Only one insect signal exceeded 600 Hz (663Hz) within the entire track.

This high-frequency gap was analyzed by a X^2 test to assign a probability of finding no high-frequency (over 600 Hz) signals in the Kmart parking lot if its signal distribution were in fact identical to the Dansville old-field or the Rose Lake Flooding

Table 2. Time of day each 10 min recording was made at each site on each date in year 2000

Site	Date	Time
Dansville Field	July 20th	12:59 pm
	August 16th	12:43 pm
	Sept. 6th	3:35 pm
Rose Lake Flooding	July 20th	2:08 pm
	August 16th	4:30 pm
	Sept. 6th	11:10 am
Kmart Parking Lot	July 20th	11:36 am
	August 16th	11:35 am
	Sept. 6th	1:36 pm

sites. With the old-field serving as the expected values, the probability of finding no signals above 600 Hz in the Kmart parking lot proved < 0.001 ($X^2 = 11.39$, df = 1). I thus conclude that parking lots are devoid of high-frequency fliers. When Rose Lake flooding served as the expected values for the Kmart comparison, there should have been a 99.9% chance of finding at least one signal > 600 Hz ($X^2 = 20.0$, df = 1), if the these distributions were not different.

At the old-field sites and all wetland sites, I visually observed some Odonata and Lepidoptera flying over the sensor throughout the season. Their signals, known to fall below 100 Hz, were mostly undetectable by Canary 1.2.4. , that could display harmonics for signals only as low in frequency as 78 Hz. Unfortunately, this suggests that the OFIDIS sensor is not well-suited for detecting insects with wing-beat frequencies below 80 Hz. This includes most aquatic insects (except for Diptera), Lepidoptera, some Coleoptera and Odonata.

When the distribution data in (Figures 18-20) are compared to known wing-beat frequencies for all insects (Figure 2, Chapter 1), the OFIDIS sensor appears to be primarily a flying Hymenoptera and Diptera detector. Nevertheless, it is still a highly useful instrument that may allow scientists to gain some perspective on the vast complexity of pollination systems, natural enemy densities, and insect migration.

In *The Forgotten Pollinators*, (Buchmann and Nabhan 1996), the complex and mutualistic relationships between plants and their pollinators are well described. The bear-claw poppy, which grows only in gypsum soils in Southern Utah, is one clear example of how unique and fragile these relationships can be with the endangered. Entomologists, such as these authors and Dr. Tepidino from Utah State University (mentioned by the authors) have attempted to determine the pollinators of these rare flowering plants. Only recently did a student of Tepidino collect the solitary bee (*Perdita merconis* Griswold) that apparently is this plant's pollinator. Further observations of the bear claw poppy indicated that this bee is truly loyal to this plant, despite all of the other flowers surrounding it.

So how does the OFIDIS sensor fit into this scenario? The wing-beat and harmonic signature of this bee could be determined, and entered into a neural network so that long hours of recorded insect data could be played and the solitary bee counted. Such data could reveal the visitation rates to the flowers and seasonality of pollination. Such information could aid efforts to restore and protect the bear-claw poppy.

Variation of harmonic patterns of insect signals from the field. Harmonic patterns for the insects used in the abundance and diversity studies were both visible and clearly separated, so that it was possible to measure primary frequencies directly from harmonic spectra. Many signals shared a common fundamental wing-beat frequency. For example, in the Dansville old-field site 40 signals fell within the 216- 217 Hz range for the dates included in the diversity test. Signals with similar frequency could then be examined (Figure 21). Indeed variation was detected within these selected signals. Some harmonic patterns revealed their darkest band (most intense harmonic) at the first harmonic, while other had their darkest band as the second or third harmonic. It is not known at this time whether these differences represent signal variation generated by a common type of insect or signals from different insect types. Although I was able to match certain signals to each other by simply listening to them and could show that matching signals showed similar harmonic patterns, I would like to see this matching process become an automated software function.

