

AN ABSTRACT OF THE THESIS OF

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Title: THE ROLE OF ACOUSTICAL SIGNALS IN THE COMMUNICA-
TIVE BEHAVIOR OF THE WATER SCAVENGER BEETLES,
TROPISTERNUS (COLEOPTERA: HYDROPHILIDAE)
Abstract approved: Redacted for Privacy
William P. Nagel

The communicative behavior of five species and subspecies of Tropisternus Solier (Coleoptera: Hydrophilidae) was studied. These aquatic beetles have stress and calling chirps (both sexes), a second male calling signal (one species), a single male courtship sound (usually), a male copulatory sound, and a female aggressive rejection buzz (three species). Males utilize visual, not acoustical, clues to orient towards females at close range. Males court females with stereotyped movements, sounds, and tactile signals while positioned dorsally on the female. The role of chemical signals in recognition of sexual readiness, sex, or species remains unclear.

A mark-release field study of T. ellipticus ruled out aggregation and territorial behavior as possible functions of acoustic signals. This species, at least, wanders freely and has no home range.

Acoustic courtship signals were stereotyped, i. e., the same signal was always produced by males in particular positions of

courtship, in T. natator and T. columbianus, but variable in T. ellipticus and two subspecies of T. lateralis.

Experiments using models of beetles indicated that visual stimuli can release a calling chirp response and an approach in both sexes, or a courtship sound and an approach in males. Results of experiments utilizing broadcasts of tape recorded signals established that Tropisternus can hear water-borne acoustic signals even though auditory receptors are as yet unknown in Coleoptera. Males of T. natator and T. lateralis nimbatus discriminated female calling chirps from other acoustic signals. Female calling chirps stimulated males to emit calling chirps, to cease feeding and begin swimming, and to approach models of beetles (searching behavior).

Males of T. ellipticus and T. lateralis limbalis, species from populations in western Oregon, did not respond predictably to acoustic signals. T. natator and T. lateralis nimbatus, which responded to calling chirps, are from populations in southern Michigan, where four species of Tropisternus share breeding sites. In contrast, the three species in western Oregon probably breed in different microhabitats. The lack of mating interference between species in western Oregon may have allowed the loosening of stereotypy in courtship seen in T. ellipticus and the degeneration of the calling function of acoustic signals seen in T. ellipticus and T. lateralis limbalis.

The Role of Acoustical Signals in the Communicative
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Tropisternus (Coleoptera: Hydrophilidae)

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THE ROLE OF ACOUSTICAL SIGNALS IN THE COMMUNICATIVE
BEHAVIOR OF THE WATER SCAVENGER BEETLES,
TROPISTERNUS (COLEOPTERA : HYDROPHILIDAE)

I. INTRODUCTION

Recently, Mayr (1974) discussed the importance of natural selection theory and comparative studies in explaining animal behavior, and suggested that all behavior should not be treated typologically, but should be classified as either communicative or non-communicative. He also suggested that communicative behavior leading to and including courtship and mating is shaped by stabilizing selection into stereotyped or "ritualized" signal patterns (Tinbergen, 1952).

The present study is an analysis of communication in several species of water beetles in the Genus Tropisternus Solier. These beetles have a conspicuous acoustical component in their communication system, and many of the sound emissions are closely tied to courtship and mating (Ryker, 1972). This study attempts to provide insights into the biological meaning of sound production in Tropisternus, the relative role of visual, tactile, acoustical and chemical signals, the generalization of signal stereotypy, and the relative complexity of communication in Coleoptera, as compared to other insect groups.

Insect Communication: Signals and Functions

Animal communication as usually defined includes: 1) the transfer of information between organisms by some signaling mode, i. e., light, sound or vibration, or chemical. However, 2) evidence of a response by the signal recipient is necessary to demonstrate communication. Also, 3) mechanisms of signal production and reception are adaptations resulting from natural selection (Frings and Frings, 1964; Marler, 1968; Lloyd, 1971; Otte, 1974).

Signals are the units of communication. Therefore, rigorous application of the term signal requires that the behavior being described must stimulate the organism to release a demonstrable response and must have some element of specialization for the signal function. Thus defined, sounds, movements, or odors that transmit information incidental to their normal functions (chewing sounds, movements, and odors, for example) do not fall within the bounds of the definition. The majority of sounds reported from insects have been described as signals. However, the demonstrations of specific responses to these sounds by the insects are usually lacking.

If there is no explanation of the usefulness or the adaptive value, i. e., the function of the sounds, it is speculative to say that they are signals. However, as Smith (1968) points out, a basic "postulate of zoosemiotics should be that ritualization will not act to

produce functionless displays." Only the preference of the investigator determines whether he will study the behavior of the signaler and the behavioral contexts of its "signals" to categorize them, or whether he will turn to the more difficult task of demonstrating specific responses in the signal recipient. Also, it is easier to define signal meanings operationally by describing responses in the recipient than it is to attempt to ascribe functions to a signal by deciding how the signal-response interaction helps the animal meet its ecological and social needs (Smith, 1968). Thus, there are three levels of explanation being considered in insect communication: 1) descriptions of signals within specific behavioral contexts, 2) experimental demonstrations of specific responses to signals, and 3) assignment of functions within the framework of natural selection.

Two unambiguous methods of measuring responses to acoustic signals are by phonotaxis and by phonoresponse (Busnel, 1963). Phonotaxis is a directed motor response in which the responding organism orients and moves towards the source of the acoustic signal. A phonoresponse is a motor response in which the response to a signal is sound production by the responding organism. Both methods (but mostly phonoresponses) were used in this study.

Since 1950, considerable research has demonstrated that acoustic signals are important in the mating behavior of grasshoppers (Jacobs, 1953; Haskell, 1958, 1961; Perdeck, 1958; Willey and

Willey, 1969; Otte, 1970, in press), crickets (Alexander, 1957, 1961; Walker, 1957, 1973a, b), katydids (Busnel and Dumortier, 1954; Busnel, Dumortier and Pasquinelly, 1955; Alexander, 1960; Shaw, 1968), and cicadas (Alexander and Moore, 1958, 1962).

In attempting to interrelate these researches, Alexander (1967, 1968) has defined nine categories of life functions for arthropod acoustic signals, of which some common ones are calling, courtship, copulatory, aggressive, and disturbance (= stress) sounds. Calling sounds are those produced by either sex, often by isolated individuals (cricket chirps, for example), that result in the male and female coming together for mating. Courtship sounds operate after the male and female are together and are involved in recognition, stimulation and coordination of mating. Copulatory sounds occur during copulation and presumably facilitate insemination and pair-maintenance. Aggressive sounds are those that act to repel individuals (Otte, 1970) and are often associated with fighting. Stress sounds are those produced when the insect is handled or otherwise disturbed, and may have predator-avoidance or conspecific warning functions.

Sound Production in Coleoptera

Almost 100 years ago, Charles Darwin (1881) discussed the phenomenon of sound production by stridulation (friction of one specialized body part against another) in Coleoptera and suggested

that it might be important in sexual behavior. Gahan (1900) and Arrow (1904) enumerated a great many families and genera of Coleoptera presumed to produce sounds on the basis of having stridulatory organs. In 1942, Arrow summed up the information on sound production in Coleoptera and stated that no evidence existed to support Darwin's suggestion that stridulation might have sexual functions.

In the last 15 years, aggressive and/or courtship sounds have been described for beetles of the families Cerambycidae (Michelsen, 1966), Trogidae (Alexander, Moore and Woodruff, 1963), Passalidae (Schuster and Schuster, 1971), Scolytidae (Wilkinson et al., 1967; Rudinsky, 1968, 1969, 1973; Barr, 1969; Rudinsky and Michael, 1972, 1973; Michael and Rudinsky, 1972; Rudinsky et al., 1974; Libbey et al., 1974), and Hydrophilidae (Van Tassell, 1965; Ryker, 1972). The calling signals of beetles are most commonly chemical signals called pheromones (Karlson and Butenandt, 1959; Schneider, 1966; Wood, 1970). Several authors have suggested that acoustic signals in Coleoptera are generally limited to low amplitude, short range sounds, and that longer range, calling signals represent a more recent evolutionary advance in Orthoptera and Cicadidae, one not obtained in most Coleoptera (Alexander, Moore and Woodruff, 1963; Alexander, 1967, in press).

Possible acoustic calling signals have been reported in Trox suberosus Fabricious (Trogidae) (Alexander, Moore and Woodruff,

1963), and several species of Tropisternus (Ryker, 1972). Males and females of the plum curculio, Conotrachelus nenuphar (Herbst) (Curculionidae), were shown experimentally to move to cages containing stridulating beetles of the opposite sex, but not to cages containing silenced beetles (Mampe and Neunzig, 1966). How this species uses its acoustic signals to facilitate mate-finding in nature is unexplained, however.

Sound Production in Aquatic Insects

Sound production has been noted in many families of aquatic Heteroptera--Corixidae, Naucoridae, Nepidae, and Notonectidae--and aquatic Coleoptera--Amphizoidae, Dytiscidae, Haliplidae, and Hydrophilidae (Frings and Frings, 1960). Wilcox (1970) showed that Notonectidae of the Genus Buenoa use a combination of visual and acoustical signals in calling and courtship situations.

Jansson (1973) showed experimentally that Corixidae of the Genus Cenocorixa use acoustical signals in calling and aggressive situations. Cenocorixa are able to orient to and locate the source of broadcast sounds underwater. Females will respond to the calling sounds of conspecific males, but not to other signals. Males answer and approach almost any stridulatory source, copulating with sexually receptive females, and nudging and chasing other males.

Van Tassell (1965) analyzed stridulations of 12 species of aquatic beetles of the Genus Berosus (Hydrophilidae), and showed that both sexes produce stress sounds. Premating sounds (presumably courtship) were recorded in four species. Ryker (1972) studied the Genus Tropisternus in the same family and described male and female stress chirps and calling sounds, and male courtship sounds. No experimental verification of function was provided for the calling sounds, which were named on the basis of context alone.

Research Objectives

This study had five general objectives:

1. Describe the patterns of communicative behavior of the three common species of Tropisternus in western Oregon, particularly comparing the acoustical signals associated with mating.
2. Using representative species, determine the respective roles of acoustical, visual, tactile, and chemical signals in the mating behavior of Tropisternus.
3. Experimentally determine the stimuli that release the acoustical signals of a representative species of Tropisternus.
4. Experimentally determine the typical responses as well as the range and variability of responses to each acoustical signal of a representative species of Tropisternus.

5. Using the available evidence from associating signals and responses, describe the life functions of acoustical signals of a representative species of Tropisternus.

II. MATERIALS AND METHODS

Laboratory Studies

Live beetles were maintained either singly or in small groups in pint jars with screen lids. A small rock, aquatic plants, and tap water were kept in the jars, and crumbs of dried dog food were added every several days (Young, 1958). The jars were scrubbed out with a brush once a week. A combination of incandescent and fluorescent light was used with various light cycles. In 1974, holding jars were placed outdoors in water tables 1.2 x 1.2 m and 10 cm deep. Cold tap water was slowly run through the tables to maintain jar temperatures below 31°C in the midday sun.

Larvae of Tropisternus were reared in small paper cups or baby food jars, and vegetation or asbestos screening allowed the larvae access to the water surface for breathing and feeding. First instar beetle larvae were hand fed with mosquito wigglers. Older instar larvae were either hand fed with mosquito wigglers or provided with rattailed maggots (Syrphidae, Eristalis spp.). Containers of moist sand were used for pupation (Young, 1958).

Adult beetles were photographed in positions of courtship and copulation with a Besler Topcon D-1, 35 mm camera, and a 16 mm Bolex H-16 motion picture camera using a zoom lens, film speed of 24 frames/second and Tri-X Reversal film.

A plexiglas chamber (Fig. 1a) was used for all experiments involving visual and acoustical stimuli. The chamber floor and one of the two side walls were covered with two layers of asbestos screening oriented at 45° to each other to provide walking surfaces for the beetles and to minimize sound reflection. One end of the chamber was used for tests and observations and separated by two screens from the other end, which was filled with aquatic plants to dampen sound reflection from the end wall. The microphone and speaker were placed in the center space between the two screens.

Males and females of Tropisternus were silenced by covering their plectra with a coating of glue. For tests of the effects of chemical signals, silenced beetles were housed in a small isolation chamber (Fig. 1b), while another beetle was being tested with visual and auditory stimulation. A double layer of asbestos screening covered the isolation chamber, which was constructed from a plastic pill vial by cutting the sides out, and obscured (visually) the beetle within the small chamber. However, the free flow of water through the screening permitted chemical stimuli released by the silenced, isolated beetle to reach the beetle in the larger chamber. Aquatic plants and dog food were provided for the isolated beetle held within the small chamber, and a pebble at the bottom provided ballast, allowing the cage to remain upright as it hung from the water surface.

