

**DEVELOPMENT OF VOCAL SIGNATURES AS A METHOD FOR ACCURATE
IDENTIFICATION OF FREE-FLYING BATS IN NORTHERN ARIZONA**

By

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IDENTIFICATION OF FREE-FLYING BATS IN NORTHERN ARIZONA**

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SUMMARY

A study was conducted to test the efficacy of the Anabat II bat detector and analysis system for obtaining reliable vocal signatures for species identification in northern Arizona. A total of 17 locations in northern Arizona, representing the range in elevations and associated habitat types, were sampled acoustically. An additional six locations in Utah, Nevada, and Wyoming were selected in order to sample species which could not be accurately separated under faunal conditions experienced in Arizona. Select localities were sampled seasonally in an attempt to determine temporal variations in vocal repertoire.

Anabat II provides a powerful tool by which to examine the structure of a bat community and assess levels of activity and patterns in habitat use. Eighteen of the 20 species known to occur in the region were identified by recognizable differences in the time/frequency characteristics of echolocation calls. An additional species, *Eumops perotis* was identified acoustically and later verified visually, which represents an extension of the known range. The use of acoustic sampling with this equipment yielded significantly more species compared to conventional netting and trapping. The present study suggests that the efficacy of performing inventories will increase with the determination of better sampling procedures and the development of a comprehensive library of vocal signatures.

It is important to recognize the limitations of the equipment. As with any tool, it can not be reasonably expected to function with all species under all conditions. It appears that Anabat is not suited for sampling "whispering" bats which use highly directional, low intensity echolocation (e.g., bats of the New World family, Phyllostomidae). Care should be exercised when sampling for and making determinations of presence of species of bats that use a gleaning foraging strategy or commonly negotiate cluttered environments. These species tend to use low intensity calls and could be missed acoustically. Likewise, bats that use low frequencies may not be detected due to hardware filtering of low frequencies to eliminate insect and other background noise from obscuring bat vocalizations.

Further work is warranted to establish a comprehensive library of vocal signatures, including descriptions of the range of variation inherent in each species, both temporally and geographically. It is also critical to develop various sampling strategies to better apply acoustic sampling to as many situations as possible. Such work, aided by future technological development, will allow greater latitude in the study of bats.

INTRODUCTION

Because of the increased general awareness of bats as an important biological resource, they are receiving increased state and federal agency scrutiny for listing as threatened or endangered. Of the 28 bat species known to occur within Arizona, 20 occur within the extreme northwestern portion of the state designated as the Arizona Strip (Hoffmeister, 1986). This portion of Arizona lies north and west of the Colorado River and the Grand Canyon. The Arizona Strip presents a relatively small geographic region that contains most of the elevations, and consequently habitat ranges, found in the state. As such, it shares most of the bat species known to occur in the contiguous states (Hall, 1981). Current knowledge, or lack thereof, has resulted in candidate status for listing as threatened or endangered for four species by the state (AGFD, 1988) and 15 species federally (USFWS, 1994). All of these species are known or are expected to occur within the Arizona Strip region (Hoffmeister, 1986).

Much of the biology of bats, away from roost sites, has been determined using nets and traps. In the past, verification of a bat species' presence was primarily through capture at roost sites, water holes, or along foraging flyways (see Kunz and Kurta, 1988 for a review). Not all bat species and not all individuals within a species are equally susceptible to capture. To compound the sampling problem, a given location may not be used every night by the same species assemblage. Effective netting requires that nets be tended constantly, thus limiting the number of locations that can be sampled simultaneously. The increasing use of double-frame harp traps in place of, or in conjunction with, nets over the past two decades has allowed increased sampling efficiency for some species in some habitats. The use of traps reduces the need for constant monitoring because the traps contain a collecting bag which holds captured individuals for later processing and with reduced chance of mortality. The restriction of sampling to nets and/or traps has limited the ability to effectively study free flying bats.

Electronic acoustic devices (bat detectors) have been developed to allow investigators to hear and/or visualize the ultrasonic echolocation calls of bats (see Fenton, 1988 for a review). Many species of bats produce echolocation calls that appear to be distinctive (Simmons, Fenton, and O'Farrell, 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). In Sweden, a similar attempt provided an acoustic guide to the identification of bats of Europe (Ahlen, 1990).

Limited studies have been conducted using echolocation calls as a means to characterize feeding behavior (Barclay, 1982, 1988; Fenton, 1982; Fenton and Bell, 1979). The relation between echolocation call design and foraging behavior has been used in an ecomorphological examination of bat communities (Saunders and Barclay, 1992). General habitat use and bat faunal assemblages also have been examined with the aid of bat detectors (Bell, 1980; Crome and Richards, 1969; Fenton et al., 1977; Fenton, Merriam, and Holroyd, 1983; Storz, 1995). The studies suggest that echolocation calls can be an extremely valuable tool for accurately defining the structure of local bat faunas and describing patterns of habitat use.

The sonar repertoires of at least some bat species may be quite variable (Simmons et al., 1978; Simmons and O'Farrell, 1977). Studies on intraspecific variation indicate that individual differences in certain aspects of call structure exist (Brigham, Cebek, and Hickey, 1989). Geographic variation in "dialects" may exist. Seasonal changes in echolocation strategy are also expected.

A number of southwestern bats are known to be active throughout the year, even in sub-freezing conditions (O'Farrell and Bradley, 1970). Active bats may only be in search of water under the coldest conditions (O'Farrell and Bradley, 1977). Information on auditory-evoked potentials of *Myotis lucifugus*, from the eastern United States, indicate a decrease in response to high frequency sound with decreasing temperatures, reaching total deafness below 12°C (Harrison, 1965). The sonar repertoire of some species may change depending on the search image required and auditory nerve sensitivity at reduced temperatures.

The emergence of different insect groups through the year provides opportunistic bats variation in prey size, shape, and behavior. Intraspecifically, echolocation signals vary from narrow bandwidths in uncluttered habitats to wide bandwidths in cluttered situations (Kalko and Schnitzler, 1993). The presence of conspecifics results in changes in call duration and cadence (Obrist, 1995) the presence of other species may influence behavior and vocalizations (Barclay, 1982; Obrist, 1995). However, temporal, geographic, and species composition influences in defining identifiable echolocation calls have not yet been adequately addressed.

The equipment used in previous studies has been varied and the output generalized. A standardized system with easily obtainable field equipment and a reference library of vocalizations containing the range of variation that may be encountered in each species' vocal repertoire has not yet been achieved. The detailed study of bat echolocation calls requires the use of ultrasonic

detectors, tape recorders, period meters, oscilloscopes, and a range of analytic procedures (see Fenton, 1988 for an overview). Major problems with early devices involved cost, availability, and ease in analyzing recordings. Technological advances within the past few years have generally resolved analysis concerns but cost and availability are still a problem. Currently, all bat detectors are manufactured outside the U.S. Costs are high for most detectors capable of providing sufficient sonar information for species identification. Not all detectors are available with compatible signal analysis systems. For example, the QMC Model S200 (England) costs approximately US\$1,200, requires a moderately expensive tape recorder and does not have a signal analysis system available. The D940 (Sweden) costs approximately US\$1,700, requires a similar high quality tape recorder, and comes with a signal analysis package that costs an additional US\$1,400.

