USE OF ECHOLOCATION CALLS FOR THE IDENTIFICATION OF FREE-FLYING BATS

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ABSTRACT: I studied the efficacy of the Anabat II detector for obtaining reliable call structures for the identification of some southwestern bat species. A total of 20 locations in northern Arizona, southwestern Utah, and southern Nevada representing the available range in elevations and associated vegetation types, were sampled acoustically. The Anabat II in conjunction with a laptop computer provides an instantaneous output of echolocation call structure. Select sequences can be saved directly to the hard drive. Nineteen of the 22 species known to occur in the study region were identified by recognizable differences in the time/frequency characteristics of their echolocation calls. Species that forage in the open appear to use loud calls that can be detected at a greater distance than species that forage in clutter and use calls of low intensity. The present study suggests that the efficacy of bat inventories will increase with the establishment of better sampling procedures and the development of a comprehensive reference library of call structure incorporating the range of variation inherent within and between species.

Key words: Anabat, Arizona, bats, echolocation, identification, monitoring, Nevada, Utah.

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The recent awareness of bats as an important biological resource has resulted in increased state and federal agency scrutiny for listing as threatened or endangered. There are 28 bat species known to occur in the western United States, 2 of these are federally listed as endangered and 13 are identified as species of special concern. Currently, Arizona lists 4 species as candidates for threatened or endangered status, Nevada provides protection to one species, and New Mexico has 4 species given threatened or endangered status. California lists 13 species of special concern. The nocturnal and volant nature of bats has limited establishment of an adequate base of knowledge concerning their basic biology.

The study of bats, away from roost sites, has relied primarily on direct capture via nets and/or traps (Kunz and Kurta 1988). However, not all bat species and not all individuals within a species are equally susceptible to capture. The small relative size of collecting surfaces and the varying ability of bats to detect these collection devices further limit their effectiveness. These direct capture techniques are limited to roost sites, water holes, or along foraging flyways where bats tend to concentrate. To compound these sampling problems, a given location may not be used every night by the same bat species assemblage. Standard capture techniques require relatively expensive equipment and constant tending, limiting the number of localities that can be sampled simultaneously.

Electronic acoustic devices (bat detectors) have been developed that allow investigators to hear and/or visualize the ultrasonic echolocation calls of bats (Fenton 1988). Echolocation calls of many bat species appear to be distinctive (Simmons et al. 1979). Some insectivorous bats in the western United States have been characterized by the frequency-time structure of search and feeding calls, providing a basis for species recognition for free-flying individuals (Fenton and Bell 1981).

Bat detectors hold the promise of effective surveys for bats that are difficult to capture and in areas that are difficult to sample with standard capture techniques. A standardized system with easily obtainable field equipment and a reference library of calls containing the range of variation that may be encountered within species has not yet been achieved. Presently the detailed study of bat echolocation calls requires the use of ultrasonic detectors, tape recorders, period meters, oscilloscopes, and a range of analytic procedures (Fenton 1988). Major problems with many devices involves cost, availability, and ease in analyzing recordings.

Recently, a relatively inexpensive bat detector and analysis system has become available, the Anabat II (Titley Electronics, Ballina, Australia). The system allows direct interface between the detector and a laptop computer. Echolocation calls can be observed while being generated and the observer may select which sequences to save directly to the computer hard drive. Because of the zero-crossings method of analysis, information on amplitude and harmonics are lost. However, the structural detail of the dominant harmonic of individual calls may provide the necessary information for species identification.

The purpose of my study was to test the efficacy of the Anabat II system and to establish a preliminary baseline of species-specific echolocation calls for bats in northern Arizona, southwestern Utah, and southern Nevada.

STUDY AREA AND METHODS

Twenty locations (Utah 1; Nevada 3; Arizona 16) were sampled from May 1994 through August 1995. An attempt was made to sample the available range of elevations and associated vegetation types found within the region. Middle and high elevations predominate in the study area, thus 13 of the 20 sampling locations were at elevations greater than 1219 m.