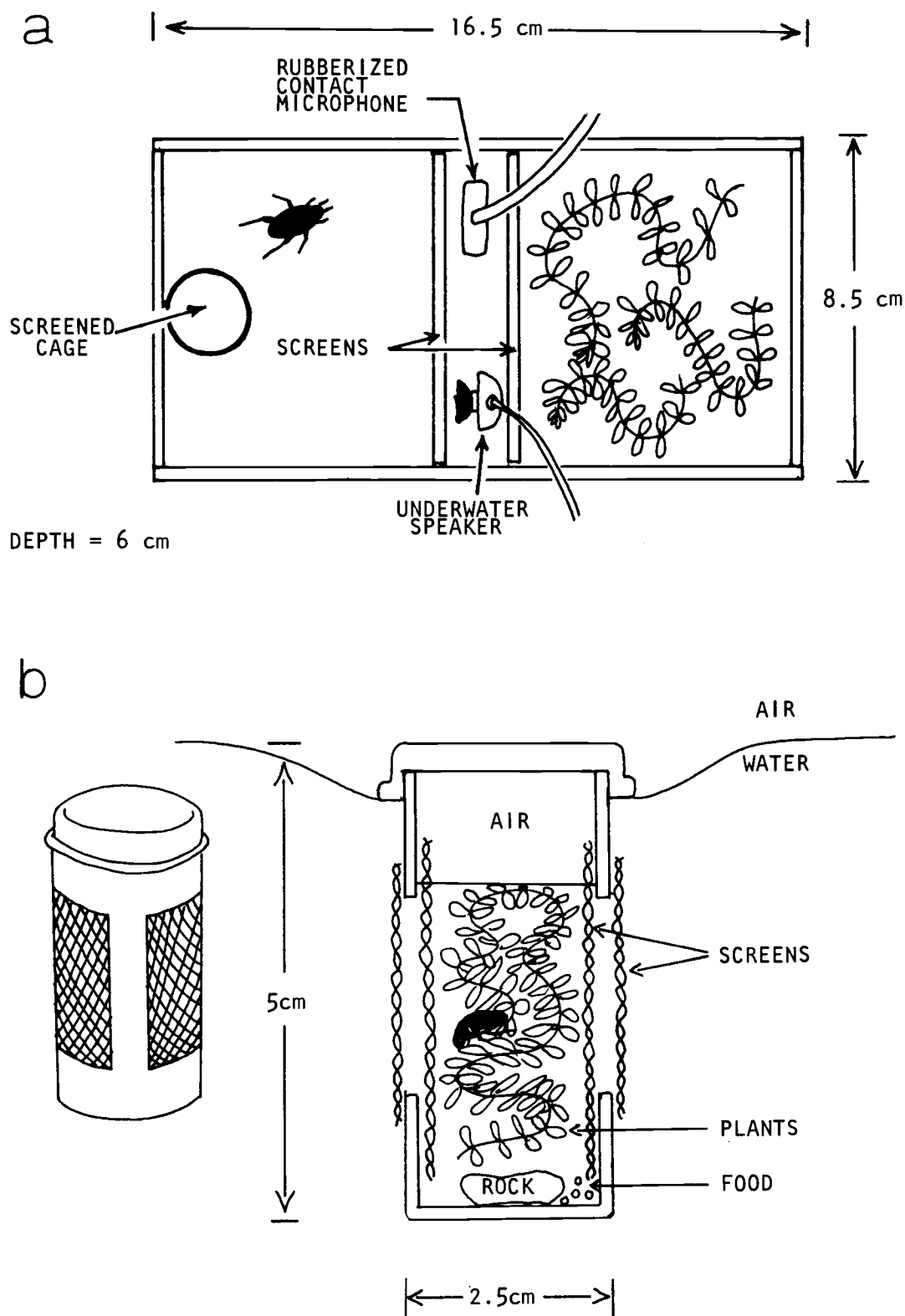


Figure 1. Experimental chambers. a) plexiglas chamber, top view; b) screened cage in which silenced males or females were placed.

Sound Recording, Playback, and Analysis

Sounds were recorded with a Uher 4000L portable tape recorder at a tape speed of 19 cm/second (7.5 ips), using either a Sennhauser MD405S microphone made waterproof with a condom and dipped about 6 mm into the water, or a clip-on contact microphone for guitars, coated with rubber, and completely immersed in the water (Kellogg, 1960). Playback for analysis and broadcasting sounds underwater was also with a Uher 4000L. The measured frequency response for the recording Uher was 50-10,000 Hz (\pm 3 db).

The structure of stridulatory sounds of Tropisternus was examined oscillographically, and most measurements of sound parameters were taken from sonographs. Oscillographs were recorded by Polaroid photographs of sounds displayed on a Tektronix Type 547 dual beam oscilloscope. Sounds were also examined on a storage oscilloscope, Tektronix Model 5103N. Sonographs were made with a Kay Sona-Graph 6061A, 85-8000 Hz Spectrum Analyzer, using the Wide Band Filter for precision in timing parameters of sounds. The Sona-Graph voltage unit level was kept at -7 or below during both Record and Reproduce phases of analysis.

Tape recorded sounds were played back on a Uher and broadcast through a Stenorette earphone modified as an underwater speaker by coating it with rubber. Sonographs of beetle sounds broadcast under

water through the speaker and re-recorded with a second Uher showed a rather narrow band of frequencies at 4-5 kHz (Fig. 2a, left). Sounds of live beetles, however, show frequencies both higher and lower than this. The exoskeleton of an adult Tropisternus was glued over the speaker opening to broaden the frequency output. When a sound pattern was played through the speaker, the sound vibrations excited the beetle exoskeleton, which vibrated to emit a broader band of frequencies (Fig. 2a, right).

The analysis of chirp structure and the pulse structure of other types of stridulations (buzzes, ticks, etc.) were examined to determine which parameters were important or possible to measure. Analysis of a chirp of a columbianus female by making sonographs at slower and slower recorder input speeds shows an apparent pulse structure (Fig. 2b-d). Close examination of the "pulses" shows that they are not unitary. The multiple nature of pulses within chirps is general, found in chirps of both sexes of all species of Tropisternus examined.

Oscillographic analyses of buzzes (Figs. 3a-d, 4c-d) and chirps (Figs. 3e-h, 4a-b, e-g) also show complex structure within and between pulses. Pulses within buzzes contain four or more spikes (Figs. 3d, 4d). Many lower amplitude spikes fill the spaces between the apparent pulses in the chirp of limbalis (Fig. 4b). Analysis of the fine structure of stress chirps of a male (Fig. 3h)

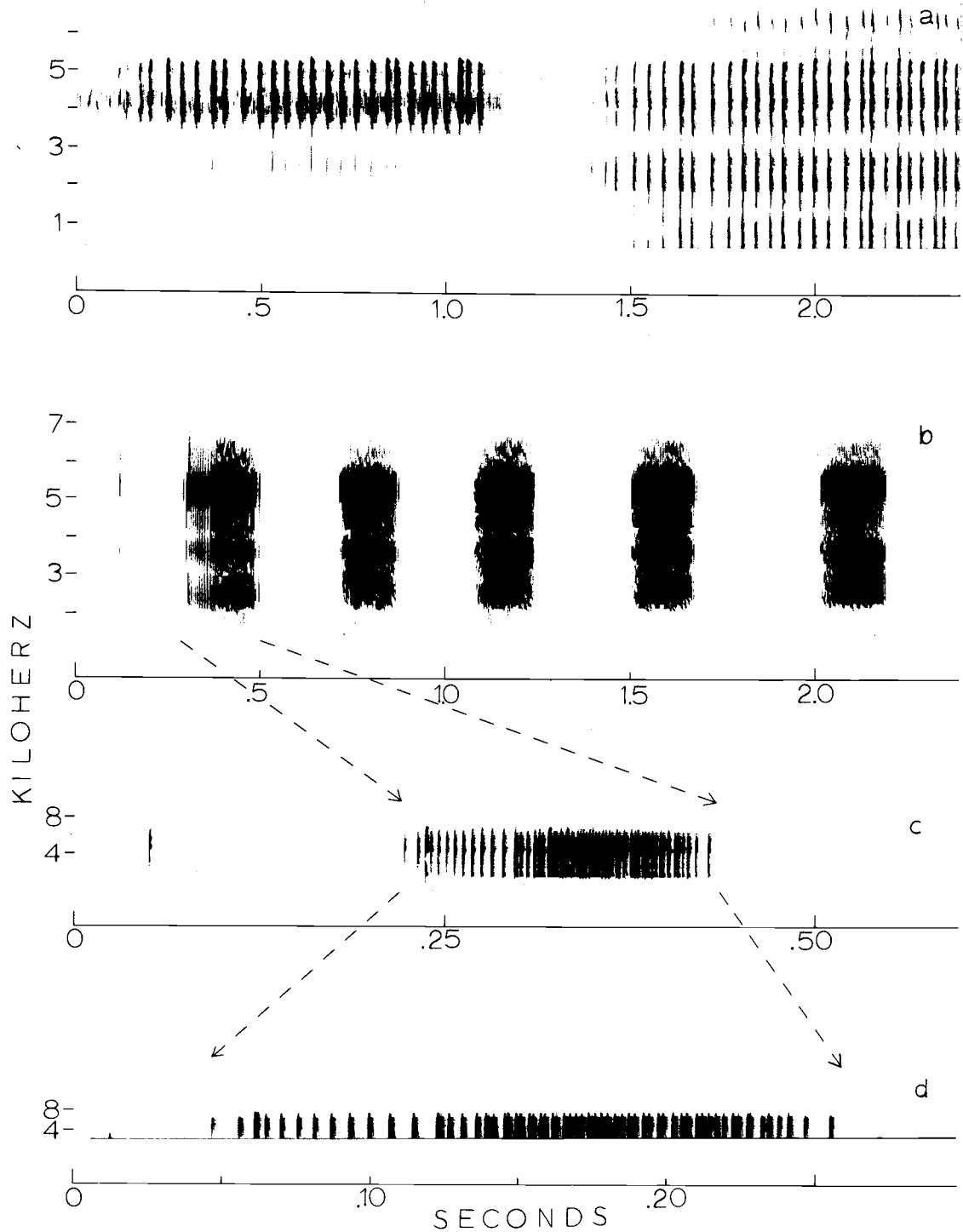
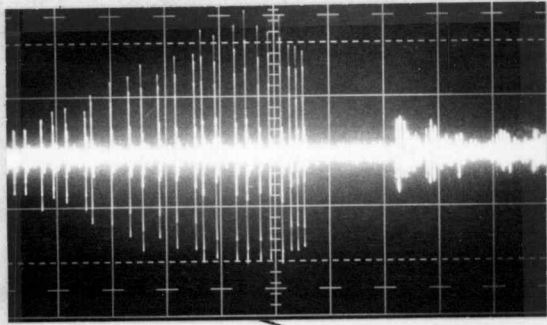
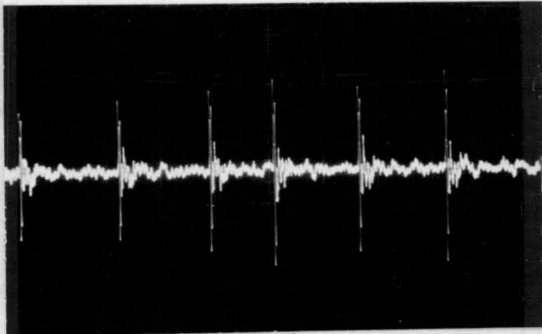


Figure 2. Sonographs illustrating structure of Tropisternus stridulations: a) re-recording of tape recorded buzzes broadcast underwater through speaker only (left) and with beetle exoskeleton glued on (right); b-d) detailed analysis of one stress chirp of Tropisternus columbianus female showing that pulses are complex (bottom).

