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AN ELECTRONIC TECHNIQUE FOR MONITORING THE TEMPORAL ASPECTS OF ACOUSTIC SIGNALS OF CAPTIVE ORGANISMS

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ABSTRACT

We introduce an inexpensive electronic technique for monitoring the temporal aspects of any captive animal's acoustic signals. The electronic apparatus, attached to a data acquisition unit and personal computer, compares microphone output to a pre-set level and stores calling/non-calling data to disk. Total time calling and temporal signaling patterns of up to 256 individuals can be monitored for indefinite lengths of time. Sampling rate is adjustable, with a maximum rate of 6 samples/microphone/second. The capabilities of the system are illustrated with the field cricket *Gryllus integer*. Temporal aspects of acoustic signaling are discussed in terms of monitoring time scale and recognition of individual variation, energetics research, and hypothesis testing of the costs and benefits associated with mating success and predation.

Key words: temporal, calling, patterns, electronic, technique

INTRODUCTION

Animals often use acoustic signals to attract mates. Despite the important implications of the temporal aspects of acoustic signaling for energetics, predator avoidance and mating success, how individuals distribute their calls over time has received limited attention, in part because of the time required to track calling individuals in the field (e.g. French and Cade 1987,1989, Cade and Cade 1992, Runkle et al. 1994, Bertram et al. 1996). Therefore, researchers have turned to electronic devices to monitor captive individuals (Cade 1984, 1991, Kidder and Sakaluk 1989). However, these electronic techniques either assess total time calling but not its temporal distribution (e.g. Cade 1984, 1991), or they detect both aspects of calling but only monitor a few individuals at a time (e.g. Kidder and Sakaluk 1989). Here we introduce an efficient electronic device capable of monitoring the

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temporal call parameters of large numbers of captive individuals. We illustrate the capabilities of the system with the field cricket *Gryllus integer*, a well-studied acoustical species.

ELECTRONIC MONITORING APPARATUS

This device monitors the temporal calling pattern of acoustically signaling organisms by continually determining whether an animal is calling or not, and recording the information to a data file on the computer's hard-drive. The data can be used to determine the exact onset of acoustic signaling, the temporal pattern of calling behavior, and how much time an individual spends signaling over any time scale. Any organism that produces acoustic signals 10 dB louder than the background noise, in the frequency range of 20–16,000 Hz, and longer than 168 msec in duration can be monitored using this technique.

The sensing apparatus consists of a central printed circuit board (PC board) from which 32 wires lead to microphones (Electret condenser microphones) distributed among acoustically sound-muffled cages housing individual organisms. The sensing apparatus is attached to a data acquisition unit (DaqBook 120, IOTech, Cleveland, OH) and a personal computer (133 MHz Pentium Processor, 32 MB of RAM, 2.1 GB hard-drive). The data acquisition unit (programmed using C^{++}; Appendix 1) sends a specific signal to the PC board, resulting in the selection of a microphone. The selected microphone is turned on and reads the cage's noise level. The PC board then conditions the microphone's reading (Figure 1) by amplifying it, converting it from an AC to a DC signal, filtering it, then comparing it to a pre-set adjustable level. If the conditioned signal's level is greater than the pre-set level, a 1 representing calling is saved to the data file on the hard-drive. Otherwise, a 0 representing non-calling is saved. The microphone is then turned off and the next microphone in the series is selected via a change in the data acquisition unit's signal. Only one microphone per apparatus is turned on at a time. The process takes 5 msec per microphone, set by the PC board's ability to accurately compare the conditioned output of the microphone to the pre-set level. The sampling speed is adjustable but due to the minimum processing time has an upper limit of 6 samples/microphone/second.