Each site was monitored using an Anabat II bat detector linked to an IBM-compatible laptop computer. Simultaneous with acoustic sampling, bats were captured at all but 2 locations using mist nets or a combination of mist nets and double-frame harp traps (Austbat Research Equipment, Victoria, Australia). The intensity of capture effort was directly influenced by the physical characteristics of each location.

Each location was acoustically sampled by placement of the detector at a central location. Although it was desirable to have an observer continually monitoring the acoustic equipment, it was not always possible due to the need to remove bats from collecting devices and process them. During constant monitoring of the acoustic equipment each echolocation call was examined and the best representative samples that reflected various behaviors (e.g., search, pursuit, capture) were saved.

Species identification of specific calls was achieved in several ways. Visual recognition was occasionally possible by illuminating free-flying, individual animals with a hand-held spotlight while they were being monitored acoustically. Some vocalizing individuals were actually followed into a net and identification obtained immediately. When possible, acoustic sampling was conducted near known roost sites in order to follow target species immediately upon the evening dispersal. Captured animals were held for later release under controlled conditions. When activity declined so that released bats could be monitored without extraneous input from nontarget bats, captured bats were released individually and followed to obtain as many confirmed vocalizations as possible. Some individuals were light tagged with Mini-light Sticks (Chemical Light Inc.) following the methodologies described by Barclay and Bell (1988) and recorded while foraging in the area.

Identification of calls of several species was accomplished with less direct methods. Comparative files of calls recorded for *Eumops perotis* and *Myotis yumanensis* were obtained from California (C. Corben, pers. comm.). *Euderma maculatum* calls were obtained from free-flying individuals emitting the characteristic human-audible pulses which fit the signals described by Leonard and Fenton (1984). Finally, calls from *Nyctinomops macrotis* were identified by visual comparison with those presented by Simmons et al. (1978) and later by free-flying bats.

Calls from each species were identified subjectively using the following criteria. Calls verified by capture were designated as known species and were cataloged. All saved files were compared visually with known, cataloged calls. Basic aspects of call structure, including maximum and minimum frequency, duration, and shape were used as reference points but no attempt at quantification was made. It is important to recognize that not all calls or sequences of calls can be used for identification purposes. I examined all calls obtained but used only those sequences that contained frequency range and structural characteristics known to be exhibited by a particular species. If there was doubt or overlap with other species, sequences were disregarded.

Examination of the frequency/time characteristics of echolocation calls reveals patterns in shape. The frequency range and duration of calls vary within species depending on behavioral mode (e.g., orientation, foraging) and between species depending on foraging strategy (e.g., gleaning, aerial hawking). Either case can be physically affected by distance and angle of the individual bat to the detector.

RESULTS

The following figures, taken directly from the Anabat computer, depict the frequency-time structure for the species found during the present study. Quantitative examination of call structure features was beyond the scope of the present study. The figures simply provide visual evidence for the capability of separating species by call structure. The call sequences presented have been carefully selected to incorporate the clearest, and most definitive group of individual calls representative of each species. It is critical to stress that these illustrations are for general comparison and not to be used as a definitive reference base. More detailed studies are required to establish the range of variation in call structure within each species.

Species of *Myotis* tend to have vocalizations of short duration (< 3 msec) resulting in relatively linear, perpendicular call patterns. The genus *Myotis* is sufficiently diverse that there are generally multiple species present at any single locality. Separation of coexisting conspecifics appears possible from data collected in the present study. Species of *Myotis* that hunt in the open (i.e., aerial hawking) produce calls that have relatively narrow frequency range (Fig. 1A, B, C, D; M. *californicus, M. ciliolabrum, M. volans,* and *M. lucifugus,* respectively). *Myotis* that hunt for and glean insects from vegetation surfaces produce calls with relatively wide bandwidths (Fig. 1E, F, G; *M. auriculus, M. evotis,* and *M. thysanodes,* respectively). *Myotis yumanensis* tends to forage in the open but in close proximity to water surfaces (Fig. 1H).