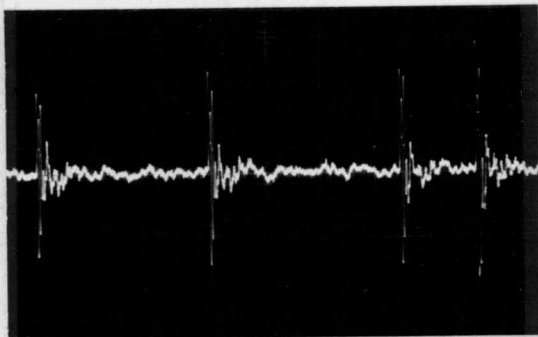
Figure 3. Oscillographs illustrating structure of Tropisternus stridulations. Buzz of T. ellipticus; abscissa time scale a) 1 sec., b) .2 sec, c) .1 sec., d) .02 sec. Stress chirp of male of ellipticus; e) 1 sec., f) .5 sec., g) .2 sec., h) .02 sec.



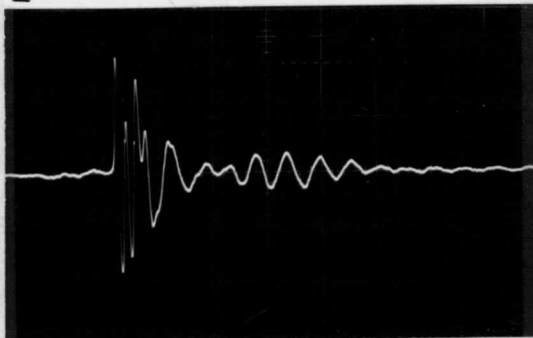
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b

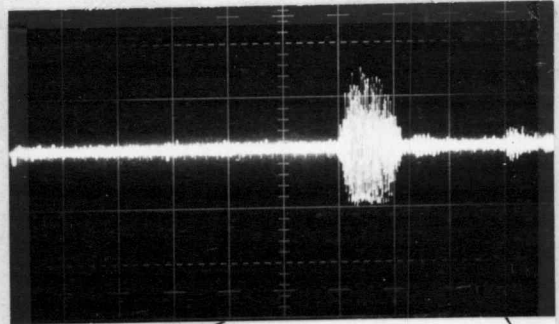


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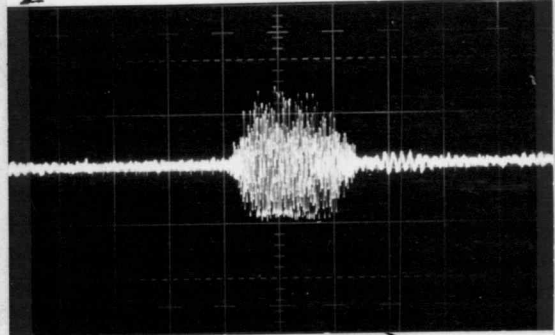


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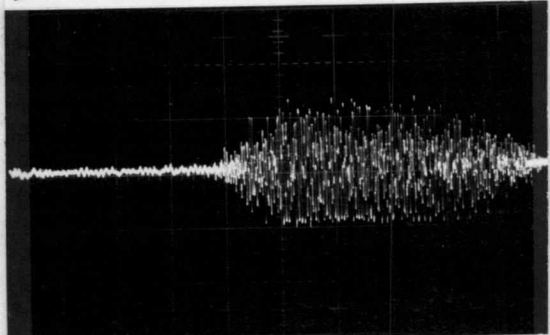
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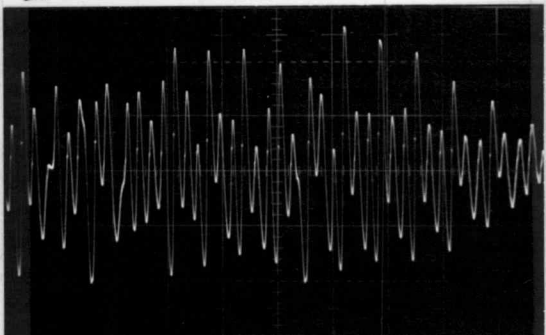
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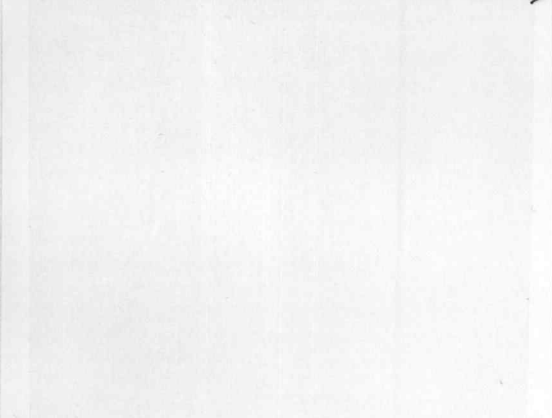
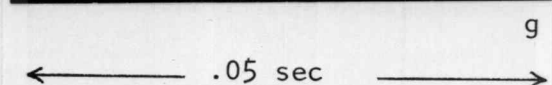
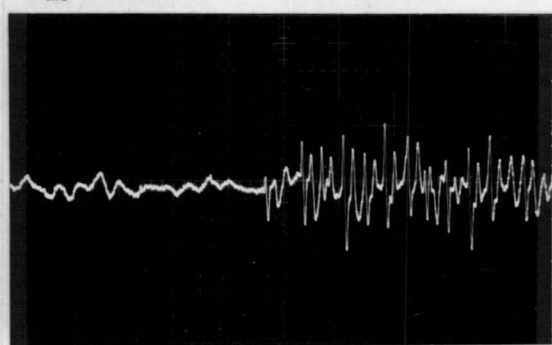
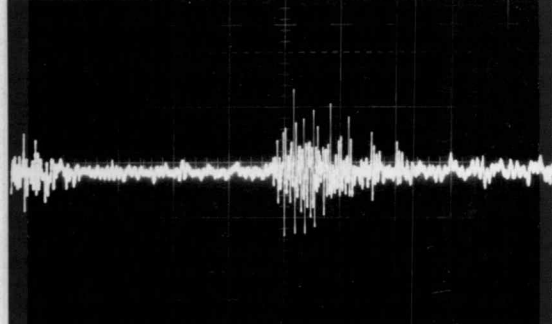
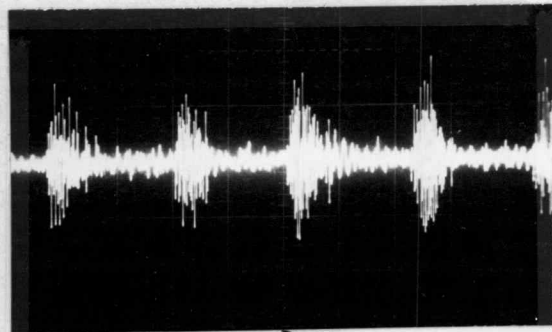
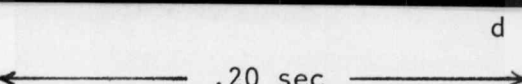
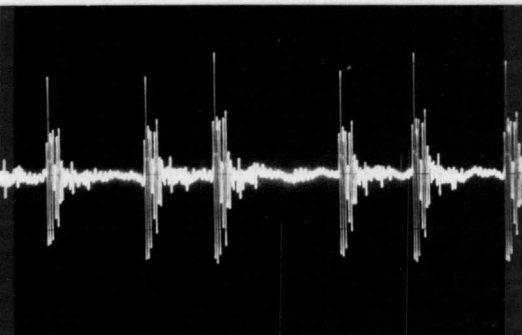
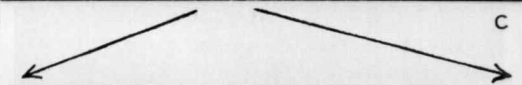
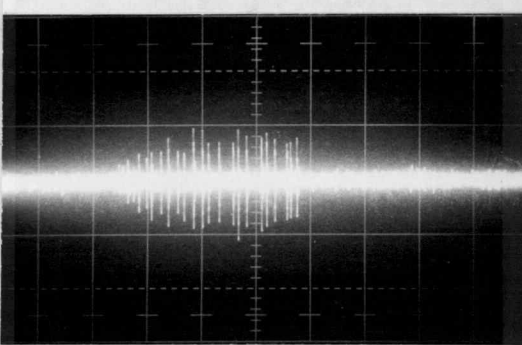
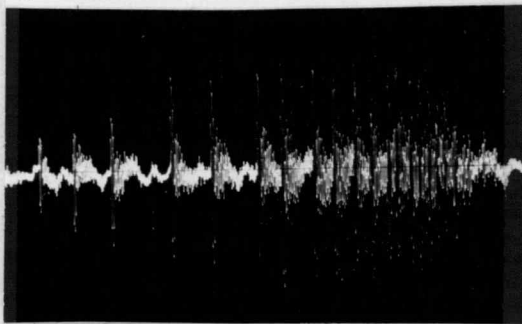
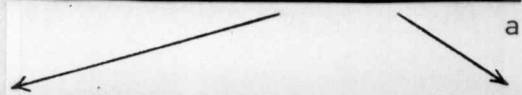
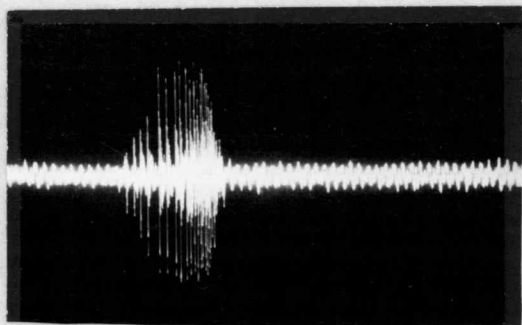
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Figure 4. Oscillographs illustrating structure of Tropisternus stridulations. Chirp of T. limbalis; abscissa time scale a) 1 sec., b) .2 sec. Buzz of ellipticus; c) 2 sec., d) .2 sec. Stress chirp of female of ellipticus; e) .5 sec., f) .2 sec., g) .05 sec.



and female (Fig. 4g) of ellipticus shows that the pulses are so close that they overlap, with no space in between. Upward and downward movements of spikes begin before the preceding spike has completed its up and down circuit, giving the spike pattern a zig-zag appearance. The overlapping pulse structure of chirps of Tropisternus is to be expected because of the thousands of engaging structures in the stridulatory surfaces (Ryker, 1972 and see Ch. V.). Because pulses cannot be counted within chirps, and because it is improbable that the beetles are able to extract pulse information that the oscilloscope cannot, only durations of chirps were measured. Pulse rates were not estimated for chirps, but the complex "pulses" of buzzes were measured, operating on the assumption that the group of spikes within each apparent pulse was heard as a single unit by the beetles.

A Uher was operated at 2.38 cm/second with .5 mil audiotape to record the diel sound activity of three species of Tropisternus. Individual beetles were recorded using the rubberized contact microphone in a pint holding jar. The jars were placed either in a small, sound absorbing chamber with a floodlamp shining down through a plexiglas window on top, or outdoors in a water table. In 1974, the Uher was plugged into a timing clock, which turned it on to record 15 minute sound activity samples each hour. For analysis, the tapes were played back at eight times the recording speed, and chirps were

counted with the aid of an oscilloscope. Chirps and buzzes make easily recognizable patterns on the screen, which correlate with the characteristic sounds.

III. GENERAL BIOLOGY AND BEHAVIOR

Beetles of the Genus Tropisternus occur throughout the Western Hemisphere, typically in quiet water habitats. They are generally 8-11 mm long, have a metasternal keel extended posteriorad into a sharp point below the abdomen, and produce stridulatory sounds when handled (Spangler, 1960; Ryker, 1972). Although the larvae are predaceous on small aquatic insects, the adults graze and scavenge under water, consuming algae, detritus, and animal remains (Young, 1958).

Due to the close relationship between sound production and mating, several aspects of reproduction were studied, viz, copulation, clutch size and timing of egg laying. Because of the desirability of obtaining virgin females to allow the study of communicative signals and responses related to mating, I reared the three western Oregon species in the laboratory. Developmental time is presented for the egg, three larval instars, and pupa under two sets of environmental conditions. Mating behavior, diel periodicity of stridulatory activity, territoriality and home range, and dispersal were also studied to gain insight into the adaptive value of sound communication in this genus.

Life Cycle

Development of Tropisternus includes the egg stage, three larval instars and a quiescent pupa. The females of the three western Oregon species studied lay an average of 13-14 eggs (Table 1) in a silken case. The female constructs the case just beneath the water surface, using a pair of terminal spinnerets. Egg laying and spinning have been observed both day and night, with the female fastening the case to floating or emergent vegetation.

Table 1. Mean and range of clutch sizes of three western Oregon species of Tropisternus.