Chapter 4

Summary of thesis research outcomes and suggestions for building upon them

I. Research summary

The research presented in this thesis has significantly advanced understanding of the performance and utility of the Model 2000 Optical Flying Insect Detection and Identification System (OFIDIS) under field conditions using sunlight. The sensor was maximally sensitive when operating under full sunlight. Under a cloudless sky, the active space for shadowed signals proved to be a cylinder extending from the sensor face some 25 meters toward the face of the sun. That for reflected signals was characterized as a cone extending widely from the face of the sensor and likely beyond 25 meters. Flying insect populations were successfully sampled during 10 min intervals at multiple times during Year 2000 under a protocol where this sensor was pointed directly upward toward the unobstructed and cloudless sky at replicated wetland, old-field, and parking lot sites in Central Michigan. Data were analyzed using commercially available wave-analysis software packages (TRex 2.0 and Canary 1.2.4) to yield insect counts and fundamental wing-beat frequencies accurate to ± 2.5 (SD) Hz. Plots of fundamental frequency of insect signals against abundance revealed statistically discernable differences in insect composition as well as abundance between some sites. As expected, insect abundance and diversity were lowest at large parking lots, selected to serve as negative controls. However, all locations achieved a diversity index of 0.69 or greater, suggesting even over parking lots, many different flying insects are present. On average, insect abundance and diversity proved equivalent for the wetland and old-field sites. In addition to fundamental frequencies, patterns of harmonics were obtained for many of the insect signals above but not below 100 Hz. However, because the identities of the insects generating the field signals were known in only a few cases, information from these harmonic records could not be assessed for correlations to particular taxa.

Collectively, this research validates, for the first time, the concept that OFIDIS or similar optical tachometer systems can be used to quantify insect abundance and diversity under field conditions. Moreover, this equipment is judged to have high potential for operating remotely. This study yielded an extensive database of insect signals rich in harmonics. However, until further work is done relating these signals to identified insects, little can be concluded here about the utility of OFIDIS as an identification system for insects generally. When multiple records were captured from defined oscillators (tooth brush, tuning fork, or defined digital electronic signals), sufficient variation in harmonic patterns due to signal duration and intensity was found to raise caution about over-interpreting subtle differences in harmonics between individual records. According to its designer, the main intent of OFIDIS was detection and identification of particular insects of interest (e.g., key insect pests) for which extensive background on specific flight signals (including their variations) could be generated to serve as a reliable reference for identifications. Nevertheless, the current research shows that the OFIDIS system can also capture quantitative data of broader ecological interest. As demonstrated here, this system is appropriate for capturing data of the type useful to conservation biology and management of habitat quality.

II. Suggestions for improving OFIDIS

The following suggestions are offered for possible improvements in or expansion of OFIDIS utility for studies such as this one: 1) Because the fundamental wing-beat frequencies of many insect Orders fall below 100 Hz (see Figure 1, p. 5), the sensitivity of the sensor and/or the responsiveness of analysis software to low frequencies might be improved. The OFIDIS equipment used here appeared to be better suited to detecting higher- rather than lower-frequency signals. Perhaps filter systems could be implemented to better separated low-frequency signals from noise, which seemed to be more prevalent at lower than higher frequencies. 2) If it were difficult to design one sensor optimally sensitive to all insect signal frequencies, it might be desirable to employ one low- and one high-frequency sensor simultaneously to cover the full range of insect wing-beat frequencies with higher confidence. 3) Design sensors that are range-specific to reduce the amount of extraneous data collected. This would save time during signal analysis, especially if signal recording were triggered only by certain frequencies. 4) Same signals vary in their intensity, which showed evidence of producing variation in their harmonic spectra. Finding a way to standardize all captured signals prior to harmonic analysis might reduce this variation. 5) A powerful and long-range artificial light source should be developed as a substitute for sunlight, which in most locations varies widely over time. For example, an ordinary laser pointer was found promising in preliminary trials; its beam projected over many meters to generate a large active space for the sensor both during daytime as well as night. Clearly, it would be advantageous for OFIDIS users to be able to sample flying insects consistently over 24 h, under varying weather conditions. A laser beam appropriately configured with the OFIDIS sensor could extend wing-beat sampling to shade-seeking (e.g., mosquitoes) as well as nocturnal insects (e.g., moths), in addition to those flying in full sunlight as sampled here.