Recently, an Australian system (Anabat II) has become available. The detector costs about US\$450, the hardware interface and signal analysis software costs about US\$400. Many systems use circuitry which divides the frequency of the raw sonar pulses (generally 120 to 20 kHz) by a fixed ratio to generate human audible signals which are thus available for recording on conventional tape recorders. Most use a division ratio of 10, which requires a tape recorder with a frequency response of 12 kHz. Recorders with a 12 kHz capability cost in excess of US\$500. The Anabat II uses a base-2 division ratio with detector settings that can generate output frequencies below 6 kHz. Most tape recorders under US\$100 will suffice to record sounds up to 6 kHz. The Anabat II also allows direct interface between the detector to a laptop computer. Vocalizations can be observed while being generated and the observer may select which sequences to save directly to the computer hard drive.

The purpose of this 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 the habitats found within the Arizona Strip. Seasonal sampling provides additional data on variation within the echolocation calls of the bat fauna of this region.

STUDY AREA

Initially, sampling locations were selected based on past collecting localities listed in Hoffmeister (1986) and input from biologists with the Arizona Game and Fish Department (AGFD) and Bureau of Land Management (BLM). An attempt was made to sample the range of elevations and associated habitats found within the Arizona Strip. Several locations outside the

designated study area were visited in order to sample target species known to occur in the study area but whose calls might be confused with sympatric congeners. Recording a target species in a geographic area without potentially confusing species present presumably furnished a baseline for examination of the more diverse bat fauna in northern Arizona. Therefore, eight of the 20 sampling locations occurred in Arizona south of the Colorado River, southern Utah, central Nevada, and Wyoming (Table 1).

Middle and high elevations predominate in the study area, thus 13 of the 20 sampling locations were greater than 1219 m elevation. A total of four major habitat types occur within the Arizona Strip (Hoffmeister, 1986). At some sampling sites certain riparian influences were present, particularly in wash settings. Mohave desertscrub occurred at the low elevation sites, a mix of Great Basin desertscrub and juniper-pinyon woodland occurred through the middle elevation valley settings, and high elevations contained montane conifer forest.

MATERIALS AND METHODS

Each site selected for acoustic sampling was monitored using an Anabat II bat detector, usually linked to an IBM-compatible laptop computer. Simultaneous with acoustic sampling, a representative sample of bat species was collected at all but two locations (see Table 1). Lees Ferry Landing was specifically targeted for the Yuma myotis, based on the prevalence of this species foraging low over the water surface (M. Rabe, personal communication). Wind conditions were severe enough to preclude setting nets at Limekiln Canyon. The remaining sites were sampled with mist nets or a combination of mist nets and double-frame harp traps (Austbat Research Equipment, Victoria, Australia). The intensity of collecting effort (see Table 1) was directly influenced by the physical characteristics of each specific location.

In general, each location was acoustically sampled by placement of the detector at a central location. Although it was desirable to have an observer monitoring the acoustic equipment constantly, it was not always possible due to the demands of removal of bats from collecting devices and subsequent processing of captured individuals. When the equipment could not be constantly monitored, the detector was propped at a 45°-angle from perpendicular and whenever time allowed, the computer screen was examined for vocal sequences and appropriate series were saved. The procedure used during constant monitoring of the acoustic equipment entailed an evaluation of the quality of the signals and saving of the best representative samples of vocal sequences that reflected various behaviors (e.g., search, pursuit, capture).

Table 1. Reference number (REF NO), location, latitude, longitude, elevation (ELEV), and collection effort (surface area) for sampling sites in Arizona (AZ), Nevada (NV), Utah (UT), and Wyoming (WY).

REF NO	LOCATION	LATITUDE (N)	LONGITUDE (W)	ELEV (m)	DATE	NETS (m ²)	TRAPS (m ²)
1	Stone House, Beaver Dam Wash, Mohave Co., AZ [*]	36° 53' 51"	113° 55' 24"	488	10 MAY 1994 18 MAY 1995	84.4 148.2	4.5 -
2	Forest Road 700, Stock tank, Coconino Co., AZ	35° 04' 05"	111° 40' 30"	2103	24 JUN 1994	65.3	4.5
3	Frehner Cabin Pond, Elbow Canyon, Mohave Co, AZ [*]	36° 45' 51"	113° 53' 28"	1506	30 JUN 1994	65.1	4.5
4	Parashant Field Station, Stock tank, Mohave Co., AZ [*]	36° 08' 14"	113° 35' 01"	1859	20 JUL 1994 21 JUN 1995 9 AUG 1995	53.8 282.6 109.8	- 4.5 4.5
5	Cram Ranch Road, Stock Tank, Houserock Valley, Coconino Co., AZ [*]	36° 41' 26"	111° 54' 05"	1516	29 AUG 1994 1 SEP 1994	84.4 84.4	- -
6	Sunshine Trail, Stock tank, Mohave Co., AZ [*]	36° 57' 49"	113° 27' 29"	914	4 FEB 1995	115.1	-
7	Cottonwood Wash, near Buckhorn Spring, Mohave Co., AZ [*]	36° 19' 17"	113° 58' 47"	488	23 FEB 1995	92.2	-
8	Site 2, Fort Pearce Wash, Mohave Co., AZ [*]	36° 59' 53"	113° 26' 35"	945	11 JUL 1994 24 AUG 1994 1 JUN 1995	76.4 19.1 23.1	4.5 - -
9	Tank 3, Buffalo Ranch, Houserock Valley, Coconino Co., AZ [*]	36° 23' 51"	111° 57' 49"	1829	11 JUN 1995 12 JUN 1995	232.7 232.7	4.5 4.5
10	Tank 4, Buffalo Ranch, Houserock Valley, Coconino Co., AZ [*]	36° 25' 56"	111° 56' 20"	1737	12 JUN 1995	120.2	-

Table 1. (Continued).

REF NO	LOCATION	LATITUDE (N)	LONGITUDE (W)	ELEV (m)	DATE	NETS (m ²)	TRAPS (m ²)
15	Lees Ferry Landing, Coconino Co., AZ*	36° 51' 58"	111° 35' 03"	951	13 JUN 1995	-	-
16	Limekiln Canyon, Mohave Co., AZ*	36° 39' 47"	114° 00' 38"	1859	14 JUN 1995	-	-
11	Trail's End, Black Rock Mountain, Mohave Co., AZ*	36° 46' 12"	113° 47' 53"	2256	20 JUN 1995	85.6	4.5
12	Pot Hole Tank, Hualapai Reservation, Coconino Co., AZ	35° 51' 54"	113° 10' 41"	1707	26 JUN 1995	176.4	4.5
13	Diamond Creek, Hualapai Reservation, Mohave Co., AZ	35° 45' 28"	113° 21' 36"	427	27 JUN 1995	42.2	4.5
14	Oak Grove Tank, Coconino National Forest, Coconino Co., AZ	34° 50' 49"	111° 33' 22"	2042	30 JUN 1995	61.3	-
17	Murphy Wash, Snake Range, White Pine Co., NV	38° 45' 16"	114° 18' 29"	2073	21 SEP 1994	76.4	9
18	Swallow Canyon, Snake Range, White Pine Co., NV	38° 50' 44"	114° 21' 53"	2073	22 SEP 1994	96.0	9
19	Shingle Spring, Egan Range, Lincoln Co., NV	38° 32' 24"	114° 56' 07"	1951	17 JUN 1995	38.2	4.5
20	Fort Pearce, Fort Pearce Wash, Washington Co., UT	37° 00' 28"	113° 24' 42"	884	10 APR 1995	76.4	4.5
21	Hot Hole Caves, Wind River Canyon, Hot Springs Co., WY	43° 30' "	108° 10' "	1341	5 JUN 1995	11.6	-
22	Seedskadee National Wildlife Refuge, Sweetwater Co., WY	41° 50' "	109° 50' "	1939	6 JUN 1995	38.2	-

* Locations on the Arizona Strip

Identification of the source of specific vocalizations 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 vocal input, captured bats were released individually and followed to obtain as many confirmed vocalizations as possible. In the latter stages of this study, some individuals were affixed with light tags (Mini-light Sticks, Chemical Light Inc.) glued on the fur, either dorsally or ventrally. Animals with light tags were followed to obtain vocalizations from a known species.