Species	No. eggs		Range	No. clutches sampled
	\bar{x}	\pm SE		
<u>columbianus</u>	13.5	\pm .39	7-19	62
<u>l. limbalis</u>	12.7	\pm .43	7-17	35
<u>ellipticus</u>	14.0	\pm .52	10-18	24

The precaceous first instar larvae develop within five to seven days and remain an additional day within the silken case with no sign of cannibalism. Within a day after emergence, the larvae begin feeding on small aquatic invertebrates. Hunting behavior does not seem to depend on visual cues; stimulation of the sensory bristles of a larva elicits a rapid lunge and closure of its mandibles. The larva holds the prey above the water surface for mastication, and discards the exoskeletal remnants.

The results of the laboratory rearing of the Oregon species at 22°C (16L/8D light cycle) is summarized in Table 2. One Culiseta spp. mosquito larva was fed by hand per day (Lab. conditions A) or at about 12.5°C (dark) to 31°C (light)(16L/8D) with five mosquito larvae available per day as food for 1st instar larvae and five Eristalis spp. rattail maggots per day for 2nd and 3rd instar larvae (Lab. conditions B, T. ellipticus only). At 22°C and with limited food supply, developmental time is about 56 days for T. columbianus, 59 days for T. limbalis, and 68 days for ellipticus. Most of the growth takes place in the 3rd stadium, which is correspondingly longer in duration. By increasing the daytime temperatures and decreasing nighttime temperatures to approximate those measured in the breeding pools for ellipticus, in addition to providing about five times the larval body weight in food per day (Eristalis), the developmental time was shortened by 21 days to about 46 days. Under conditions A, most adults died within two or three days of pupal eclosion, whereas under conditions B, about 50% of the larvae reached the adult stage and were used experimentally.

Pupation time is actually somewhat shorter than the values in Table 2 because the adult beetles do not emerge from their pupal chambers in the moist sand for a day or so after they are fully formed. However, the delay is consistent and can properly be considered part of the developmental time.

Table 2. Developmental times of eggs, larvae, and pupae of three western Oregon species of Tropisternus.

Species	Average duration in days (\pm SE)					
	Egg	Instar 1	Instar 2	Instar 3	Pupa	Total
<u>Laboratory conditions A¹</u>						
<u>columbianus</u>	7.5 \pm .43	7.3 \pm .42	6.6 \pm .57	22.0 \pm 1.9	12.4 \pm .31	55.7
n =	(31 clutches)	(7)	(7)	(7)	(13)	
<u>l. limbalis</u>	5.8 \pm .48	6.2 \pm .20	10.0 \pm 1.0	24.5 \pm 1.5	12.5 \pm .50	59.0
n =	(4 clutches)	(5)	(4)	(4)	(8)	
<u>ellipticus</u>	7.9 \pm .47	8.5 \pm .49	8.5 \pm .58	27.8 \pm 1.2	14.8 \pm .47	67.5
n =	(16 clutches)	(33)	(30)	(26)	(22)	
<u>Laboratory conditions B²</u>						
<u>ellipticus</u>		4.9 \pm .29	8.0 \pm .78	13.5 \pm .94	11.9 \pm .52	46.2
n =		(8)	(8)	(8)	(8)	

¹Laboratory conditions A were a 16L/8D light cycle at about 22°C with one Culiseta spp. larva per day as food, June-September, 1972.

²Conditions B were a 16L/8D light cycle at about 12.5°C (dark) to 31°C (light) with five Eristalis spp. rattle maggot per day as food for 2nd and 3rd instar larvae, August-September, 1974.

Because physiological readiness to mate in insects is generally a prerequisite for mating behavior and for sexual responsiveness in both sexes (Haskell, 1961), and because of the close association between sound production and mating in Tropisternus (Ryker, 1972), an attempt was made to find out how long a female beetle can continue to lay fertile eggs without re-mating. If, say, it was found that a female could lay fertile eggs for ten days, then it would be a simple matter to isolate each female for a ten-day period while she became sexually responsive and then to test her response to various acoustic stimuli. By this method, for example, Jansson (1973) was able to show that females of Cenocorixa (Heteroptera, Corixidae) would emit a stridulatory response to the calling sound of the male, but only if they were isolated from males for at least one week.

The egg laying frequency of individual females of the Oregon species of Tropisternus is shown in Fig. 5. These females were observed in successful copulation after a prior period of separation from males, and then were placed singly in pint jars. Egg cases (C = case; F = case with fertile eggs) were recorded daily and, when possible, checked after five days to see if development was proceeding normally. If so, the case is represented by the letter F. These examples show that, in all three species, a female can lay fertile eggs for at least 42-44 days, and possibly longer. This means that isolating a field collected female would not necessarily bring her into

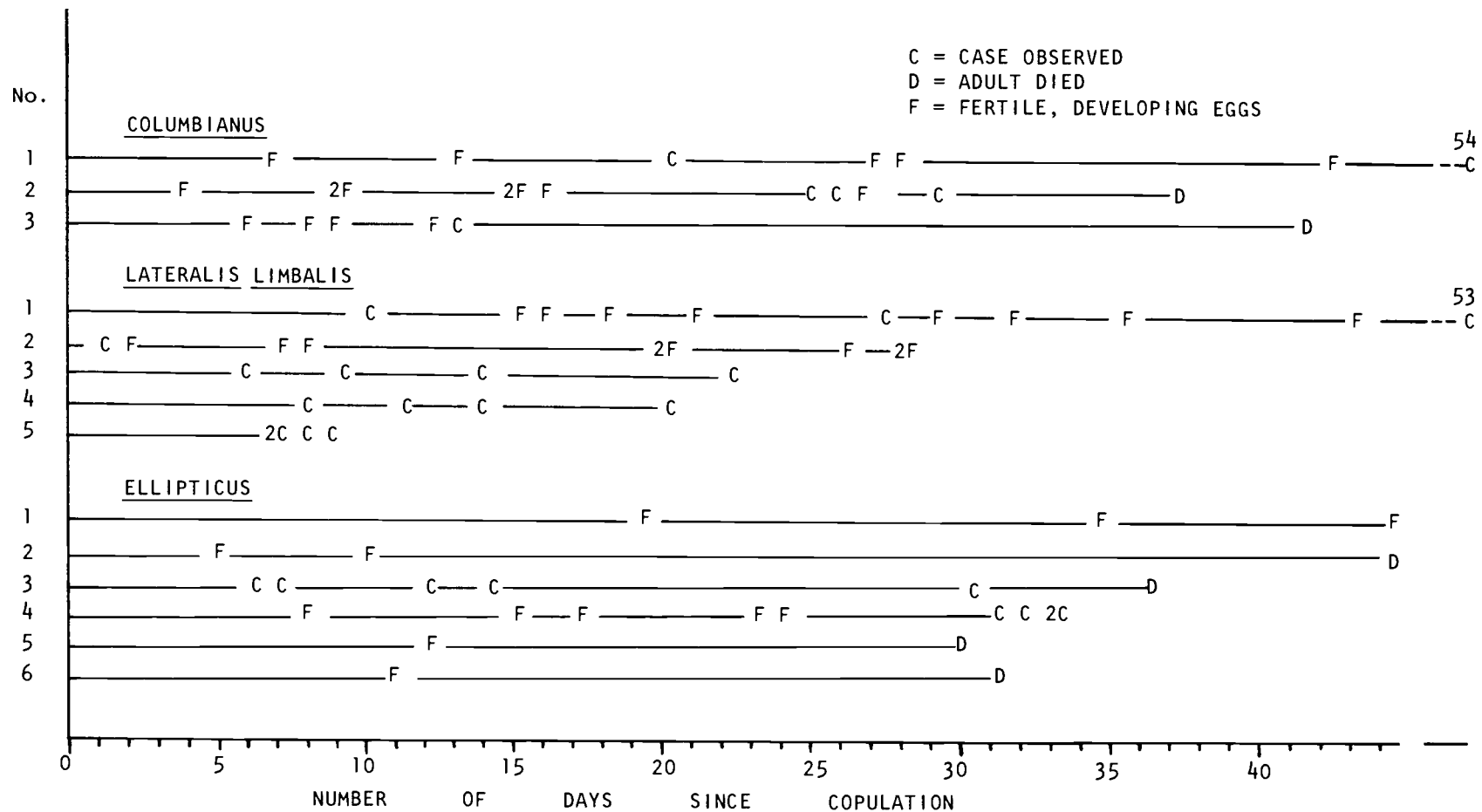


Figure 5. Pattern of egg laying of mated, isolated females of Tropisternus in pint holding jars outdoors in a water table, June-July, 1974.

a sexually responsive state within a reasonable time, making it difficult to work with females experimentally, unless rearing is possible. Males, however, are sexually responsive at almost any time that light and temperature conditions are favorable.

The elapsed time between copulation and egg laying was generally four to seven days (Fig. 5), but only one day in a limbalis female isolated for 45 days prior to mating. T. columbianus and T. limbalis produced a clutch every four days, on the average, and ellipticus about every nine days. However, the clutches were also frequently at longer intervals and in pairs, either on the same day or one day apart.

Spermatophores were collected by separating males that had just begun copulation from the females (Fig. 6). This technique was used when acoustic signals associated with mating were being recorded in order to determine whether a pair were actually in copulo. Once copulation has begun, the male begins a series of rhythmic contractions that expel the spermatophore. Males are unable to stop this process once begun, and will expel the spermatophore while being held between the fingers. The structure is irregularly bi-globose, with the appearance of a membranous bag containing two spheres one on top of the other. It is 1-2 mm long, and may or may not have a sleeve tailing at one end.

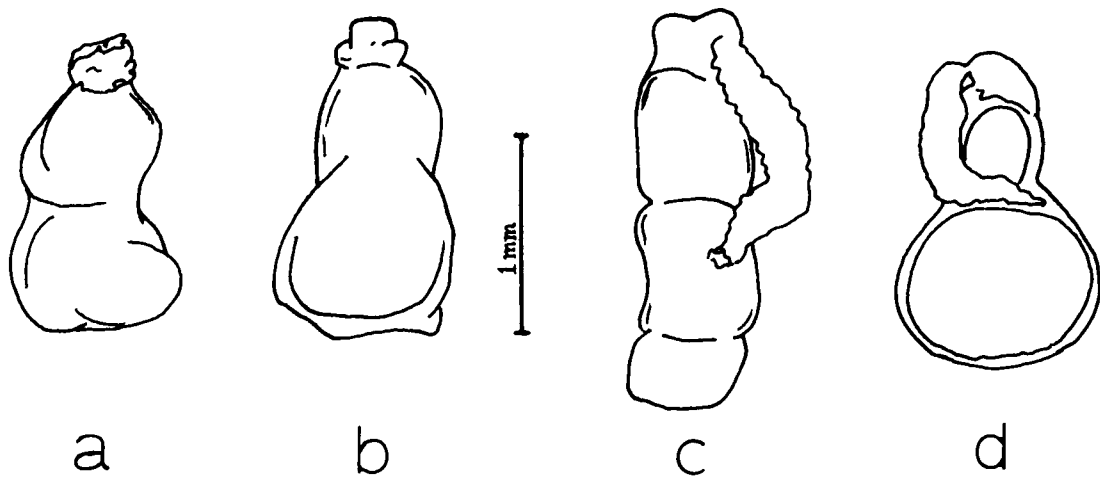


Figure 6. Spermatophores of *Tropisternus*: a) natator; b) T. limbalis; c and d) ellipticus.

Habitats and Dispersal

Little is known about differences in micro-habitat preferences between the four species of Tropisternus studied at Ann Arbor, Michigan, T. lateralis nimbatus (Say), natator (D'Orchymont, glaber (Herbst), and mixtus (LeConte). However, I have collected all four species from the same ponds and pools (Ryker, 1972) during the summer. Apparently, all four species commonly breed in the same pools, and at the same times.

The three western Oregon species, T. lateralis limbalis, columbianus, and ellipticus, may not breed in the same habitats. T. ellipticus, particularly, overwinters in ponds in a non-breeding condition (dissected females have no eggs developing in their ovaries), but disperses in April and May and can be found breeding in temporary pools that dry up in June. Their progeny, the first summer generation, disperse into rock pools along rivers, where they can be found breeding from late May through August.

T. lateralis limbalis also overwinters in ponds, but in April and May can be found breeding in flooded grassy swales and muddy ditches of cultivated fields. Later in the summer, they are commonly found in ponds that have shallow, muddy edges, where they can be collected in large numbers. Congeners of l. limbalis are seldom collected in such habitats.

T. columbianus, the third western Oregon species, also overwinters in permanent ponds. This species is commonly collected only from the edge habitat of permanent ponds and probably breeds there.