Other genera of bats in the area demonstrate greater variation in the shape of calls. In general, aerial hawk-

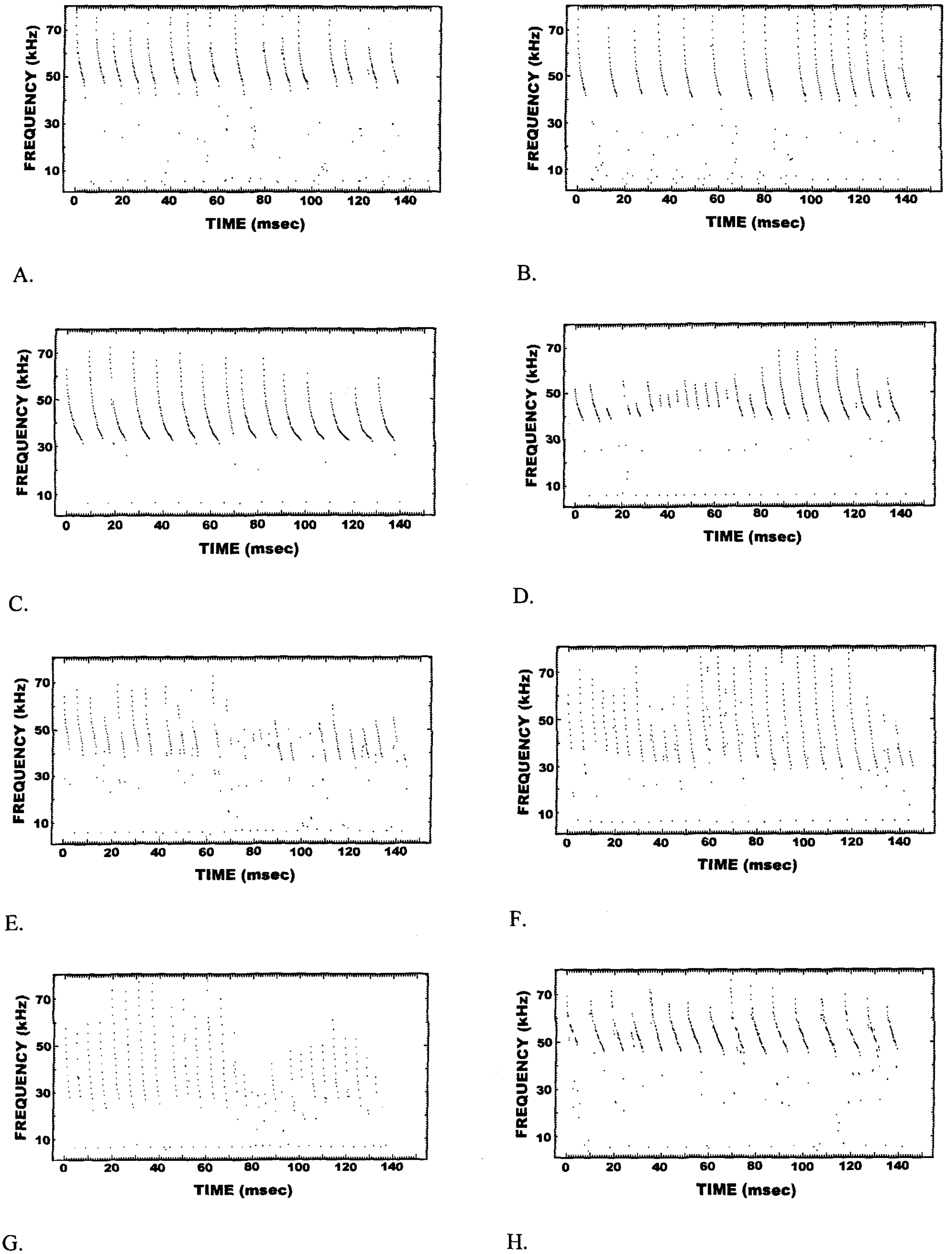

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Fig. 1. Frequency/time display of echolocation calls from *Myotis californicus* (A), *M. ciliolabrum* (B), *M. occultus* (C), *M. volans* (D), *M. auriculus* (E), *M. evotis* (F), *M. thysanodes* (G), and *M. yumanensis* (H).