III. The core question revisited

The OFIDIS system is a tremendous advancement in insect detection. As a device that can successfully converts subtle insect flight cues into electrical information that is rich in specific information about each insect, it has proven to be a useful tool of the modern electric age. I believe that this non-invasive method of insect detection will prove valuable to conservation biology researchers aiming to monitor specific insects and those who use insects to measure environmental quality. It will also be useful to the pest-management community protect both crops and human health.

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Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2001-01

Title of thesis or dissertation (or other research projects):

Evaluation and field application of an optical sensor that detects flying insects

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Meghan French

Date August 3, 2001

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 5 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Coleoptera: Cerambycidae *Typocerus velutinus* (Olivier)	Rose Lake Wildlife Research Area 31-Jul-00						1		
Lepidoptera: Lymantriidae *Lymantri dispar* (Linneaus)	Rose Lake Wildlife Research Area 29-Jul-00						1		
Neuroptera: Chrysopidae	Rose Lake Wildlife Research Area 29-Jul-00						1		
Odonata: Coenagrionidae	MSU Pond 20-Jun-00						1		
Lepidoptera: Pyralidae *Paraponyx sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Diptera: Tabanidae *Chrysops sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Meghan French

Date 17-Aug-01

Voucher No. 2001-01

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

17 Aug 2001

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 2 of 5 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Lepidoptera: Pterophoridae *Platyptilia sp.*	Rose Lake Wildlife Research Area 23-Jun-00						1		
Odonata: Coenagrionidae	MSU Pond 20-Jun-00						1		
Odonata: Coenagrionidae	MSU Pond 20-Jun-00						1		
Hemiptera: Miridae *Lygus sp.*	Rose Lake Wildlife Research Area 29-Jul-00						1		
Diptera: Sciomyzidae *Tetanocera sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Coleoptera: Curculionidae *Apion centrale* Fall	Rose Lake Wildlife Research Area 31-Jul-00						1		
Hymenoptera: Apidae *Bombus sp.*	MSU Pond 22-Jun-00						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Meghan French

Date 17-Aug-01

Voucher No. 2001-01

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 3 of 5 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							
		Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Hemiptera: Miridae	Rose Lake Wildlife Research Area 31-Jul-00						1		
Hymenoptera: Andrenidae *Andrena sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Diptera: Syrphidae *Platychierus sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Diptera: Syrphidae *Toxomerus marginatas* (Say)	MSU Pond 22-Jun-01						1		
Hymenoptera: Halictidae *Halictus sp.*	MSU Pond 20-Jun-00						1		
Hymenoptera: Sphecidae *Ectemnius maculosis* (Gmelin)	Rose Lake Wildlife Research Area 29-Jul-00						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Meghan French

Date 17-Aug-01

Voucher No. 2001-01

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 4 of 5 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Diptera: Syrphidae *Toxomerus marginatas* (Say)	Rose Lake Wildlife Research Area 29-Jul-00						1		
Hemiptera: Lygaeidae *Zeridoneus sp.*	Rose Lake Wildlife Research Area 23-Jun-00						1		
Diptera: Calliphoridae	Rose Lake Wildlife Research Area 29-Jul-00						1		
Hymenoptera: Anthrophoridae	MSU Pond 20-Jun-00						1		
Hymenoptera: Apidae *Apis mellifera* Linnaeus	MSU Pond 20-Jun-00						1		
Hymenoptera: Halictidae *Augochlorella sp.*	MSU Pond 20-Jun-00						1		
Hymenoptera: Apidae *Bombus sp.*	MSU Pond 20-Jun-00						1		
Homoptera: Cichidelidae	Rose Lake Wildlife Research Area 29-Jul-00						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Meghan French