Small pools, such as the rock pools frequented by ellipticus and the ditches and pond edges used by other species, characteristically have temperature variations approximating ambient temperatures if exposed to full sunlight in summer, but pool temperatures may remain warmer than ambient on cool nights (Pajunen and Jansson, 1969). The change in temperature in a small rock pool between 1000 and 1600 hours on a sunny May day is illustrated in Fig. 7. Slightly cooler temperatures were found within the pool in the shade of bottom rocks.

All three western Oregon species, and probably all species of Tropisternus, are good fliers and disperse readily when ponds and pools start to dry up (Spangler, 1960; Miller, 1963). Dispersal flight may be triggered by the higher temperatures developing in pools that are drying and becoming shallower (Zimmerman, 1959). Adults of all three species crawl out onto surface vegetation and take flight when the temperature of holding jars reaches 31°C.

Territoriality and Home Range

One possible communicative function of acoustic signals is

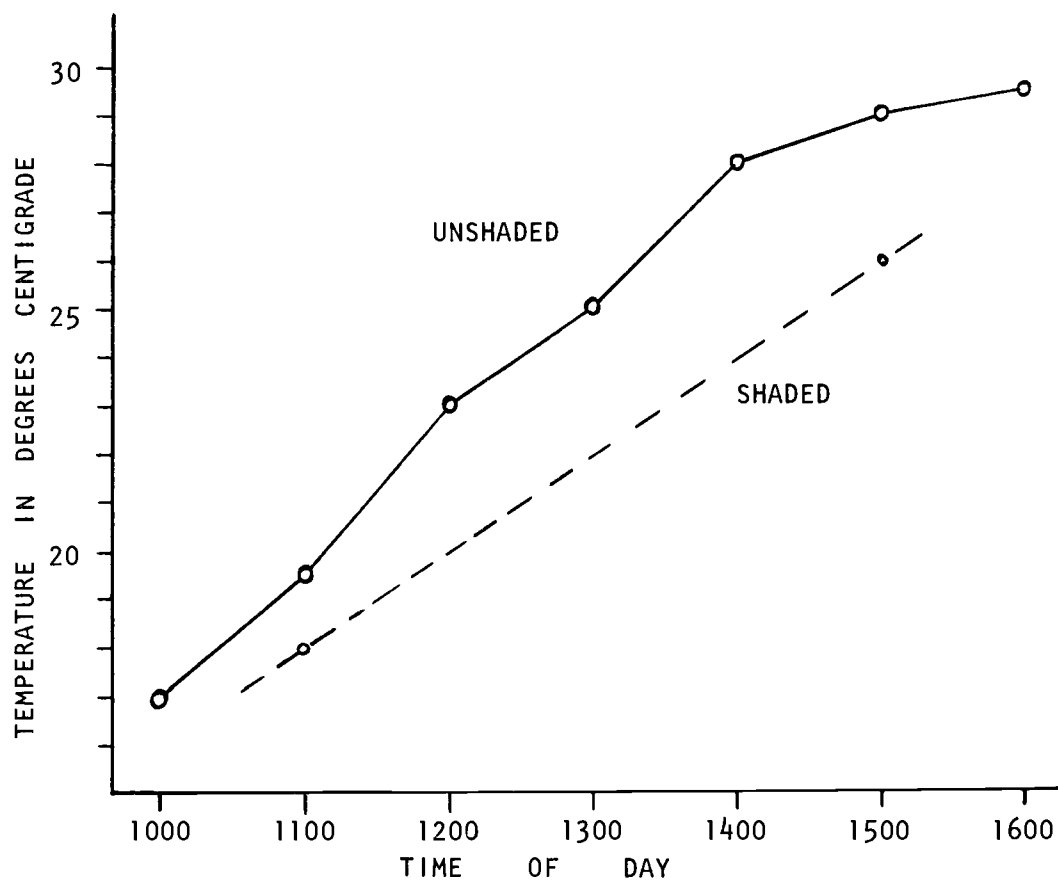


Figure 7. Water temperature of a small rock pool at Waterloo, Oregon, South Santiam River on May 18, 1973. Temperatures measured at 30 cm depth; shaded water was beneath rock.

territorial defense and aggression, as demonstrated by Alexander (1961) in field crickets. Jansson (1973) found that water boatmen males (Cenocorixa) respond to stridulation of nearby males with nudging and chasing behavior, such that each male maintains a defended personal space. Rudinsky and Michael (1973, 1974) have shown that males of several species of bark beetles (Dendroctonus) have specific rivalry chirps associated with male-male aggressive behavior, and that the clicking sounds of a female will repel other females from the immediate area of her entrance hole. I selected T. ellipticus for a field study of territoriality and home range because this species occurs in summer in easily accessible rock pools that contain vegetation no more complex than Spirogyra algae, making the beetles easy to find and observe.

The study site was a series of rock pools at Waterloo Falls, South Santiam River, Linn County, Oregon, within a reasonable distance of Corvallis. The rock pools formed in May in 1973 as the summer dry period lowered the river level and persisted until the fall rains. The rock pools were mapped (Fig. 8), and 487 beetles were marked with individual numbers (scratched into the elytra), which were recorded on maps for each collecting date. Marked beetles were released within a few feet of their capture location. Beetles were collected either by netting swimming individuals or by removing

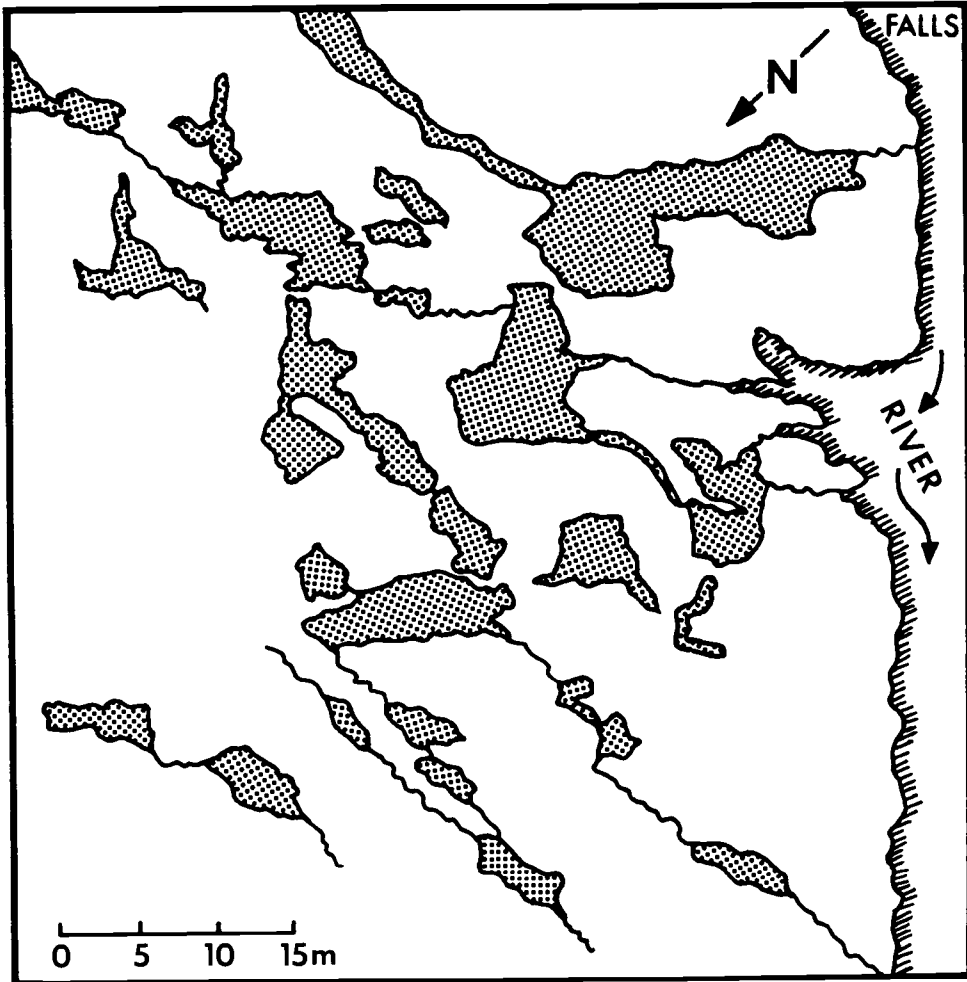


Figure 8. Map of summer study site for *Tropisternus ellipticus* at Waterloo Falls, South Santiam River, Linn County, Oregon. Dotted areas are rock pools; white area is rock.

floating Spirogyra mats and feeling for the beetles. All pools were sampled on each visit.

Beetles were usually seen swimming from place to place along the rock bottoms of the pools unless the water was about 20°C or colder, under which conditions they became rather torpid. Of the beetles marked, 140 (29%) were recaptured, and some of these were captured five or six times over a period of several months. Of the beetles recaptured, 26% had changed pools. However, of 28 beetles captured within two days, 27 (96%) were still in the same pool. Typically, beetles recaptured within the same pool were caught at a different location in the pool. Thus, movement seems to be the normal behavior, and evidence does not support the possibility of territorial behavior nor the existence of a home range.

General Pattern of Mating Behavior

Young (1958) published the first description of the mating behavior of Tropisternus species from observations of T. lateralis nimbatus, natator, and striolatus. Later I showed that acoustic signals were an integral part of the mating behavior of four Michigan species (Ryker, 1972), of which l. nimbatus and natator are studied experimentally here.

The behavioral sequence of mating can be broken down into steps:

- Step 1. Alone. The male or female is not receiving stimuli from other beetles, but it may be stridulating intermittently.
- Step 2. Acoustical Contact. The behavior of a sexually responsive beetle is affected by acoustical stimuli from other beetles.
- Step 3. Visual Contact. The behavior of a sexually responsive beetle is affected by visual stimuli from other beetles.
- Step 4. Mounted at Head. A male is mounted on the back of another beetle, situated forward above its head. Palpal-touching occurs in this position.
- Step 5. Probing. A mounted male situated back over the elytra of another beetle probes its genital area with extended aedeagus.
- Step 6. Copulation. Copulation follows probing with the male in the same position.

Steps 1-3 involve calling signals and the formation of male-female pairs. Steps 4-5 are courtship behavior (Fig. 9), which culminates (with responsive individuals) in copulation, Step 6 (Alexander, 1967). I am delineating these steps for ease of interspecific comparisons of acoustical and other signals involved in mating behavior. Species may differ in their behavior at each step in the sequence either by remaining silent or by emitting a structurally distinct acoustical signal or a distinct tactual signal. These comparisons will appear in later sections of the thesis.

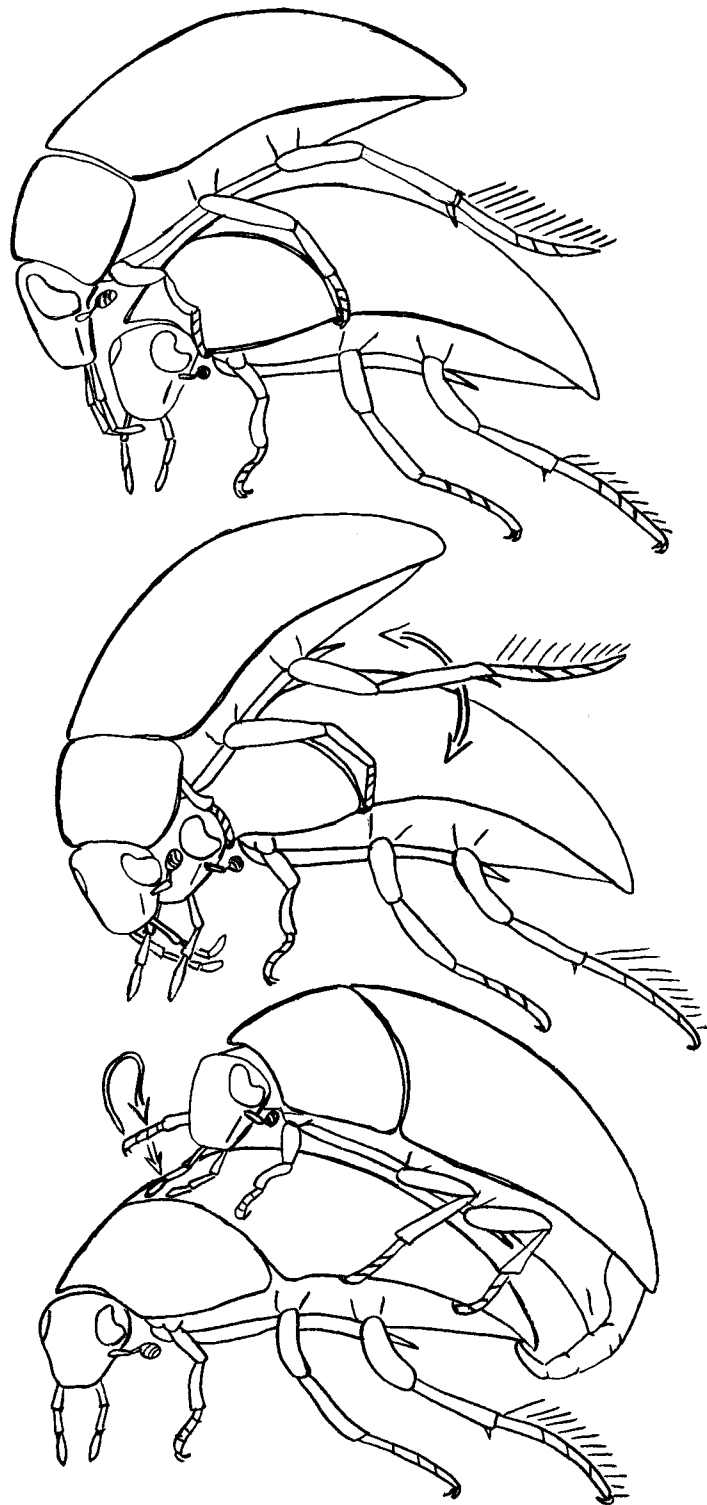


Figure 9. Courtship positions of Tropisternus. The male is mounted and touching palpi in the top two drawings, probing in bottom drawing.