ing species produce orientation/search calls of relatively narrow frequency range and long duration (> 5 msec). Upon detecting a potential prey item, the pulses increase in frequency range and repetition rate and decrease in pulse duration. During the latter portion of the pursuit phase, pulses decrease in bandwidth and duration, increase in repetition rate, and end in a "feeding buzz". *Pipistrellus hesperus* and *Eptesicus fuscus* represent this basic pattern (Fig. 2A and B, respectively). Variations in the pattern may be wide. Initial search/orientation pulses may be relatively constant shifting to a more variable, higher frequency, wide band pursuit/approach phase (Fig. 2C, D, and E; *Lasionycteris noctivagans*, *Nyctinomops macrotis* and *Eumops perotis*, respectively). At the other extreme, search calls may be variable, alternating in frequency then shifting to more constant, higher frequency wide band approach calls (Fig. 2F, G, and H; *Lasiurus cinereus* and *Tadarida brasiliensis*). A portion or all of the calls emitted by *N. macrotis* and *E. perotis* contain low frequencies that can be heard by the unaided human ear. Evidence also suggests that *T. brasiliensis* may produce orientation sounds that are human-audible.

Species that use a gleaning foraging strategy or actively hunt close to vegetation or rock surfaces on a regular basis demonstrate different echolocation strategies. The most unusual and distinctive calls are produced by *Idionycteris phyllotis*. During apparent orientation/search behavior, long sequences of low frequency, frequency modulated (FM) pulses can be heard with the unaided ear (Fig. 3A). However, during foraging activity, a long quasi-constant frequency component is interjected (Fig. 3B and C). Fig. 3B illustrates a single vocal pulse (time scale at 10 msec intervals) and Fig. 3C shows a sequence of those pulses (time scale at 50 msec intervals). A variation of the low frequency FM pulse appears to comprise the entire vocal repertoire of *Euderma maculatum* (Fig. 3D).

Remaining species that use a gleaning strategy present more of a problem in characterization. Existing vocal files for *Corynorhinus townsendii* (Fig. 3E) are fragmentary and suggest the use of 2 strong harmonics, providing a relatively wide-band pulse. There is indirect evidence that *Antrozous pallidus* has a highly variable vocal repertoire that depends on immediate surroundings. Monitoring orientation calls of *A. pallidus* in a room revealed that most pulses were narrow in frequency range and short in duration. Similar signals were detected from hand-released individuals in the field. Once flight was achieved and the pallid bat continued activity in the vicinity of the monitor, a wider range of relatively broadband, short duration signals were observed (Fig. 3F). Other bats may use similar calls in confined situations.

DISCUSSION

The ability to acoustically distinguish some species of free-flying bats has been well documented (Ahlen 1990, Bell 1980, Fenton and Bell 1979, 1981). Ultrasound equipment has evolved dramatically in the past 15 years and has become less expensive and more in the reach of most field investigators. A variety of ultrasonic detectors are available and have been used for determination of general bat presence, activity, and sometimes identification of species present. The range of approaches and limitations were discussed in detail by Fenton (1988). The Anabat II system that I used is relatively inexpensive and has the advantage of providing instantaneous displays of call structure with high resolution and detail.

In general, echolocation data have been available in two forms: (1) visual representation of the time-frequency structure of a single call; and, (2) verbal descriptions of certain structural characteristics (e.g., maximum and minimum frequency). Photographs of sound spectrograms (Fenton and Bell 1979) or apparently hand-drawn sound spectrograms (Fenton and Bell 1981) at varying scales make comparison difficult. Anabat II allows examination at a variety of time and frequency scales. The clarity of calls is generally better than those published earlier due to finer resolution on the frequency axis. This is particularly evident with the early description of use of a long constant frequency for *Idionycteris phyllotis* (Simmons and O'Farrell 1977), which Fig. 3B shows to be far more complex.