Identification of vocalizations of several species was accomplished with less direct methods. Comparative files for *Eumops perotis* and *Myotis yumanensis* were obtained from California (C. Corben, personal communication). Calls from *Euderma maculatum* were obtained at a known locality (Poché, 1975) from free-flying individuals emitting characteristic human-audible pulses which consistently fit the signals described from Canada (Leonard and Fenton, 1984). Finally, calls from *Nyctinomops macrotis* were identified by comparison (Simmons et al., 1978) and by deduction. Audible calls, consistent with past personal experience, that were definitely molossid in nature but conspicuously not *Eumops* or *Tadarida* were ascribed to *N. macrotis*, when the presence of this species was known for a location from prior collections.

RESULTS

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.

The following figures depicting the frequency-time display for the species found during the present study are simply for gross generalization and comparison. No attempt was made to perform a quantitative examination of call structure features. This was beyond the scope of the present study. The figures are presented solely to provide visual evidence supporting the capability of separating species by call structure. All figures are taken from screen captures directly from the Anabat program. The vertical axis is frequency (kHz) and the horizontal axis is time in major increments of 10 ms. 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 one locality. Separation of co-existing 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 bandwidths (Figures 1-4; *M. californicus*, *M. ciliolabrum*, *M. volans*, and *M. lucifugus*, respectively). *Myotis* that fly in clutter and glean insects from vegetation surfaces produce calls with relatively wide bandwidths (Figures 5-7; *M. auriculus*, *M. evotis*, and *M. thysanodes*, respectively). *Myotis yumanensis* tends to forage in the open but in close proximity to water surface (Figure 8).

Other genera of bats in the area demonstrate greater variation in the shape of calls. In general, aerial hawking species produce orientation/search calls of relatively narrow bandwidth and long duration (> 5 msec). Upon detecting a potential prey item, the pulses increase in bandwidth 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 (Figures 9 and 10, respectively). Variations in the pattern may be wide. Initial search/orientation pulses may be relatively constant shifting to a more variable, higher frequency wideband pursuit/approach phase (Figure 11-13; *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 wideband approach calls (Figures 14-16; *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 was gathered that suggests that *T. brasiliensis* may also produce orientation sounds that are human-audible.

Other species that use a gleaning foraging strategy or negotiate clutter on a regular basis demonstrate some different vocal strategies. The most unusual and distinctive vocalizations 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 (Figure 17). However, during foraging activity, a long quasi-constant frequency component is interjected,

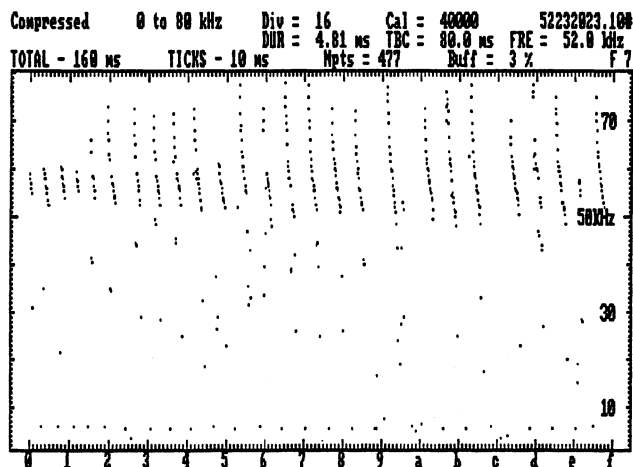


Figure 1. Vocal sequence for *Myotis californicus*.

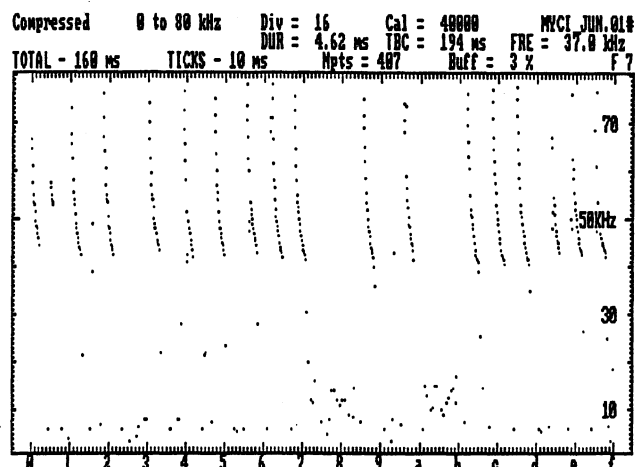


Figure 2. Vocal sequence for *Myotis ciliolabrum*.

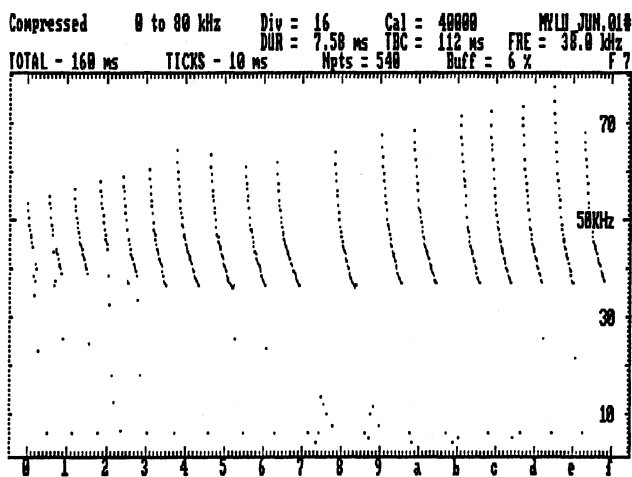


Figure 3. Vocal sequence for *Myotis lucifugus occultus*.

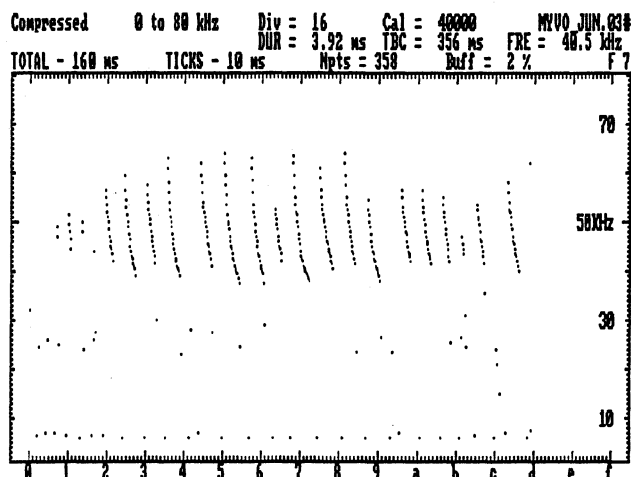


Figure 4. Vocal sequence for *Myotis volans*.