Sixteen sensing devices can be connected allowing a total of 512 microphones to be monitored. When multiple systems are used microphones sharing the same number are monitored simultaneously. For example, microphone #5 from devices A and B are simultaneously selected and their respective conditioned outputs are stored sequentially in the data file on the computer's hard-drive. The sensing devices

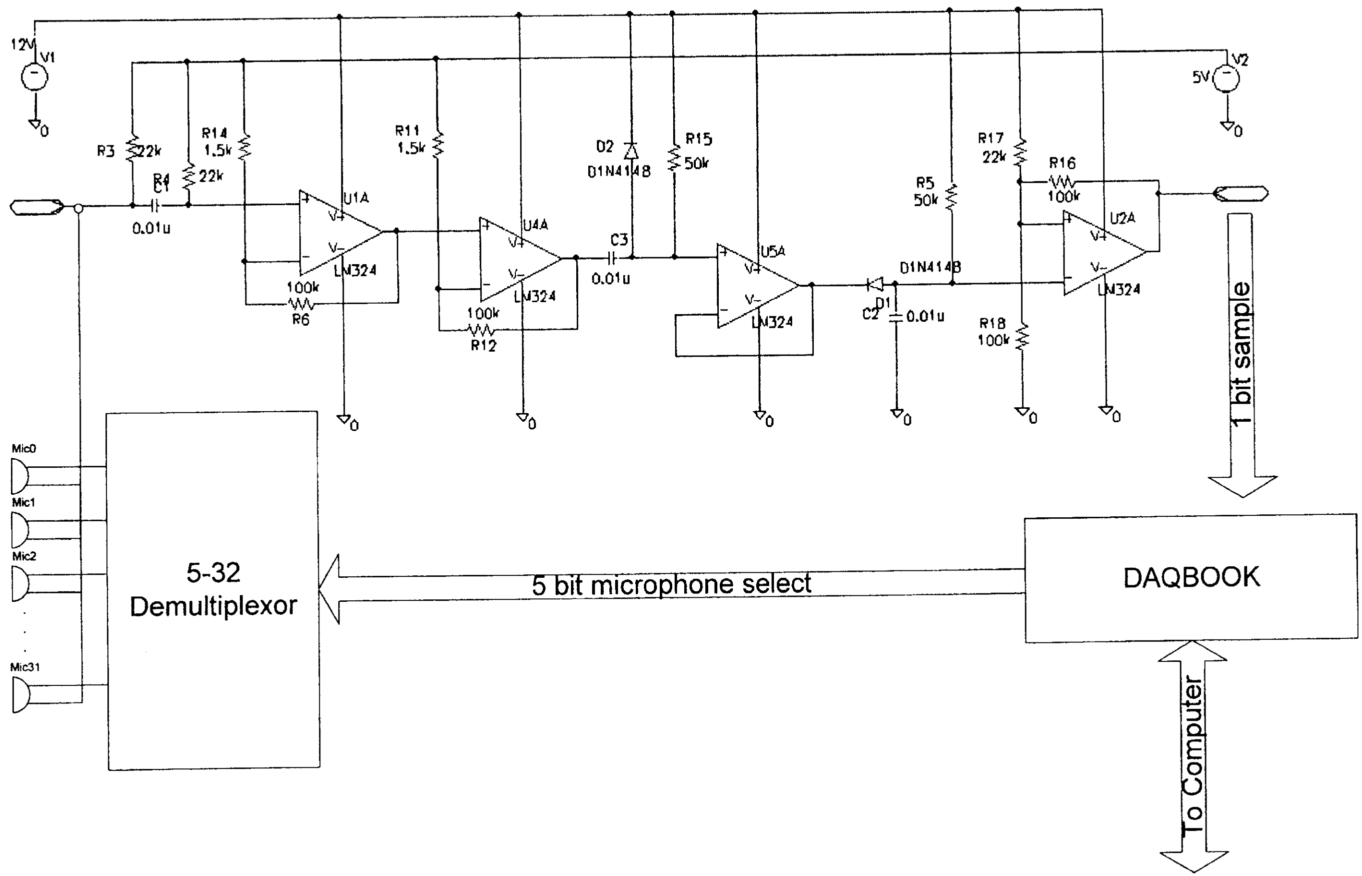

Figure 1. Schematic diagram of the sensing apparatus.

are powered by an independent 5 and 12 volt 80 watt source. The apparatus require a personal computer with at least a 33 MHz processor, a 800 MB hard-drive, and a printer port for the attachment of the data acquisition unit. Any data acquisition unit capable of outputting and receiving signals and writing to disk can be used.