One criticism of the Anabat zero-crossings analysis is that harmonic information is lost; thus, the frequency/time display derived will be represented by the harmonic with the greatest energy. The inclusion of harmonic information provides a detailed description of a vocalization while furnishing some indication of possible habitat use and/or feeding strategy (Simmons and Stein 1980). Incorporation of harmonics in a vocalization and consequent increase in effective bandwidth results in sharpening an image while nullifying the masking effects of background clutter. Although certain sonar information may be lost, all harmonic information may not be necessary for the reliable identification of bats by call structure. In fact, a comparison of equipment versus output for *Pipistrellus subflavus* (MacDonald et al. 1994) shows a fundamental frequency with a strong second harmonic recorded with an instrumentation tape recorder but only the second harmonic was visible with the Anabat system. If only the fundamental frequency

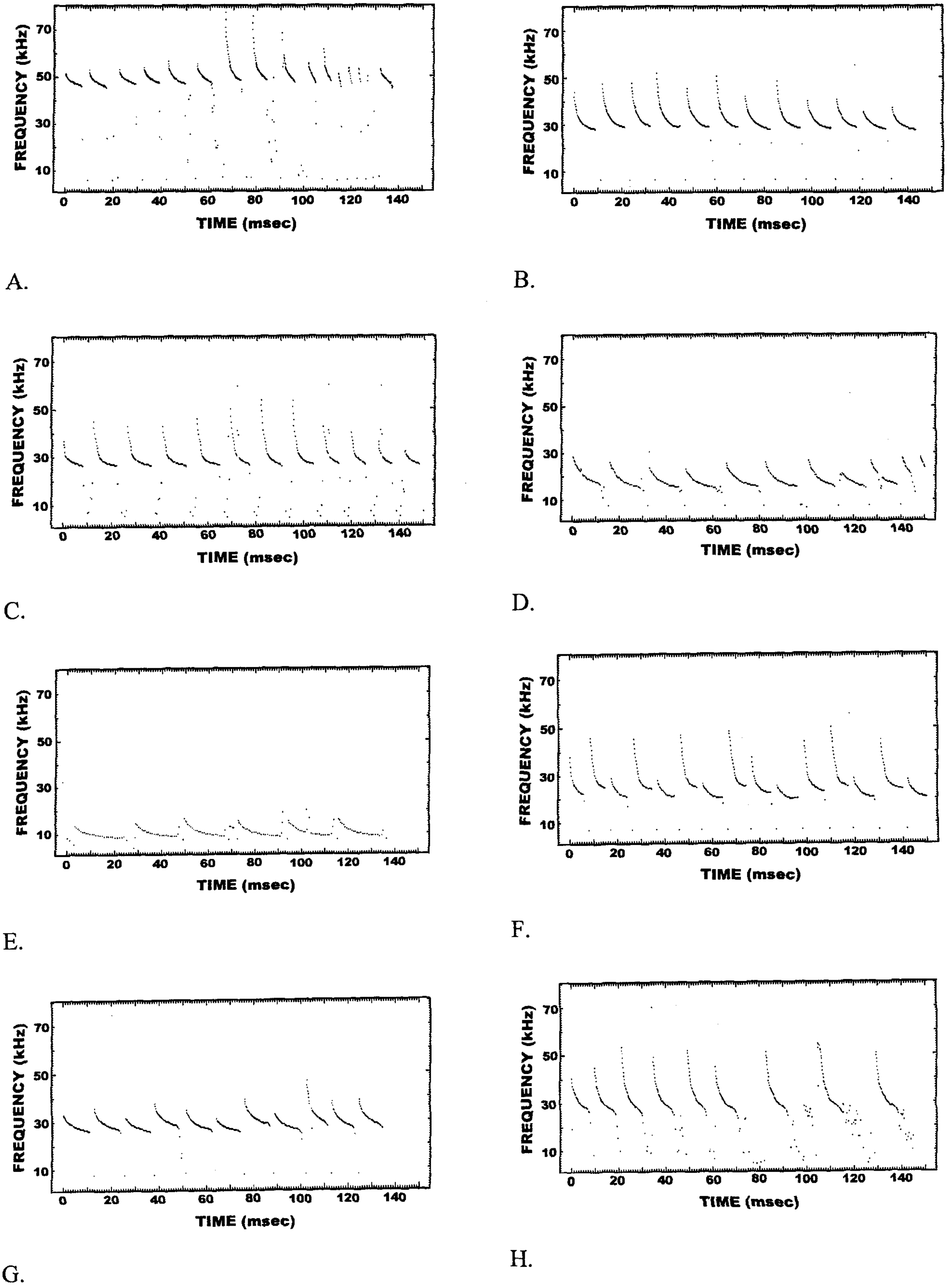

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Fig. 2. Frequency/time display of echolocation calls from *Pipistrellus hesperus* (A), *Eptesicus fuscus* (B), *Lasionycteris noctivagans* (C), *Nyctinomops macrotis* (D), *Eumpos perotis* (E), *Lasiurus cinereus* (F), *Tadarida brasiliensis* (G and H).