Date 17-Aug-01

Voucher No. 2001-01

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

Appendix 1.1

Voucher Specimen Data

Page 5 of 5 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Diptera: Dolicopodidae *Condostylus sp.*	MSU Pond 22-Jun-00						1		
Hymenoptera: Sphecidae *Tachytes sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Hymenoptera: Halictidae	Rose Lake Wildlife Research Area 31-Jul-00						1		
Diptera: Dolichopodidae *Dolichopus sp.*	Rose Lake Wildlife Research Area 31-Jul-00						1		
Diptera: Dolichopodidae *Condostylus sp.*	MSU Pond 22-Jun-00						1		
Diptera: Chironomidae	MSU Pond 22-Jun-00						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Meghan French

Date 17-Aug-01

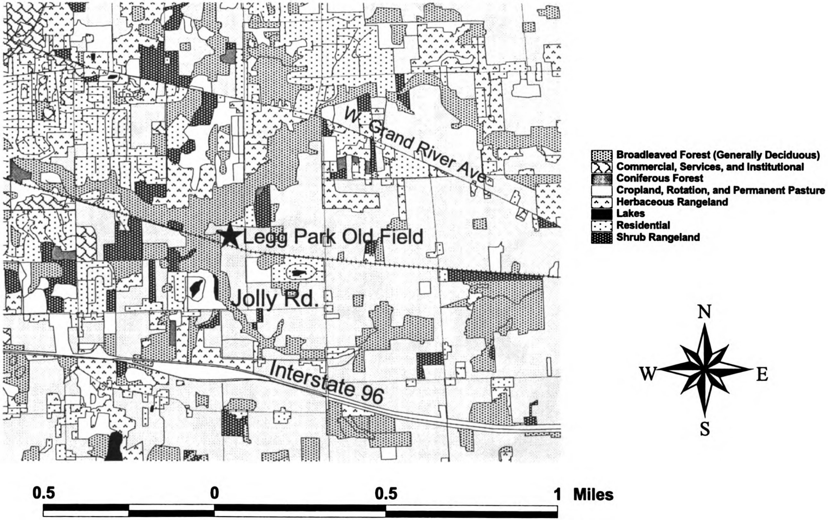
Voucher No. 2001-01

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

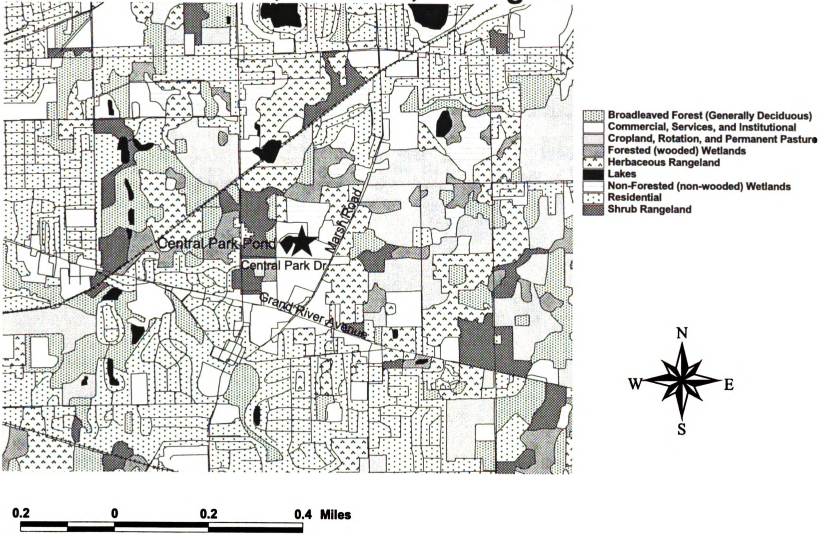
Curator Date

Appendix 2

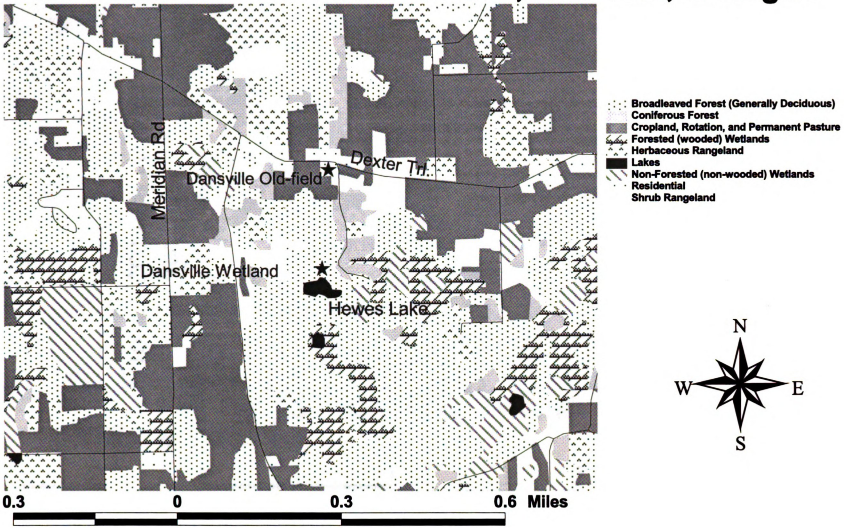
Legg Park Old-field, Okemos, Michigan



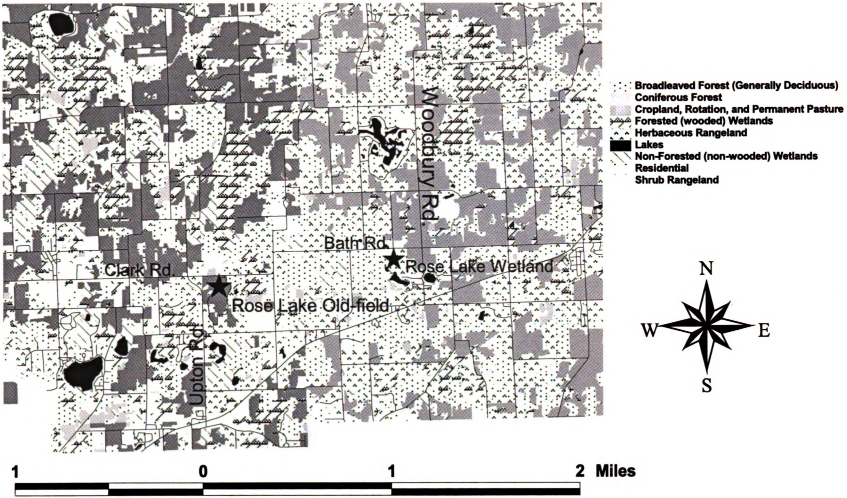
Central Park Pond, Okemos, Michigan



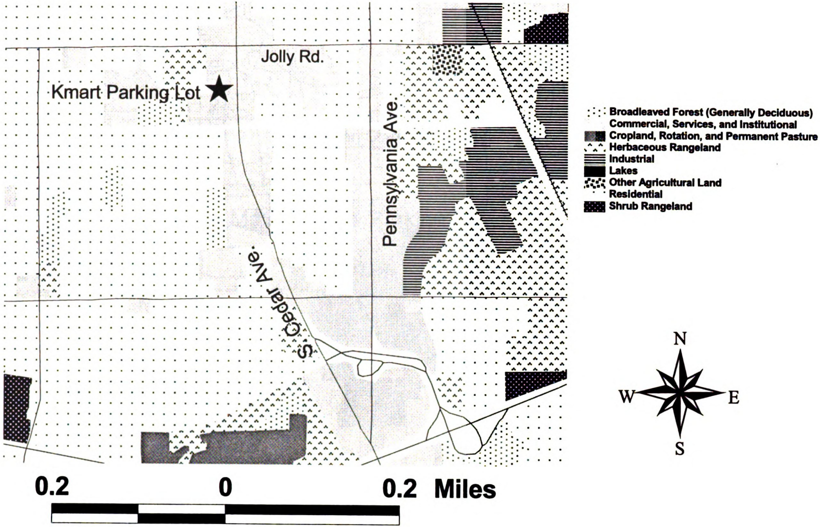
Hewes Lake and Dansville Old-field, Dansville, Michigan



Rose Lake Flooding and Old-field, Michigan



Kmart Parking Lot, Lansing, Michigan



Hannah Plaza Parking Lot, E. Lansing, Michigan