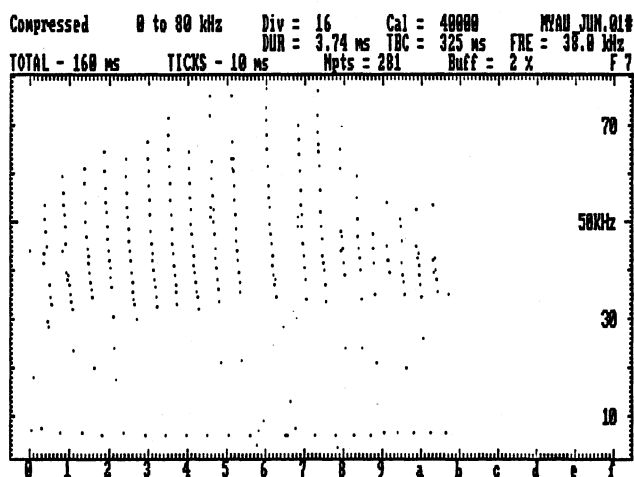


Figure 5. Vocal sequence for *Myotis auriculus*.

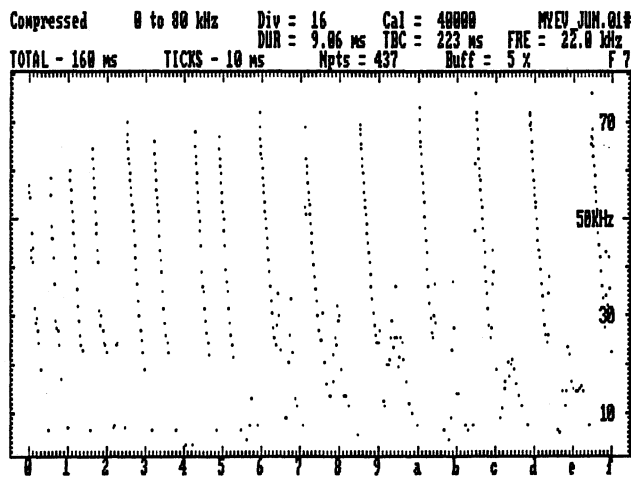


Figure 6. Vocal sequence for *Myotis evotis*.

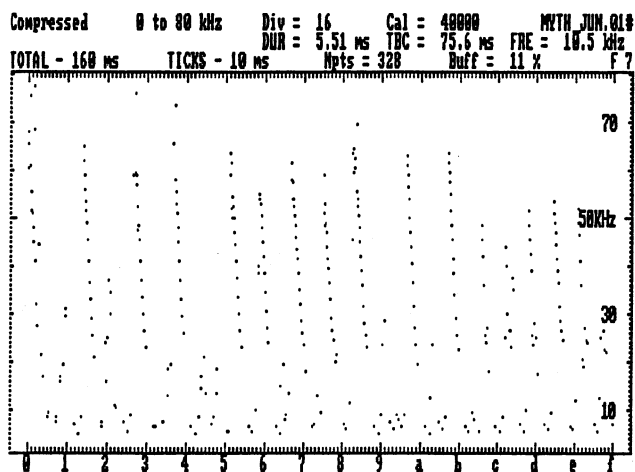


Figure 7. Vocal sequence for *Myotis thysanodes*.

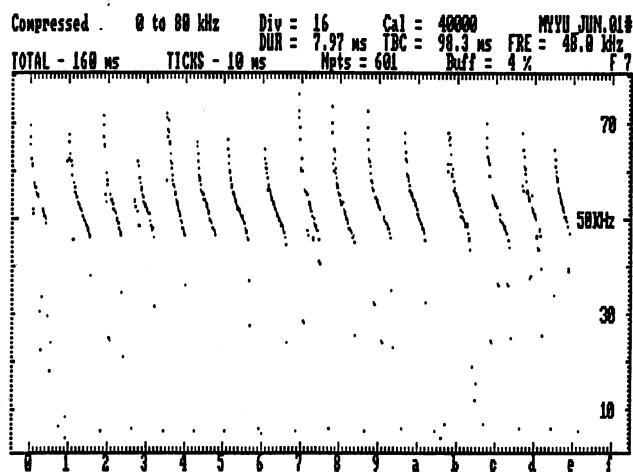


Figure 8. Vocal sequence for *Myotis yumanensis*.

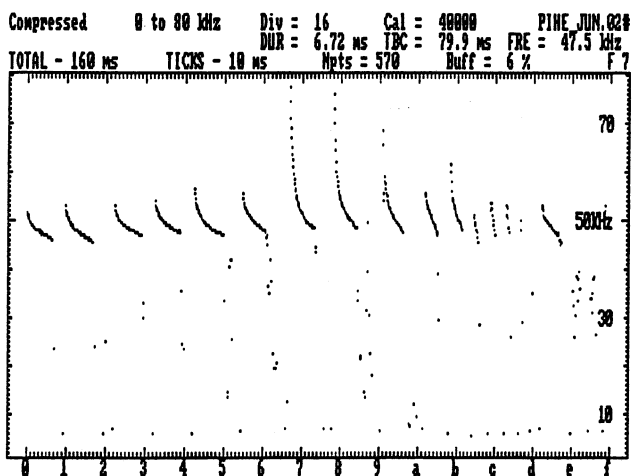


Figure 9. Vocal sequence for *Pipistrellus hesperus*.

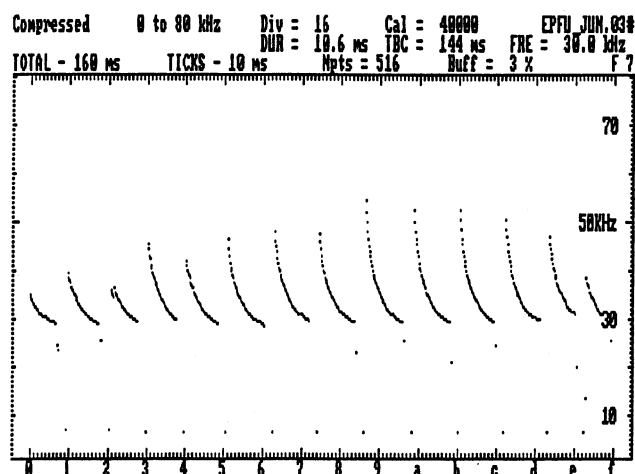


Figure 10. Vocal sequence for *Eptesicus fuscus*.

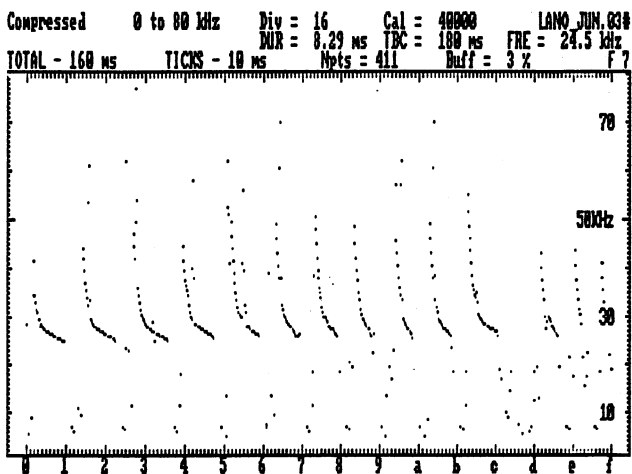


Figure 11. Vocal sequence for *Lasionycteris noctivagans*.