Methodology

Thirty-two microphones, grouped into pairs, were used to monitor the calling behavior of sixteen male crickets. Microphones were attached to two electronic devices (1 microphone/pair attached to each apparatus) that recorded calling/non-calling sampled 6 times/second/ microphone. Microphone pairs were hung 3 cm from the top, inside glass containers (250 ml preserve sealer). The hole through which the microphones were hung was sealed by a rubber stopper drilled with air holes. Crickets were placed in their own container with food, water and shelter. Fresh food and water was provided every five days. Cricket calling was monitored over ten days using a 12 hour light/dark cycle.

The field cricket *Gryllus integer* is an ideal organism to illustrate the operation of this system. Males call in the laboratory, and previous work has shown males to vary in the amount of time they spend calling through a night (Cade 1991). *Gryllus integer* calls consist of a series of short pulses strung together into a trill. The calls have an average (SE) frequency of 5.28 (0.0065) kHz, pulses have an average duration of 450 (7.98) msec, and are followed by a silent interval of 164 (2.75) msec prior to the next pulse (n = 686; Bertram and Montoya, pers com). Call amplitude averages 76.5 (2.27) dB in the field (Cade 1979), thus falling within the sensing apparatus' frequency and amplitude specifications. Further, as the apparatus samples at a rate of 6 times/second, it counts all the pulses of a call, resulting in complete temporal calling patterns.

RESULTS

The acoustic monitoring apparatus is an accurate means of assessing time spent calling and signal partitioning. Data from each container's two microphones, summarized into one hour segments, were virtually identical in 150 of 160 cases (16 crickets × 10 days: regression showing average(SE): df = 23, p = 0.0001(5.959×10^{-5}), r_{adj} = 0.9416(0.0093), slope = 0.9923(0.0171), intercept = 0.111(0.0315)). In 10 cases, one of the two microphones' functioning was interrupted by a layer of dried feces, resulting in nonsignificant correlations. For all subsequent results, the presented data are from one microphone of the pair.

Individuals varied in the time they called per day. Males ranged from a mean calling time of 0 (0) hr to 2.66 (0.546) hr per 24 hr period (Table 1). Variation also occurred within individuals. Thirteen of the sixteen crickets failed to call on at least one night (Table 1), and individuals that called every night called more on some nights than on others. This variation was not dependent on date.

Animals that called for the same amount of time per night differed in the temporal distribution of their calls. For example, crickets 4 and 8 did not differ across the gross scale of total time spent calling through the 10 day monitoring period; however, they consistently partitioned their signals differently, calling for different lengths of time each day (Figure 2A) and at different times of the night (Figure 2B and 2C).

DISCUSSION

This apparatus efficiently tracks the onset of calling, temporal calling patterns, the amount of time called over set periods, as well as the cessation of calling. This apparatus does not, however, record the sounds produced by animals, so any other call parameters that may be of interest (inter-pulse interval, decibel level, dominant frequency or harmonics) require another device. Several hundred individuals may be independently monitored and their variation examined. However, as the apparatus only monitors the cage's sound level, the device is incapable of discriminating among individuals that are housed together. Field applications would be limited to organisms spatially separated to the extent that each microphone only picks up one individual's calls. The length of time that individuals are monitored is limited only by the size of the computer's hard-drive. However, as the data are 95% compressible a tremendous amount of data can be collected and stored. The problem caused by dried feces interfering with microphone function can be circumvented by hanging the microphones at the top of the cage and using a screen barrier to separate the subjects from the microphones. To ensure accuracy, however, we suggest two microphones be used for each individual monitored.