The visual limit of an adult Tropisternus under water is about 2 cm. A beetle of either sex will often approach and touch its palpi to another beetle that moves within visual range, particularly if the former has been isolated from other beetles. Beetles grazing in the same container often ignore each other. Males isolated for several days may swim to and mount other beetles on sight, and this approach may be accompanied by stridulation, or may be silent. Whether a male approaches silently or stridulates while approaching depends on previous stimulation, particularly acoustical, as will be shown below for each species.

A species of Tropisternus may have either one or two calling sounds. In species with two calling sounds, one is usually chirp-like and is the signal most often given by an isolated individual of either sex. In those species that have it, the second calling sound is restricted to males and is usually given by a male that has been stimulated by a conspecific calling chirp, or the sight of another beetle. Males isolated for long periods sometimes give both the chirp call and the secondary call while alone.

Sexually responsive males mount to the back of other beetles regardless of sex, orient so that they are head to head, walk forward and lean down over the head of the mounted beetle, and touch palpi with it (Fig. 9). While in this position the male shifts from one side of the head to the other several times, pausing at each side, and in

some species, stridulating. After shifting from side to side several times, the male steps backwards and probes the female's genital area with his extended aedeagus. He is unable to copulate unless the female lowers her abdomen to receive him. Stridulation may occur in either the probing step or during copulation. The entire sequence of palpus-touching and probing may be repeated numerous times, and courtship lasting three to ten minutes is common.

Females at times shake vigorously from side to side after males have mounted them, and in some species this behavior is accompanied by stridulation, usually a rattley buzz. I interpret female shaking and buzzing as rejection behavior, as it usually terminates courtship.

Diel Periodicity of Sound Production

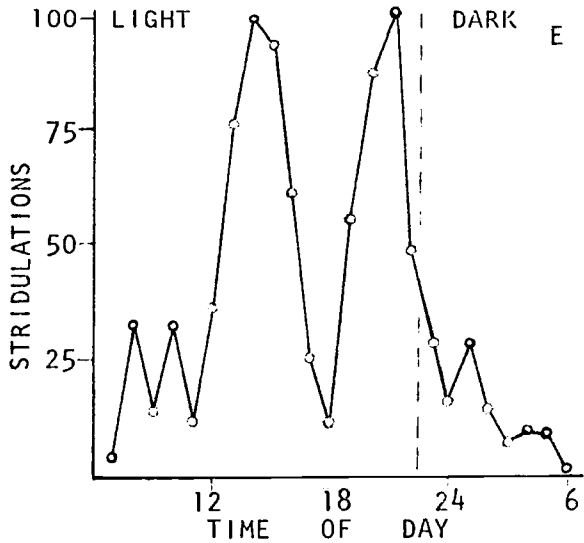
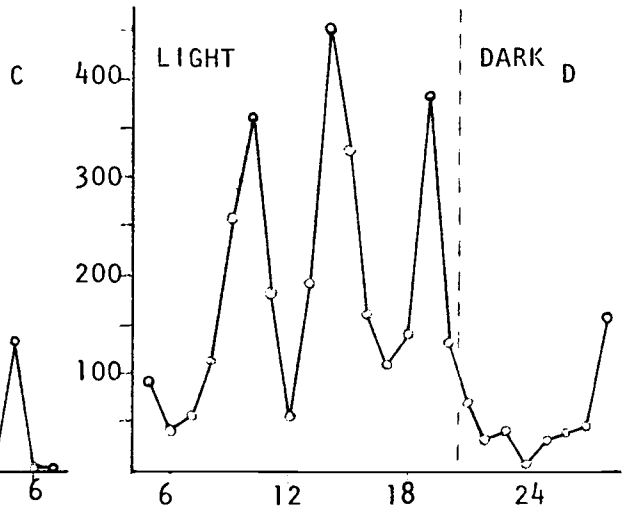
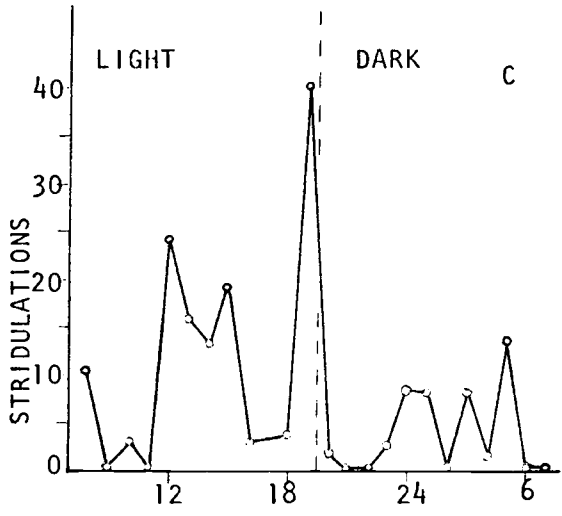
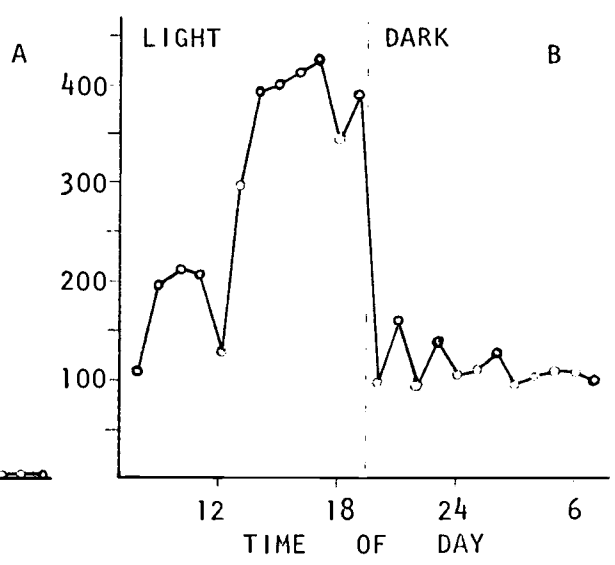
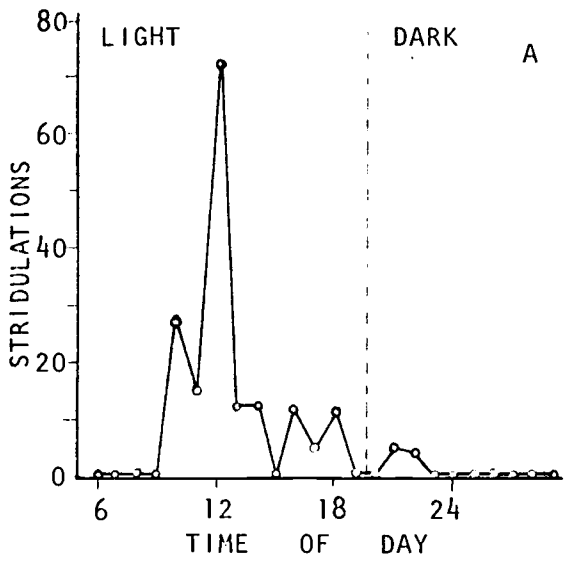
The diel periodicity of activity, as indicated by the chirping of isolated males, was studied to determine whether species of Tropisternus are diurnal or nocturnal and to allow experimental work to be planned accordingly. Recording acoustic behavior at night is advantageous because of the reduced level of extraneous activity, including machine noises, of colleagues, etc. So it was important to find out if the beetles are normally active at night or some other time.

The hourly number of stridulations of four males of columbianus collected May 4, 1974 and tested during the next week in pint holding jars sitting in a water table outdoors is shown in Fig. 10A. Maximum high and low temperatures averaged 27°C and 10°C. These males stridulated very little, but did so primarily at midday.

The hourly number of stridulations of one male of l. limbalis collected in late fall, 1973, held at 15°C under a 12L/12D light cycle for five months, and tested in a pint jar in an acoustic chamber is shown in Fig. 10B. Environmental conditions were 12L/12D with 29°C (light) and 21°C (dark) temperatures. Although this male stridulated both day and night, an analysis of variance F-test indicates that stridulation activity is significantly greater during the light period ($\alpha = .01$). However, daytime temperatures were also higher, and may have affected activity. Temperatures in this test were higher throughout most of the 24 hour period than in the test on columbianus.

The hourly number of stridulations of six males of ellipticus collected March 21, 1974 and tested in the next two weeks in pint holding jars in an acoustic chamber is shown in Fig. 10C. Environmental conditions were 12L/12D with 27°C (light) and 21°C (dark) temperatures. Four of the beetles were silent most of the time, and beetles No. 1 and 2 stridulated both day and night. There was more acoustic activity ($\alpha = .05$) during the light period than the dark period,

Figure 10. Hourly counts of stridulations of males of Tropisternus under different environmental conditions: A) T. columbianus May 4-11, 1974, outdoors in a water table, 10°-27°C; B) T. l. limbalis March 3-5, 1974, in chamber, 29°C light/21°C dark; C) T. ellipticus March 23-April 3, 1974, in chamber, 27°C light/21°C dark; D) T. ellipticus June 8-13, 1973, outdoors in water table, 14°-30°C; E) T. ellipticus, November-December, 1972, in chamber, 24.5°C light/22.5°C dark. 1972-73: entire hour counted; 1974: 15 minute samples counted hourly.



using hourly totals as observations. However, temperatures were higher during the light than the dark period, and this test does not separate the effects of the two factors.

To simulate natural conditions of light and temperature, a nine day test was run in June with three males of ellipticus each in a pint holding jar sitting in a water table outdoors (Fig. 10D). Minimum nighttime water temperatures were about 14°C, and the daytime maximum was 30°C. Hourly water temperatures were not recorded. Dawn came between 0400 and 0500 hours, and darkness between 2000 and 2100, giving an approximate 16L/8D light cycle. The totals of hourly stridulation activity were significantly different in daytime and nighttime hours ($\alpha = .01$). To see if the daily temperature fluctuations contributed to the difference between day and nighttime activity, hourly air temperatures during the nine day test were summed and averaged, and the number of chirps per hour (sums) was regressed against the average air temperature per hour. The F-test was significant ($\alpha = .01$), indicating that a certain portion of the variability in chirp activity ($r^2 = .31$) can be attributed to variation in temperature.

An attempt was made to eliminate the influence of temperature so that the influence of the light/dark cycle could be assessed. A 12 day test was run in late November and December, 1972, with four males of ellipticus in an acoustic chamber (Fig. 10E). These males

were field collected in late October and held at 15°C (16L/8D) from two to four weeks before being tested. Each was given at least two days to acclimate to the test conditions before being tested for activity under 16L/8D and 24.5°C (light) and 22.5°C (dark) conditions. The 2°C difference was due to the heating effect of the light above the chamber, but this small difference probably had little effect on activity. Stridulation activity was significantly greater during the light cycle ($\alpha = .01$), in spite of the uniformity in temperature. Although males tested in December (Fig. 10E) are not strictly comparable to those tested in June (Fig. 10D) because of physiological and behavioral differences (for example, they occur in different habitat types), the implications of the two tests can stand alone, without comparison. Both tests indicate that 1) male ellipticus stridulate when alone; 2) their acoustic activity can occur both day and night; and 3) their acoustic activity is greater under daylight conditions. These conclusions are also borne out by the tests on columbianus and l. limbalis. Additionally, the summer test outdoors with ellipticus indicated that acoustic activity is dependent upon temperature. The effect of light intensity is unknown.