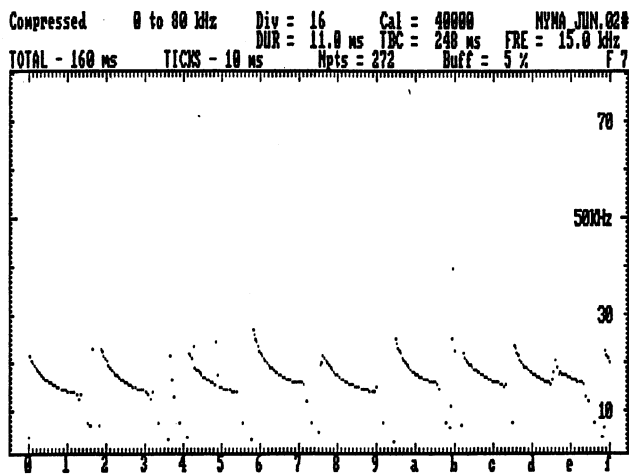


Figure 12. Vocal sequence for *Nyctinomops macrotis*.

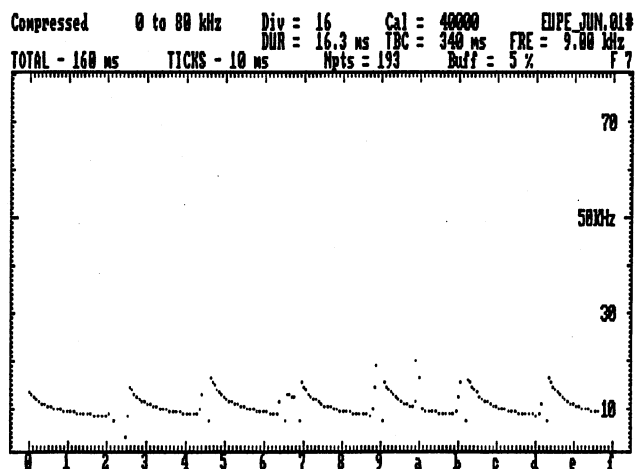


Figure 13. Vocal sequence for *Eumops perotis*.

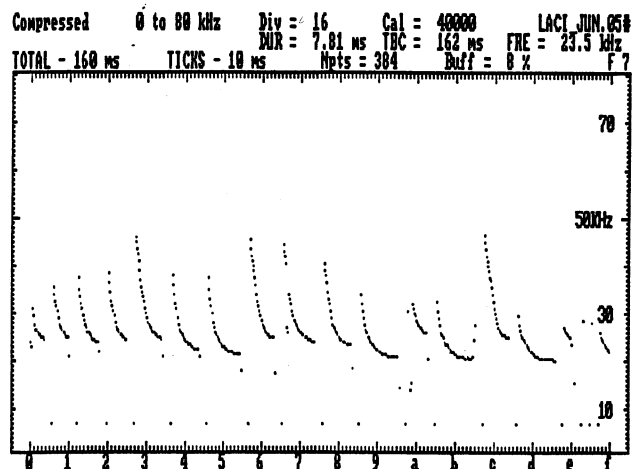


Figure 14. Vocal sequence for *Lasiurus cinereus*.

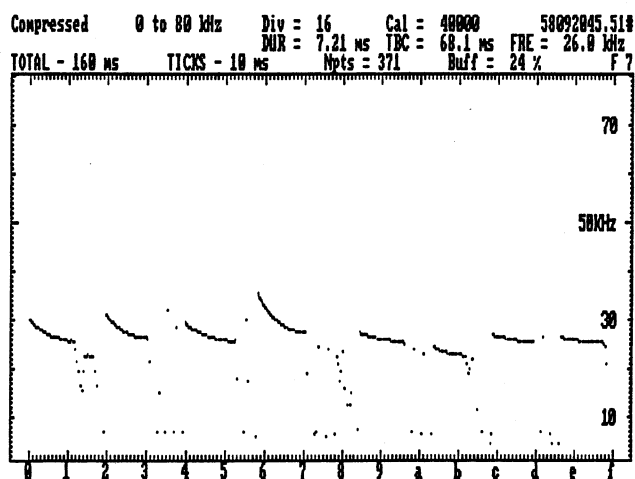


Figure 15. Vocal sequence for *Tadarida brasiliensis*.

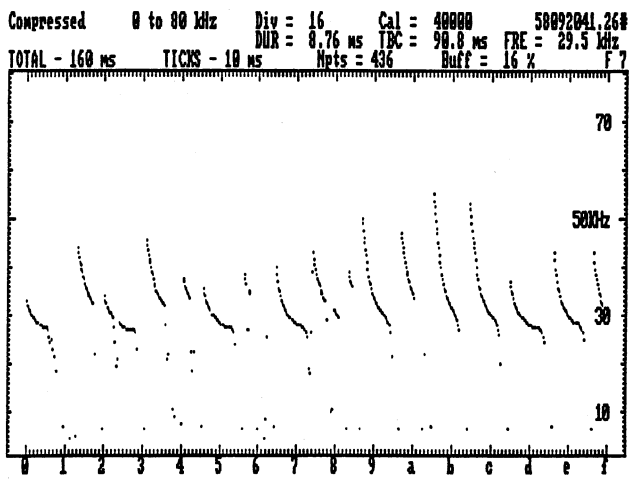


Figure 16. Vocal sequence for *Tadarida brasiliensis*.

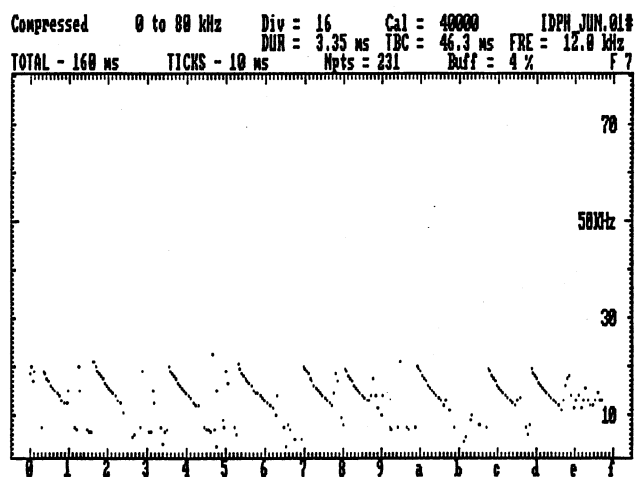


Figure 17. Vocal sequence for *Idionycteris phyllotis*.

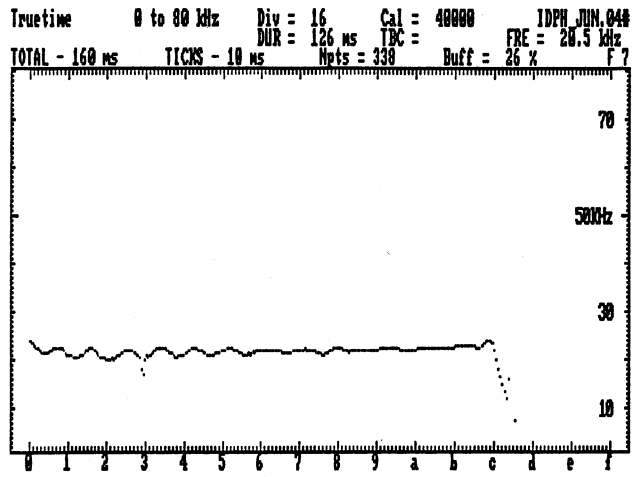


Figure 18. Vocal pulse for *Idionycteris phyllotis*.

as shown in Figs. 18 and 19. Figure 18 illustrates a single vocal pulse (time scale at 10 msec intervals) and Figure 19 illustrates 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* (Figure 20).