Our results indicate that individuals partition their calls differently and our technique provides an opportunity to investigate this variation at multiple scales. Subjects can be monitored for indefinite lengths of time, allowing the assessment of both individual variation and how signaling changes throughout the life of an individual. Variation in call partitioning can also be addressed at nightly, hourly and smaller scales. As numerous individuals can be monitored at once, our technique can be used to make heritability estimates of the temporal aspects of acoustic signaling. This research can play an

TABLE 1

Total time calling in hours for each of the 16 crickets over ten days. Crickets are in order from highest to lowest mean total time calling per day. *represents no correlation between the two microphones in the container. The time calling from one microphone per pair is shown.

	CRICKET															
DATE	4	8	2	3	16	7	5	10	12	1	15	6	14	11	13	9
22-Feb	0.47	2.52	1.55	0	0	0	0	0	0	0	0	0	0	0	0	0
23-Feb	5.68	3.47	3.87	1.33	1.08*	0.03	0.18	0.23	0	0.02	0	0	0	0	0	0
24-Feb	4.15	3.37	1.95	1.27	1.02*	0.07	0.35	0.28	0.32	0	0.07*	0	0	0	0	0
25-Feb	3.50	3.47	1.63	1.83	1.42*	0.22	1.03	0.90	0.03	0	0.18*	0	0.03	0.03	0	0
26-Feb	1.73	3.77	0.18	0.03	0.13*	1.25	0.62	0	0.32	0.12	0.1*	0	0	0	0	0
27-Feb	4.65	3.05	0.60	0.20	0.12	0.45	0.75	0.27	0.03	0.05	0	0.12	0.03	0	0.02	0
28-Feb	1.22	3.03	0.13	0.08	0.03*	0.17	0	0.47	0	0.22	0	0	0	0	0	0
1-Mar	1.37	1.00	0	0.17	0.08*	0.13	0	0	0	0.03	0	0	0	0	0	0
2-Mar	1.87	0.93	0.05	0.30	0.08	0.50	0	0.05	0.02	0.07	0	0	0	0	0	0
3-Mar	1.97	0.45	0	0.38	0.17*	0.30	0	0	0.15	0.13	0	0	0	0	0	0
avg	2.66	2.51	1.00	0.56	0.41	0.31	0.29	0.22	0.09	0.06	0.04	0.01	0.01	0	0	0
se	0.54	0.39	0.40	0.21	0.17	0.12	0.12	0.09	0.04	0.02	0.02	0.01	0	0	0	0