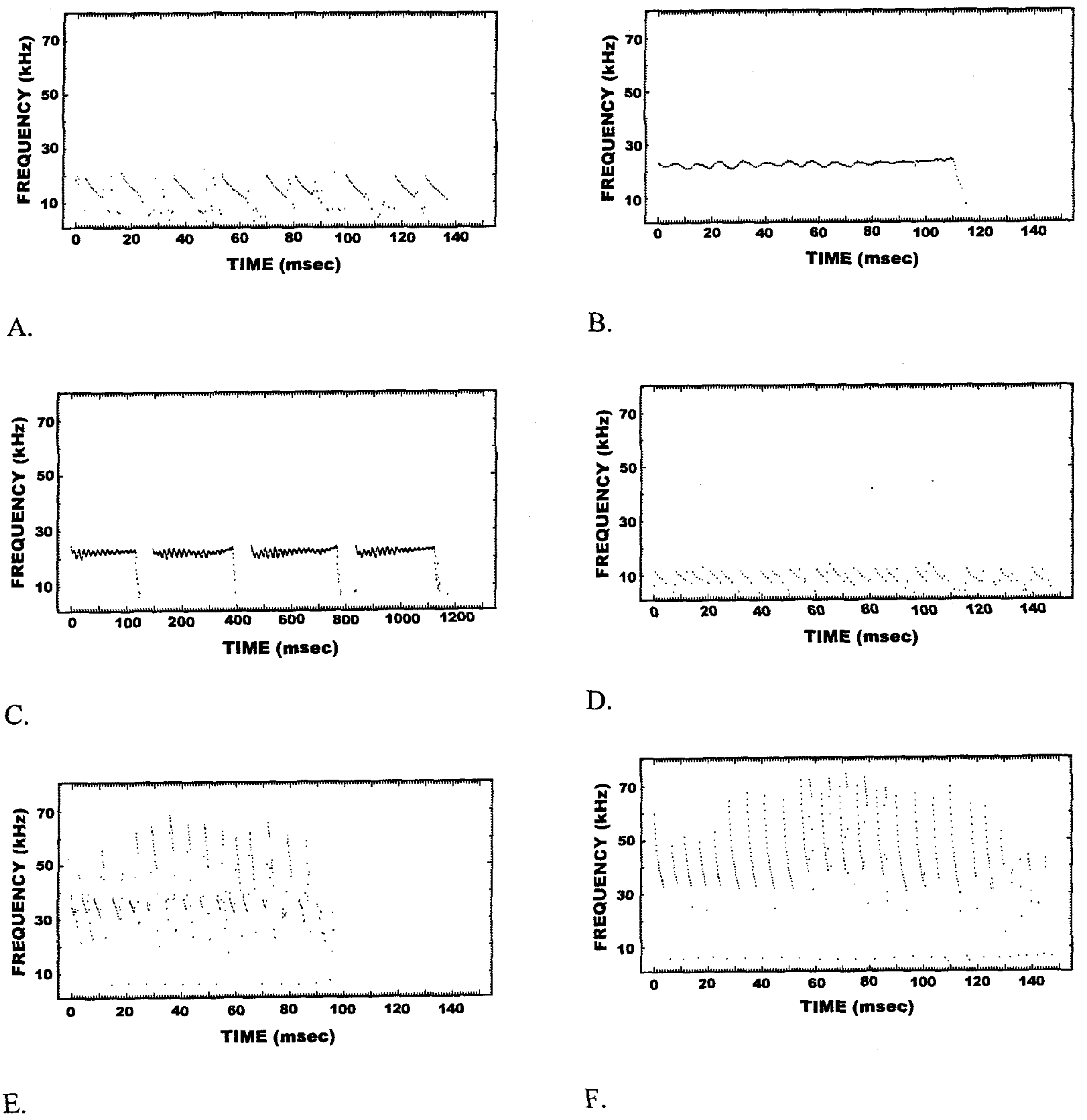

Fig. 3. Frequency/time display of echolocation calls from *Idionycteris phyllotis* (A, B, and C), *Euderma maculatum* (D), *Corynorhinus townsendii* (E), and *Antrozous pallidus* (F).

was visible, there could be confusion with a number of species using signals in the 20-30 kHz range (e.g., *Lasionycteris noctivagans* and *Lasiurus cinereus*; Fig. 2C and F, respectively).

Virtually no visual representations have been published that demonstrate the variability inherent in echolocation calls of the species studied. A single pulse is not adequate to furnish reliable identification. Although it has been recognized that signal shape changes from search through detection of a target and pursuit of a prey item (e.g., Griffin et al. 1960, Schnitzler and Henson 1980, Simmons et al. 1979), the range in variation exhibited by individual species has seldom been described. A recent study (Kalko and Schnitzler 1993) described the variation in signal characteristics for three species of European pipistrelles in relation to habitat and specific foraging behavior, providing critical information for the discrimination of species under varied circumstances.

The database gathered during my study represents a start towards a comprehensive library of echolocation calls. However, further determination of diagnostic signal characteristics will be required. The maximum and minimum frequency of a vocal sequence needs to be examined, recognizing that both are subject to variation. Duration of a signal varies with activity but average duration for a behavioral sequence may be important. Changes in shape, which incorporate both bandwidth and duration, may be a valuable tool, particularly as defined by Kalko and Schnitzler (1993). The components of signal characteristics need to be subjected to critical statistical analyses, permitting a more rigorous examination of geographic and temporal differences. I have used these general characteristics for distinguishing separate species but in a strictly subjective manner.

Quantification of specific features will be meaningful but only when performed under stringent, controlled conditions. Time intensive studies following individuals marked by light tags or radio transmitters will guarantee that all incoming calls are from the same source and will provide a context in which to interpret the sounds. Knowledge of the animal's orientation to the detector, approximate distance, and type of behavior provide additional context to assist in species identification. Some standardized procedure will be required to choose the individual calls on which to perform measurements. Not all incoming calls, even within the same sequence, are of the same quality. It is important to be selective and eliminate call fragments. Measures on fragments will definitely overlap with those of other species and introduce confounding error.

In order to achieve a comprehensive library of vocal signatures, it will be necessary to target a species and follow that species through a variety of seasons and during the range of behaviors exhibited by that taxon. Individual variation must be described. This will require the ability to follow a known individual through a variety of behaviors. Under any condition, the amassing of a comprehensive library for a given species, let alone an entire bat community, is not a trivial exercise. One may envision that such a process will be added to continually.

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