Remaining species that use a gleaning strategy present more of a problem in characterization. Existing vocal files for *Plecotus townsendii* (Figure 21) are fragmentary and suggest the use of two strong harmonics, providing a relatively wideband pulse. There is indirect evidence that *Antrozous pallidus* has a highly variable vocal repertoire that depends on immediate surroundings. Monitoring orientation calls in a room revealed that most pulses were narrow in bandwidth and short in duration. Similar signals were detected from hand-released individuals in the field. Once flight was achieved and the bat continued activity in the vicinity of the monitor, a wider range of relatively broadband, short duration signals were observed (Figure 22). Other bats may use similar calls in confined situations.

A total of 19 species of bats were captured and recorded among the 22 locations sampled (Table 2). Acoustic monitoring confirmed more species present during 17 of the total 25 sampling periods; 10 of the 17 yielded 50% or more species that did results from capture techniques. The same number of species were identified during the remaining eight sampling periods; however, the species composition was different in five of these. Not all sampling was biased in favor of acoustic monitoring. *Plecotus townsendii* were captured during nine sampling periods but were only detected acoustically during two of them. Similarly, *Myotis evotis* was captured during three sampling periods but only detected during one of them. The null hypothesis that acoustic and capture methods yield the same inventory results is rejected ($t = 6.676$; $df = 24$; $p < 0.001$).

Subjective examination revealed that the shape of echolocation calls of the bat species found in the present study did not vary by season. Likewise, no observable changes in signal shape were apparent on a geographic basis. The observed changes in signal shape appear to be related to behavior (e.g., orientation/search, pursuit) and the proximity to a cluttered background. Some species, most notably *Tadarida brasiliensis*, produce highly variable, lower frequency signals that appear to serve communication and not orientation purposes. These signals are particularly common near roost sites but are regularly observed at times when multiple conspecific individuals are present.

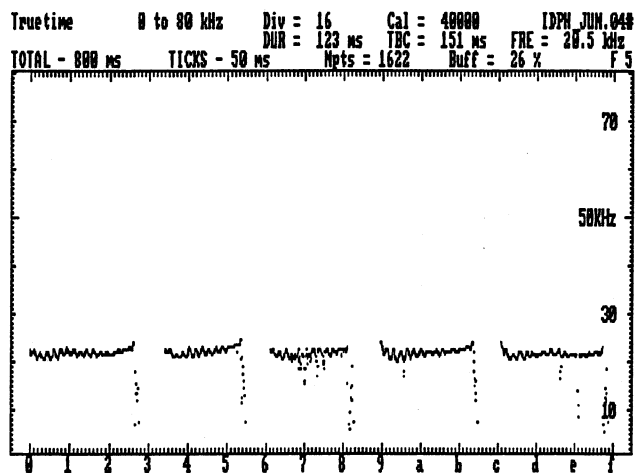


Figure 19. Vocal sequence for *Idionycteris phyllotis*.

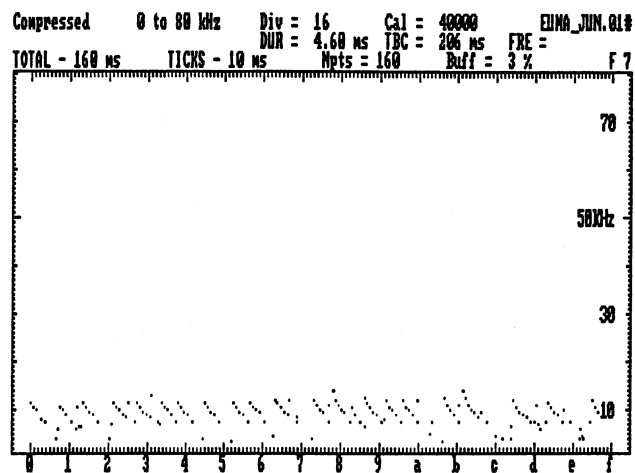


Figure 20. Vocal sequence for *Euderma maculatum*.

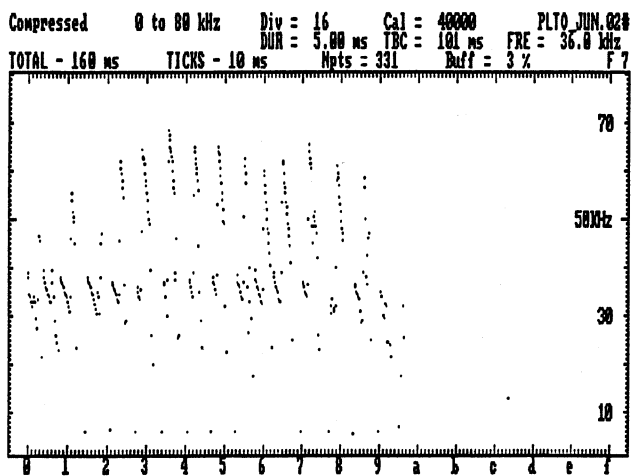


Figure 21. Vocal sequence for *Plecotus townsendii*.

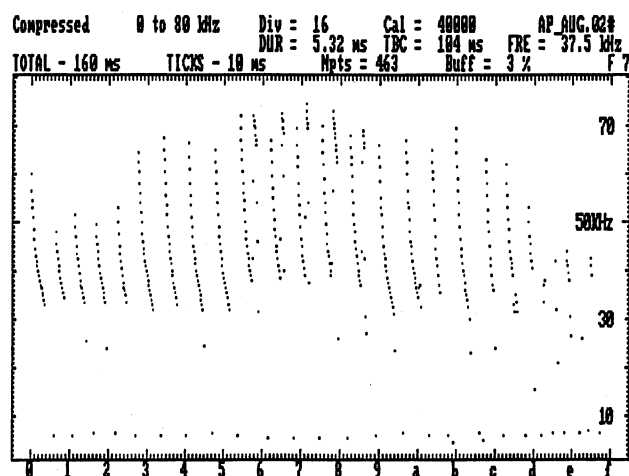


Figure 22. Vocal sequence for *Antrozous pallidus*.

Table 2. Location number (LOC), month (DATE), and number of individuals (males/females) of each species captured, presence verified by vocal signature obtained by acoustic monitoring with Anabat (A), and possible occurrence based on fragmentary vocal signatures (P). See Table 1 for detailed coordinates of each location and exact date of collection and Appendix I for the species abbreviation key.