From these tests it is apparent that even though the level of activity is somewhat lessened at night, with proper light and temperature conditions the beetles are active enough to permit study of their

behavior. Additionally, behavioral observations have shown that males will readily court other beetles, and females will copulate under day-like experimental conditions at night.

IV. NON-ACOUSTICAL MODES OF SIGNALING

Besides acoustical signals, species of Tropisternus rely on several stereotyped tactile movements, visual stimuli for orientation in pairing, and chemical stimuli either at close range or by directly tasting with palpi.

Tactile

Both sexes of Tropisternus characteristically approach and touch their palpi to other beetles as they are feeding; further, males have four stereotyped tactile movements during courtship: palpus-touching, hindleg-sweeping, foretarsi-tapping, and probing (Fig. 9, Ch. III). Females have several stereotyped movements for rejecting courtship by males: batting the male's palpi with her forelegs as he is palpus-touching; vigorously shaking her whole body from side to side in a rocking motion; and striking the male's extended aedeagus with her spiny hind tibiae.

When a male is mounted on and courting another beetle, and is positioned above the head, both palpus-touching and hindleg-sweeping occur as the male performs the side-to-side motion. In palpus-touching, the male leans down over the head of the female, either on the left or right side, and encircles one of her palpi with both of his palpi, which curve slightly backward (Fig. 9). Then, as he moves

across to the other side of the head, his outside palpus drags across her first palpus and his palpi encircle her other one. The importance of this behavior was not examined experimentally.

As the male moves from one side to the other, his body pivots so that his hind leg opposite to the side of the female that he is on is free to sweep across the surface of elytra (Fig. 9). The male sweeps two to four times per side in all species studied. As the male leans down over the right side of the female's head and encircles her right palpus, he sweeps several times with his left hind leg, and then reverses the procedure on the female's left side. Because this behavior seems to be similar in all species studied, it may be an artifact of movement by the male as he encircles one of the female's palpi, rather than a signal itself. Or it may have value as a general stimulus for mating readiness.

A male mounted on a female in the probing position performs foretarsi-tapping simultaneously with probing motions of the aedeagus (Fig. 9). Each species performs tarsi-tapping at times with both foretarsi together, at times tapping with only one foretarsus, and occasionally alternating left and right foretarsi. Although all species studied perform this behavior, the rate of tapping is different in some species. For example, columbianus taps about twice per trill chirp, or about four taps per second; l. nimbatus taps at about the same rate as its trill chirps, or about eight taps per second; and natator, which

is sympatric with l. nimbatus, taps about twice per buzz, or about three taps per second. However, I do not have comparable observations for other species; and I have made no tests of the possible signal value of this behavior.

All species of Tropisternus studied perform probing behavior during courtship. Males mounted on females, positioned far back with meso- and metathoracic claws hooked on the edges of her elytra, probe with the extended aedeagus. I have no data on rate of probing (aedeagus-tapping) for any species because of the difficulty in observing this movement, so I have no clue whether probing merely gives timing information to the female concerning copulation, or also provides information for species recognition.

In summary, of the four obvious tactile movements by the male during courtship, palpus-touching may be important in chemical recognition of species, sex, or sexual responsiveness; the rate of foretarsi-tapping may carry species recognition information; and probing may provide copulation timing and/or species recognition information. Hindleg-sweeping may have no signal value, or may combine with other movements as a mating stimulus. However, none of these possibilities is supported by experimentation.

Visual

Both sexes of the species of Tropisternus studied orient visually

to other beetles only when within about 2 cm, which seems surprising considering the large compound eyes characteristic of this genus. However, the eyes may function at longer distances in the air than in the water medium. These beetles are strong fliers and probably locate water visually, as do other species of Hydrophilidae (Fernando, 1958; Landin and Stark, 1973). On 22 occasions, at mid-day on sunny, summer days, I have observed Tropisternus ellipticus flying over small pools at Waterloo, Oregon, closing their elytra and dropping into the pools from 1-2 m height.

The importance of visual stimuli under water was studied by beetle "model" presentations. Models were made by gluing dead beetles onto slender glass rods and completely coating them with clear fingernail polish to eliminate odors. Beetles of both sexes sometimes ignored models when presented with them, but frequently they approached the models. Females typically walked up to the model, touched palpi to it, and then either turned away or ducked their heads under the model. An approaching female often came silently; however, females of all species studied would sometimes chirp while approaching (calling chirp).

Males had several possible responses to models. They either: 1) ignored the model, 2) walked to the model and touched palpi to it, or 3) leaped and swam toward the model, and mounted it. Also, they 4) either approached silently, with calling chirps, or with the male courtship sound.

Studies with T. l. nimbatus illustrate the effect of visual stimuli on males. In 16 trials, using six males, the control periods had a total of 204 chirps as compared to 359 chirps during the periods following model presentation. The Wilcoxon matched pairs, signed ranks test indicates that the number of chirps following model presentation was significantly ($\alpha = .05$) greater than the number during the control period.

In T. l. nimbatus, the approach behavior of males was affected by prior stimulation with acoustic signals. Models were presented to males following either a five minute period of silence, of calling chirp playbacks, or of ticking playbacks. Each male was presented with the model three times in each test and scored for whether he approached, and whether he chirped during the approach. The three treatment series on each male was replicated 16 times. Results are presented in Table 3.

Prior acoustic stimulation made a male more likely to approach a model. If the stimulus was a playback of calling chirps, the male was twice as likely to approach, and to chirp during his approach to the model. Stimulation by ticking playbacks also increased the likelihood of approach, but the effect was less marked. Unquantified observations on other species indicate that the increased tendency for males to approach other beetles following acoustical stimulation is general.

Table 3. Responses to presentations of models to T. l. nimbatus males with and without prior acoustic stimulation.

Stimuli	Approach		Chirp response	
	% ¹	signif. ²	%	signif.
Model	46	a	23	c
Model + ticks	71	b*	48	cd
Model + chirps	90	b**	54	d***

¹N = 48 for each percentage.

²Statistically different results are indicated by different letters.

*
 $\alpha = .06$

**
 $\alpha = .01$

 $\alpha = .05$

Chemical

Many observations of interactions between beetles at a distance and in contact, of the same or different sexes, and of the same or different species indicate involvement of some sort of chemical recognition. Attempts to support this hypothesis have not been unequivocal. A typical observation of a possible response to a chemical stimulus follows.

On July 22, 1974, a male of T. l. nimbatus was run through a series of tests, with rest periods in between, over a period of about two hours. During eight 5-minute test periods, of which three tests followed model presentations, the male chirped or ticked a total of six times. Following the tests, a female of l. nimbatus was placed into the test chamber with the male. Although she did not chirp, and the two did not come into physical contact, the male began to tick. He produced 25 ticking phrases in five minutes. When the female moved into visual range, the male very slowly walked near, stretched far forward on his legs, and touched her elytra with his palpi. Instantly after touching her, the male leaped onto her, courted, and eventually copulated with her. Similar observations in this and other species suggest that at times females release a chemical odor into the water, and that taste recognition of some kind may also operate. Present observations do not suggest whether the taste gives sex, species, or merely generic information.

I attempted to obtain quantitative evidence for the existence of chemical odor, using males of T. ellipticus and I. limbalis. These beetles were collected August 4-9, 1974, and maintained under continuous light with temperatures cycling daily from about 15° to 20°C. Males of I. limbalis were tested August 15-24; males of ellipticus were tested August 27-September 12. Females and males found to be sexually receptive in mating observations (and kept from being inseminated, in the case of the females) were silenced by coating their plectra with clear fingernail polish. During testing of another individual, either a silenced male or female was placed in the test chamber, but within a small, doubly screened container that floated just below the water surface (Fig. 1b, Methods). The screened container permitted free flow of water, chemical stimuli, and acoustic stimuli (which might have important effects on the silenced beetles), but the double screening obscured vision. Silenced beetles were acclimated for at least 30 minutes within the container, before being placed into the test chamber.

Although the males of T. ellipticus would approach females, and three of the five males would court, for the most part they were silent in response to stimuli of models and of playbacks of conspecific chirps and buzzes (Table 4). Therefore, even though visual and acoustic stimuli were presented both in the presence of a caged, silenced male and of a caged, silenced female, the lack of responses obviated drawing conclusions about chemical stimuli.

Table 4. Number of chirps of males of Tropisternus in three test situations and with either a silenced male or a silenced female present but not visually apparent.

Species	No acoustic stimuli	With acoustic stimuli	After visual stimulus	Totals
<u>ellipticus</u>				
Silent male	3	1	4	8
	ns	ns	ns	ns
Silent female	17	6	6	29
<u>l. limbalis</u>				
Silent male	58	61	13	132
	ns	ns	ns	*
Silent female	141	118	72	331

ns = not significant

* Statistically significant, $\alpha = .08$

Although in similar tests, T. l. limbalis males gave more chirp responses than ellipticus to stimuli presented (models, chirps, and ticks), the effect of the presence of a silenced male or female was not clearcut. The number of male chirps in three situations of stimulation, and the total chirps for the three, are presented in Table 4. There is no significant difference in the number of times that a male chirped in five minutes, whether a silenced female or male is present, in any of three situations: 1) with no acoustic stimuli presented, 2) with either chirps or ticks presented, or 3) with model presentation following silence or acoustic stimulation. However, by lumping the responses in all test situations into two totals (with a

silenced male, or with a silenced female present), the difference in the number of chirps is significant at the $\alpha = .08$ level. Although this result is suggestive, it is not conclusive; and the existence of chemical communication in Tropisternus remains problematical.

V. SOUND PRODUCTION AND RECEPTION

Sound Production

Species of Tropisternus stridulate by the abdomino-elytral method (Dumortier, 1963), in which specialized areas on the sides of the abdomen rub against matching surfaces on the underside of the elytra (Fig. 11) (Ryker, 1972). A similar stridulatory apparatus was reported for the Old World species, Spercheus emarginatus Schall (Buhk, 1910; Frankenberg, 1940), and for Hydrophilus piceus Geoff. and Berosus aericeps Motsch. (Brocher, 1911-12). The abdominal stridulatory surface in Berosus is developed into a definite file, illustrated by Van Tassell (1965). Previously, I described the stridulatory apparatus for Tropisternus, discussed experiments demonstrating which morphological areas were critical to sound production, and illustrated the fine structure of stridulatory surfaces with scanning electron micrographs (Ryker, 1972).

The plectrum of a stridulatory apparatus has been defined variously as the less complex part or the one that excites the vibrations in the part it rubs against (Arrow, 1904; Barr, 1969), or the moving part that plucks or strikes its opposing part (Ashlock and Lattin, 1963). The stridulatory part against which the plectrum rubs, or the more complex part, has variously been called the file or strigil (Dumortier, 1963), the stridulitrum (Ashlock and Lattin, 1963), or the pars

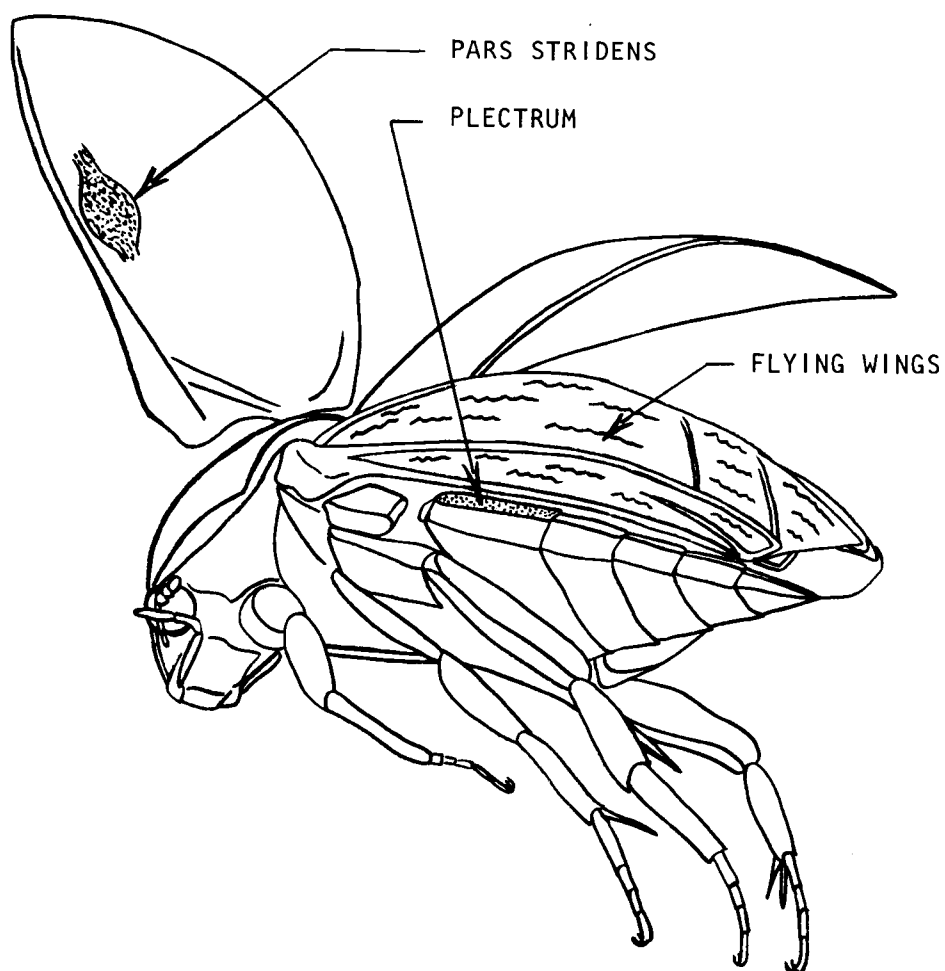


Figure 11. Location of stridulatory surfaces of Tropisternus.