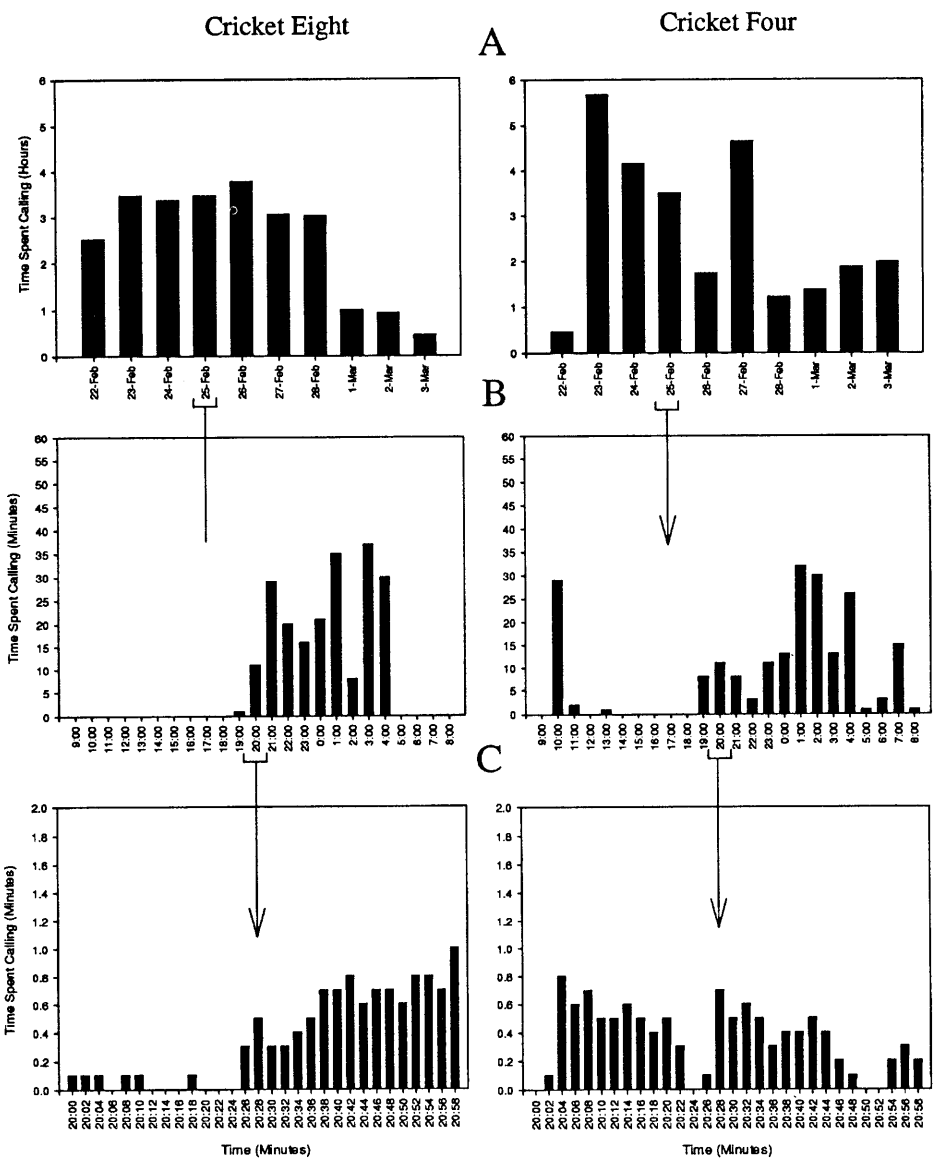

Figure 2. Comparison of calling by cricket 4 and cricket 8, two crickets that did not differ in the time spent calling over the course of the 10 day monitoring period: A) the number of hours each individual called per day; B) the number of minutes each individual called per hour on February 25, 1997, a day that both crickets had the same total time spent calling; C) the number of minutes each individual called over the hour 20:00–21:00 on February 25, 1997.

important role in testing predictions about the costs and benefits of different calling patterns.

Calling individuals often take signaling breaks (Schwartz 1991, Schwartz et al. 1995, Bertram et al. 1996), a behavior which appears to reduce mating success. Breaks may result from the high energy demands of signaling, as anurans and insects are known to have costly advertisement calls (Prestwich 1994). Our electronic technique could be easily coupled with carbon dioxide and oxygen analysers to determine the energetic costs of calling on a second by second basis. The results could be correlated with signal partitioning to aid in our knowledge of why individuals take seemingly costly signaling breaks.

Understanding how organisms partition their acoustic signals also can increase our knowledge of how selection pressures influence calling. Total calling time is positively correlated with mating success in many species (e.g. Cade 1979, Green 1990, Bertram et al. 1996). However, female preference is not based solely on total time of acoustic stimulation (Gerhardt et al. 1996). Correlating signal partitioning with data on the temporal patterns of female receptivity may help us enhance our understanding of female choice. For example, of the two crickets that called most often, cricket 4 called at dawn while cricket 8 was silent. Cricket 4 may have a higher probability of mating because he signals during the time that most female's initiate copulation (Cade 1979). Correlating signal partitioning with data on when acoustically orienting predators and parasites attack also may enhance our knowledge of calling costs. For example, crickets that call at dusk may have elevated signaling costs due to the presence of tachinid flies (Cade et al. 1996). These hypotheses can be directly tested using our technique.