LOC	DATE	MYO AUR	MYO CAL	MYO CIL	MYO EVO	MYO LUC	MYO THY	MYO VOL	MYO YUM	LAS CIN	LAS NOC	PIP HES	EPT FUS	EUD MAC	PLE TOW	IDI PHY	ANT PAL	TAD BRA	NYC MAC	EUM PER
1	MAY 94	-	3/3, A	-	-	-	0/1, A	-	A	A	A	A	A	-	0/1	-	0/2, A	A	-	-
	MAY 95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	JUN 94	2/5, A	-	1/0, A	-	0/1, A	-	-	-	A	A	-	1/1, A	-	-	A	0/1, A	-	-	-
3	JUN 94	-	6/3, A	A	-	-	-	2/0, A	-	-	A	27/31A	3/1, A	-	2/0	-	2/6, A	-	A	-
4	JUL 94	-	-	A	-	-	P	A	A	-	A	-	1/0, A	-	-	-	-	1/0, A	A	A
	JUN 95	-	-	A	-	-	-	A	1/0, A	1/0, A	A	A	0/2, A	-	-	-	-	0/1, A	A	A
	AUG 95	-	A	-	-	-	P	1/0, A	-	-	A	6/11, A	1/2, A	-	-	-	-	34/22, A	A	A
5	AUG 94	-	1/1, A	-	-	-	-	-	-	-	-	7/8, A	-	-	-	-	A	A	-	-
	SEP 94	-	1/1, A	-	-	-	-	-	-	-	-	9/16, A	-	-	-	-	A	1/0, A	-	-
6	FEB 95	-	-	-	-	-	-	-	-	-	-	2/0, A	-	-	-	-	1/0, A	-	-	-
7	FEB 95	-	A	-	-	-	-	-	-	-	-	1/0, A	-	-	-	-	-	4/0, A	-	-
8	JUL 95 [†]	-	0/1, A	-	-	-	1/2, A	-	-	-	-	16/8, A	0/5	A	-	-	1/3, A	-	-	-
	AUG 94	-	A	-	-	-	-	-	-	-	-	19/5, A	-	-	1/0	-	4/1, A	-	-	-
	JUN 95	-	1/0, A	-	-	-	-	-	-	-	-	3/14, A	-	-	-	-	2/0, A	1/0, A	-	-
9	JUN 95 [‡]	-	2/3, A	2/4, A	P	-	1/1, A	-	-	-	A	A	0/5, A	-	1/3, A	-	0/1, A	A	A	-
	JUN 95 [‡]	-	6/11	3/1	-	-	0/2	-	-	1/0	-	-	0/1	-	2/6	-	0/1	-	-	-
10	JUN 95	-	4/8, A	2/1, A	-	-	-	-	-	1/0, A	-	A	-	-	0/1	-	2/2, A	-	-	-
11	JUN 95	-	A	-	0/1, A	-	-	0/1, A	A	A	A	-	A	-	-	-	-	A	A	-
12	JUN 95	-	0/1, A	3/2, A	-	-	1/6, A	3/15, A	-	3/0, A	1/0, A	-	8/7, A	-	2/5	0/2, A	2/0, A	1/0, A	-	A
13	JUN 95	-	1/0, A	-	-	-	1/0, A	P	A	1/0, A	-	A	A	-	-	-	-	-	-	-

Table 2. (Continued).

LOC	DATE	MYO AUR	MYO CAL	MYO CIL	MYO EVO	MYO LUC	MYO THY	MYO VOL	MYO YUM	LAS CIN	LAS NOC	PIP HES	EPT FUS	EUD MAC	PLE TOW	IDI PHY	ANT PAL	TAD BRA	NYC MAC	EUM PER
14	JUN 95	0/1, A	-	A	-	0/1, A	1/0, A	-	0/1, A	A	A	-	3/1, A	-	-	-	A	A	-	-
15	JUN 95	-	A	-	-	-	-	-	A	P	A	A	A	A	-	-	-	A	A	-
16	JUN 95	-	A	-	-	-	-	P	-	-	-	A	-	-	-	-	-	-	-	-
17	SEP 94	-	0/1, A	P	3/1	-	-	28/7, A	-	-	A	-	A	-	-	-	A	-	-	-
18	SEP 94	-	10/1, A	P	1/0	-	-	8/0, A	A	-	2/0, A	-	1/0, A	-	0/1	-	A	-	-	-
19	JUN 95	-	-	1/0, A	-	-	-	0/8, A	-	-	3/0, A	-	-	-	-	-	-	-	-	-
20	APR 95	-	A	-	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-	-
21	JUN 95	-	-	2/1, A	-	A	P	P	-	-	A	-	-	P	0/5, A	-	A	P	-	-
22	JUN 95	-	-	A	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-

† Computer failure, limited files obtained using tape recorder with Anabat II

‡ No Anabat II monitoring during collection period

DISCUSSION

The ability to acoustically distinguish species of free-flying bats has been well documented (Ahlen, 1990; Bell, 1980; Fenton and Bell, 1979, 1981). However, the equipment available 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 identification of bat presence and sometimes determination of which species are present. The range of approaches and limitations have been discussed in detail by Fenton (1988). Basically, four types of systems are available: 1) a precision broadband system that allows recording the entire signal on a high speed tape recorder. This system collects all sound information but is limited in distance of detection and is extremely expensive. 2) a heterodyne system that allows only a single frequency to be monitored at one time. This system requires that a bat's vocalizations incorporate the frequency selected, provides some ability to differentiate species by tonal differences, and ranges from very to moderately inexpensive. 3) a broadband system that incorporates division circuitry that converts signals to the human audible range. These systems lose signal amplitude information thus losing harmonic information, allow limited tonal differentiation, allow visualization of the time/frequency spectrum for the harmonic with the greatest energy, and is moderately inexpensive. 4) a time-expansion system that stores signals digitally for subsequent slow speed recording on a conventional tape recorder. This system collects all sound information, eliminates the need for the high speed tape recorder, requires a lengthy analysis, provides auditory identification of bat species in the field based on perceived tonal differences, and is moderately expensive.

Anabat II is a division circuitry system, designed to enable field use with an inexpensive tape recorder. The tapes could be analyzed later in a laboratory setting. My initial experience, in late 1993, with recording sounds directly from the Anabat II detector to a portable tape recorder proved unsatisfactory. The operator either runs the tape for extended periods of time regardless of incoming vocalizations or selectively toggles the system on during bouts of vocalizations. The former strategy can result in long gaps of silence and the latter strategy results in the loss of all initial sonar pulses. During such operation, the tape recorder is only turned on after a vocal pulse is heard on the detector. Consequently, the initial vocalization is lost. Potential distortion of succeeding sounds can occur while the tape drive achieves full speed. It is essential to enter a calibration tone after each sequence of calls in order to compensate for distortion during later file examination.

A solution to these problems is the use of an Anabat II Delay Switch. This device stores incoming signals in a temporary buffer, turns on the tape recorder, downloads the buffer contents after the tape speed equilibrates, enters a time/date stamp and calibration tone after incoming signals cease, and then returns the tape recorder to standby mode. Use of the delay switch saves batteries and tape, and ensures that initial signals are not lost. However, under either scenario using a tape recorder, a large quantity of unscreened data is accumulated. Eight hours of tape recording requires an absolute minimum of eight hours of analysis time. In reality, much more time will be expended.

The development of Version 5 of the Anabat software in the spring of 1994 permitted the direct use of the Anabat II detector through the Zero Crossings Analysis Interface Module (ZCAIM) to a laptop computer in the field. This allowed for direct observation of incoming bat vocalizations and for evaluation as to species identification, general behavior (e.g., orientation, foraging), quality of sounds, and whether to save the sequence directly to the computer hard drive. There are several advantages to the new system. First, appropriate sensitivity settings can be adjusted to maximize the quality of sounds and eliminate echoes and other extraneous noise. Thus, saved files are of the highest quality possible. Secondly, representative sequences can be saved demonstrating the species composition of the sampling area and the range of behaviors being exhibited by each of the species. Active monitoring and selection of sequences dramatically reduce the quantity of time spent in reviewing and evaluating data. Lastly, the ability to obtain immediate feedback as to species identity and behavior assists the observer in making choices with respect to placement of collecting devices as well as where best to focus acoustic monitoring to yield the greatest information.

In general, the body of data developed to present has been available in two forms: 1) vocalizations presented as a representative vocal pulse; and, 2) verbal descriptions of certain 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 makes comparison with the existing data base difficult at best. For example, the vocal pulses for *Myotis californicus* represented in the two cited publications indicate signals that range in duration from 5 to 6 msec and frequencies that range from about 75 to 40 kHz, notably different from those shown in Figure 1. Published vocalizations tend to use a time scale with 2 or 5 msec intervals (Fenton and Bell, 1979; 1981) and more recently include harmonics (MacDonald, Stevens, and Fenton, 1994). Anabat II allows examination at a variety of time and frequency scales. The clarity of calls is generally better than earlier published calls due to a finer resolution

on the frequency axis.

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. 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. Although certain sonar information may be lost, there is no current indication that all harmonic information is necessary for the reliable identification of bats by vocal signature. In fact, a comparison of equipment versus output for *Pipistrellus subflavus* (MacDonald et al., 1994) shows a fundamental call with a strong harmonic recorded with an instrumentation tape recorder but only the harmonic visible with the Anabat system. Absence of the fundamental permits easy discrimination from calls of sympatric species. If only the fundamental frequency 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*; Figures 11 and 14, respectively). In other words, an abundance of detail may obscure rather than clarify species identification. Some bats apparently alternate the energy in the harmonic structure, which the Anabat displays as alternating pulses that may be helpful in providing species identification (see Figure 21 for *Plecotus townsendii*).

In the published literature, virtually no visual representations have been presented to demonstrate the variability inherent in the 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. Recently, an excellent study described the variation in signal characteristics for three species of European pipistrelles in relation to habitat and specific foraging behavior (Kalko and Schnitzler, 1993). Such studies will provide critical information for the discrimination of species identification under varied circumstances.

The utility of accurately identifying individual species based on vocal signature can best be illustrated by two examples. The first is the case of sympatric species that are virtually identical because of extensive overlap among the suite of external and internal identifying physical characters (California and small-footed myotis (*Myotis californicus* and *M. ciliolabrum*,

respectively; Bogan, 1974). Once basic vocal signatures were established in the present study, little difficulty has been encountered in distinguishing these two species while flying at the same spring at the same time (see Figures 1 and 2).

The second instance involves the inability to sample a bat fauna effectively with standard collecting techniques. One previous study compared mist nets and ultrasonic detectors for monitoring flight activity (Kunz and Brock, 1975). The acoustic system used was a traffic monitoring device incapable of species discrimination but provided data on bat presence. Nets and the ultrasonic detector yielded similar results with respect to bat activity. Table 2 provides an evaluation of collecting devices versus acoustic sampling in the present study. Overall, the Anabat II system yielded significantly more species detected at any sampling location.

These results are all the more striking in that originally the detector was placed in a central location and in many instances acoustic monitoring was intermittent in order to remove large numbers of bats from nets. Static sampling from a central location limits acoustic recognition to bats that have loud calls. Species that glean or at least forage in vegetative clutter emit calls of low intensity (Faure and Barclay, 1994) which attenuates rapidly. Therefore, if the detector is not situated near flyways or foraging areas, such species will not be identified. *Plecotus townsendii* was captured at nine locations yet was only detected twice (see Table 2). Both times, the detector was placed in strategic positions in order to maximize the opportunity to record the species. Similar problems occur with other gleaning species (e.g., *Myotis auriculus*, *M. evotis*).

It is axiomatic that bat faunas in geographic areas with abundant open water surfaces and forests, or other habitat features that inhibit effective collection techniques are generally the least known. Bats that fly and/or forage high in the open or over tree canopy are inadequately represented in collections, as illustrated in Table 2 for *Lasionycteris noctivagans*, *Lasiurus cinereus*, *Tadarida brasiliensis*, *Nyctinomops macrotis*, and *Eumops perotis*. The latter species was not known for northern Arizona, with the nearest known location over 100 airline miles to the southwest (Hoffmeister, 1986). Intensive collection efforts on the Kaibab Plateau yielded *Eumops* in August 1995 (Rabe, personal communication). Similarly, the expanse of water at Lees Ferry on the Colorado River (Location 16, Table 2) could not be adequately sampled yet cursory acoustic examination revealed eight species actively foraging over the area, including a first recordation of *Euderma maculatum* for this area despite collection efforts in August 1994 (Rabe, personal communication).

The database gathered during the present study represents a start towards a comprehensive library of vocal signatures. Further determination of diagnostic signal characteristics will be required. As a start, the maximum and minimum frequency of a vocal sequence should be examined, recognizing that both are subject to varying degrees of variation. Duration of a signal varies with activity but an average for a behavioral sequence may be important. Changes in shape, which incorporates bandwidth and duration, may be a most valuable tool particularly as defined by Kalko and Schnitzler (1993). The various aspects of signal characteristics need to be subjected to critical statistical analyses, which would permit a more rigorous examination of geographic and temporal differences. The present study used these general characteristics for distinguishing separate species but in a subjective manner. Actual quantification of specific features will only be meaningful when performed under stringent, controlled conditions. Time intensive study following individuals marked by light tags or radio transmitters will guarantee that all incoming calls are from the same source and provide some context in which to interpret the sounds. It will be important to know how the animal is oriented to the detector, approximate distance, and type of behavior. 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 imperative 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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APPENDIX I
CHECKLIST OF BATS KNOWN FROM NORTHERN ARIZONA

FAMILY/SPECIES	COMMON NAME	ABBREVIATION
Phyllostomidae		
<i>Macrotus californicus</i>	California Leaf-nosed Bat ^{*,+}	MAC CAL
Vespertilionidae		
<i>Myotis auriculus</i>	Southwestern Myotis [*]	MYO AUR
<i>Myotis californicus</i>	California Myotis	MYO CAL
<i>Myotis ciliolabrum</i>	Western Small-footed Myotis ⁺	MYO CIL
<i>Myotis evotis</i>	Long-eared Myotis ⁺	MYO EVO
<i>Myotis lucifugus carissima</i>	Little Brown Bat	MYO LUC
<i>Myotis lucifugus occultus</i>	Arizona Myotis ⁺	MYO LUC
<i>Myotis thysanodes</i>	Fringed Myotis ⁺	MYO THY
<i>Myotis volans</i>	Long-legged Myotis ⁺	MYO VOL
<i>Myotis yumanensis</i>	Yuma Myotis ⁺	MYO YUM
<i>Lasiurus blossevillii</i>	Western Red Bat ⁺	LAS BLO
<i>Lasiurus cinereus</i>	Hoary Bat	LAS CIN
<i>Lasionycteris noctivagans</i>	Silver-haired Bat	LAS NOC
<i>Pipistrellus hesperus</i>	Western Pipistrelle	PIP HES
<i>Eptesicus fuscus</i>	Big Brown Bat	EPT FUS
<i>Euderma maculatum</i>	Spotted Bat ^{*,+}	EUD MAC
<i>Plecotus townsendii pallescens</i>	Pale Western Big-eared Bat ⁺	PLE TOW
<i>Idionycteris phyllotis</i>	Allen's Big-eared Bat ⁺	IDI PHY
<i>Antrozous pallidus</i>	Pallid Bat	ANT PAL
Molossidae		
<i>Tadarida brasiliensis</i>	Brazilian Free-tailed Bat	TAD BRA
<i>Nyctinomops macrotis</i>	Big Free-tailed Bat ⁺	NYC MAC
<i>Eumops perotis</i>	Western Mastiff Bat ⁺	EUM PER

* State-listed Threatened (AGFD, 1988)

⁺ Federal Category 2 Candidate (USFWS 1994)

Nomenclature follows Jones et al. (1992).