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APPENDIX 1. The software, programmed in C++, which runs the data acquisition unit opening the file on the hard drive, sending out 5 bit binary signals, and receiving the 1 bit binary signals and saving them to disk.

```
/* tsc.c */
/* Time Spent Calling driver software */
/* by Luke A. Johnson */
/* Requires: */
/*      DaqBook*/
/*      TSC board (connected to P2) */
/*      P3:pin30 connected to P3:pinl (OSC out to Int in) */
/*      P3:pin2 connected to ground(pin 11) (IR Enable) */

#include <stdio.h>
#include <dos.h>
#include <conio.h>
#include <stdlib.h>
#include "daqbook.h"

unsigned char config, CurMic=3 1, Sample;
unsigned long LineCount = 0;
int i=0;
void _far _pascal     handler(int error_code);

void
main(void)
{
      char filename[20];
      FILE *outfile;
      struct time t;
      struct date d;
      gettime(&t);
      getdate(&d);

      daqSetErrHandler(handler);
      daqInit(LPT1, 7);

      /* Initialize Digital Ports */
      /* Port A: Output - Microphone select
         Port B: Input - Microphone sample
         Port C: Input - Unused */
      daqDigGetConf(0,1,1,1, &config);

      daqDigConf(DdcLocal, config);
      daqDigWtByte(DdpLocalA,0x00);

      /* Initialize OSC out to 100Hz Clock */
      daqCtrSetMasterMode(5,DcsF4,0,0,DtodDisabled);
```

```
sprintf(filename,"S%02d%02d.tsc",d.da_mon,d.da_day);

outfile = fopen(filename, "w+");
printf("%s\n\n ",filename);
fprintf(outfile,"%2d:%02d:%02d %d/%d/%d\n\n",
     t.ti_hour, t.ti_min, t.ti_sec,d.da_mon,d.da_dayd.da_year);
printf("Time Spent Calling data file\nstarted %2d:%02d:%02d
%d/%d/%d\n\n",
     t.ti_hour, t.ti_min, t.ti_sec,d.da_mon,d.da_day,d.da_year);
fprintf(outfile, "\nSample  00 00 00 00 00 00 00 00 00 00 11 11 11 11
11 11 11 11 11 11 22 22 22 22 22 22 22 22 22 33 33\n");
fprintf(outfile,"Number  00 11 22 33 44 55 66 77 88 99 00 11 22 33 44
55 66 77 88 99 00 11 22 33 44 55 66 77 88 99 00 1 1\n");
do {
     /* Select next microphone */
     if (++CurMic > 31) {
          CurMic = 0;
          fprintf(outfile,"\n%08d: ",++LineCount);
          printf("\n%08d : ",LineCount);
/* Extra cycle wait to filter out 31-0 swithing noise */
     daqDigWtByte(DdpLocalA,CurMic);
     /* Wait for external interrupt */
     /* Sample no counters once based on external interrupt */
     daqCtrRdNFore(0,0,0,0,0,1);
     }
     daqDigWtByte(DdpLocalA,CurMic);

     /* Wait for external interrupt */
     /* Sample no counters once based on external interrupt */
     daqCtrRdNFore(0,0,0,0,0,1);

     /* Read Microphone */
     daqDigRdByte(DdpLocalB, &Sample);
     if (Sample<0x80){
          fprintf (outfile, "1",Sample);
          printf ("1",Sample);
     } else {
          fprintf (outfile,"0",Sample);
          printf ("0",Sample);

          Sample -= 0x80;
     }
     if (Sample<0x40){
          fprintf (outfile, "1",Sample);
          printf ("1",Sample);
     } else {
          fprintf (outfile,"0",Sample);
          printf ("0",Sample);
          Sample -= 0x40;
     }
} while (kbhit()==0 || CurMic<31);
```

```
    gettime(&t);
    getdate(&d);
    fprintf(outfile,"\n\nEnded %2d:%02d:%02d %d/%d/%d\n\n",
       t.ti_hour, t.ti_min, t.ti_sec,d.da_mon,d.da_day,d.da_year);
    printf("\n\nEnded %2d:%02d:%02d %d/%d/%d\n\n",
       t.ti_hour, t.ti_min, t.ti_sec,d.da_mon,d.da_day,d.da_year);
    fclose(outfile);
    daqClose();
}
    void _far _pascal handler(int error_code)
    {
       printf("\n\n DaqBook Error: 0x%x\n\n",error_code);
       exit(l);
    }
```