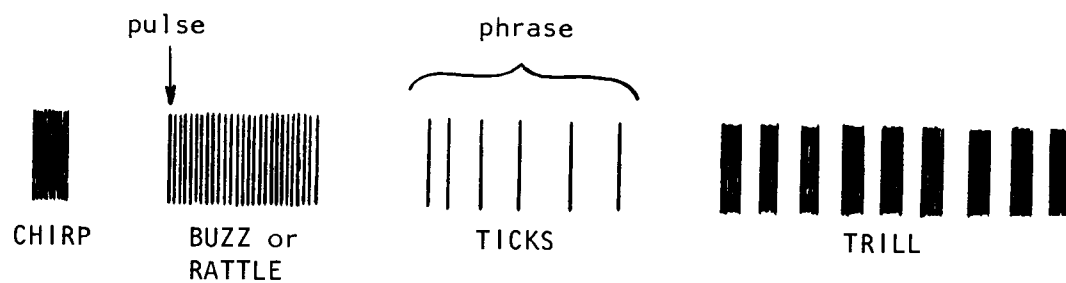


Figure 12. Diagram of types of acoustic signals of Tropisternus as they would appear in sonographs.

stridens (Dudich, 1920; Barr, 1969). In Tropisternus, both opposing stridulatory surfaces are complex, and probably both vibrate. However, the surfaces on the abdomen are the moving parts, and the major part of the vibration is probably set up simultaneously in the elytra and the thorax. Thus, I have assigned the term plectrum to each abdominal stridulatory structure, and pars stridens to the matching elytral surfaces (Ryker, 1972). Dumortier (1963), however, applied the terms to Hydrophilidae oppositely, suggesting that the elytra (which do not move) "scratch" against the moving abdomen.

The plectra lie dorsally on each laterosternite of the first visible abdominal segment (Fig. 11). They are situated laterally such that the folded wings do not interfere with stridulation, which is performed by a movement of the abdomen upward and forward against the elytra. A single forward movement of the abdomen produces a single chirp. Apparently, movement of the abdomen in the opposite direction is silent. The plectra are covered with thousands of very small, curved, prong-like setae pointing generally posteriorly. The surface of each elytral pars stridens is covered with irregular rows of tiny papillae (Fig. 13).

Stridulations of Tropisternus recorded underwater have relatively intense bands of frequencies between 1 kHz and 6 kHz, with less intense frequencies both below and above this band. However, these carrier frequencies may be restricted to a few kHz within this

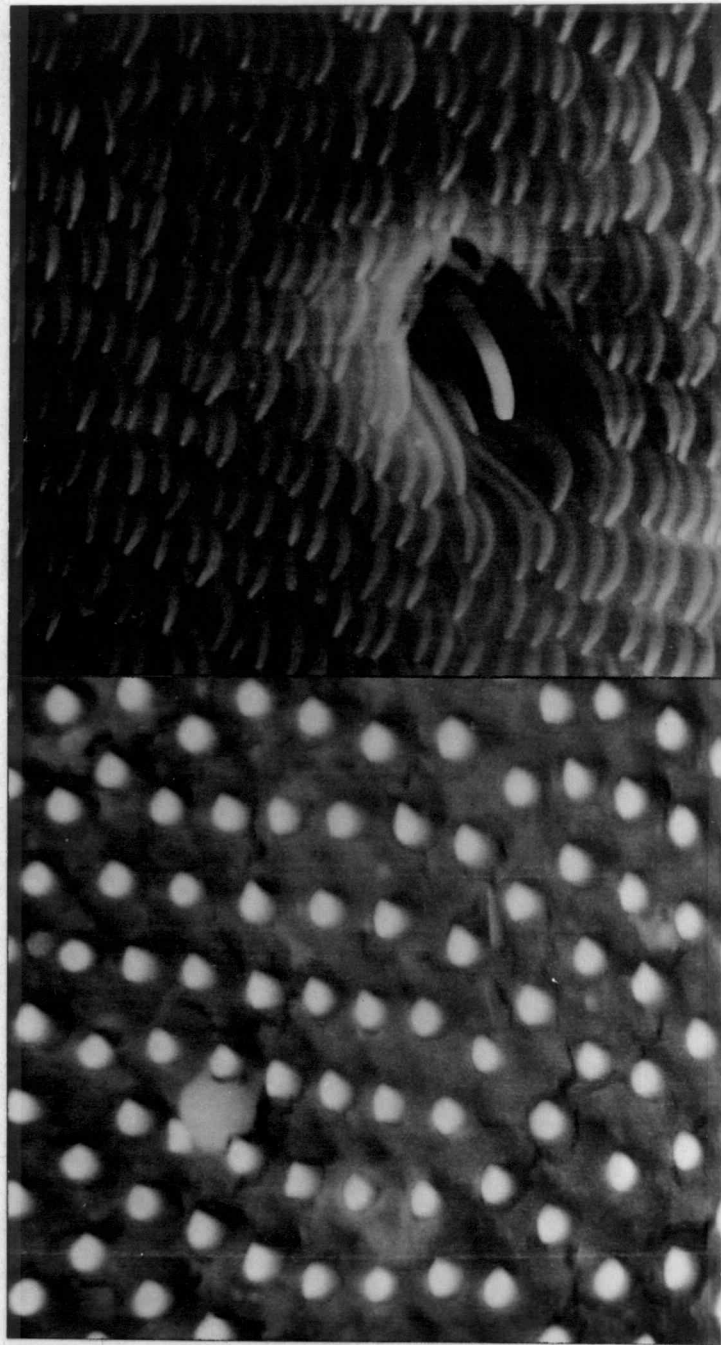


Figure 13. Scanning electron micrographs of stridulatory surfaces of males of Tropisternus. The top of each micrograph is oriented anteriorly. Top: dorsal view of part of left plectrum, on abdomen (X3000). Bottom: ventral view of part of pars stridens on right elytron (X3000) (after Ryker, 1972).

range, or fill the entire 1-6 kHz range, depending upon conditions. In general, as a beetle moves closer to the water surface or further from the microphone, the higher frequencies are emphasized (Ryker, 1972). No direct intensity measurements have been made. However, Tropisternus in jars or aquaria can be heard chirping from 20 feet in a quiet room.

Acoustic Terminology and Types of Stridulations

A short glossary is included here to avoid the confusion that exists in the literature due to variation in acoustical terminology. Subjective terms like tick, chirp, buzz, and trill are assigned for ease and clarity in discussion (Alexander, 1967).

Buzz - a long or slow stridulation in which the component pulses are individually distinguishable in sonographs but are too rapid to be counted by ear.

Chirp - a short, unitary sound composed of pulses so closely spaced in time that they are not resolvable by sonographs without time scaling.

Frequency - tone or pitch, measured in units of Herz (= cycles per second) or kiloHerz (kHz), the units on the vertical scale of sonagrams.

Intensity - the loudness or amplitude of a sound, shown in oscillograms by the vertical displacement of the sound waves, and in

sonagrams by the darkness of the mark on the paper.

Phrase - any uninterrupted group of sounds, such as a group of ticks or a group of chirps making up an unbroken trill.

Pulse - the shortest distinguishable element of a signal in sonagrams, always less than 0.01 seconds duration. Pulses may appear as complex wave trains in oscillograms.

Rattle - a buzz produced by a female while shaking from side to side; the legs striking the body impart a rattley quality to the buzz.

Tick - apparently single-pulsed sounds of less than 0.01 seconds duration, usually occurring in a group of five to eight ticks.

Trill - rapid, rhythmic trains of chirps.

Figure 12 illustrates the four types of stridulations in Tropisternus. Chirps, buzzes, and tick phrases are each produced by a single motion of the abdomen. The slow pulses of buzzes and ticks are apparently produced by repeated slipping of the abdomen. How this is done is puzzling since no file or teeth are present on either surface. Finely controlled muscle contractions may create pulses of sound by repeated slipping and stopping of abdominal motion (see Walker and Dew, 1972). Each group of pulses in a trill represents a separate forward movement of the abdomen and can be considered a chirp, so a trill is produced by a rapid series of abdominal movements.

Effect of Temperature on Acoustic Signals

Chirp duration, buzz duration, and trill rate were regressed against temperature to see what magnitude of variation is introduced by differences in temperature. Only selected signals were tested because of the requirements of similar behavioral contexts for each signal type, and of data with sufficient temperature variability.

Chirp durations of T. columbianus males responding to acoustic stimuli were regressed against water temperatures ($n = 40$), and an F-test showed no significant regression ($\alpha = .05$, $r^2 = .35$). Variability in chirp duration of this species, then, is independent of temperature variation over a 22° - 30° C temperature range.

Buzz durations of T. ellipticus males mounted on females and positioned above the head were regressed against water temperatures ($n = 57$), and an F-test was not significant ($\alpha = .05$, $r^2 = .21$). So the buzzes of this species are also independent of temperature variations from 25° - 29° C.

Courtship trill rates of probing T. columbianus males were regressed against water temperatures ($n = 46$), and an F-test showed that the regression was highly significant ($\alpha = .01$, $r^2 = .64$). For each $^{\circ}$ C of temperature, the trill rate increased approximately .08 chirps/second. The trill rate increased from about 1.8 chirps/second at 21° C to about 2.5 chirps/second at 30° C.

If these tests are representative of all the species of Tropisternus studied, as indeed seems likely, then between-species comparisons of chirps and buzzes are relatively free of temperature bias, but trill rate comparisons are not.

Sound Reception

The mechanisms of sound reception in Tropisternus have not been determined. No paired tympanal organs are present such as are found in the Homoptera, Heteroptera, and Lepidoptera, making it unlikely that Tropisternus can orient directionally to acoustic signals.

Autrum (1963) stated that subgenual organs, groups of sensory cells located inside the tibia that are frequently vibration receptors, are missing in Coleoptera. However, Coleoptera can sense ground vibration via simple chordotonal organs situated between the tibia and tarsus.

Hughes (1952) found that the simple, segmental chordotonal organs in the abdomen of Dytiscus (Dytiscidae) were sensitive to sounds of about 100 Hz. Schneider (1950), however, showed that the optimum frequencies for beetle tarsal chordotonal organs (scolopidia) are at higher frequencies in several species. Geotrupes and Melolontha (Scarabaeidae) were most sensitive to sound frequencies of 0.5-2.0 kHz. Pterostichus (Carabidae) and Silpha (Silphidae) are

most sensitive to sounds of 0.8-1.0 kHz, but show high sensitivity also at 6 kHz. Therefore, the possibility exists that chordotonal organs in Tropisternus may be adapted to receive signals within the 1-6 kHz band.

Directional orientation to underwater acoustic signals has been demonstrated in Cenocorixa (Corixidae) by Jansson (1973). Cenocorixa has paired tympani. Jansson found that males were thwarted in their attempts to orient on stridulating females by echo problems within small test chambers, but eliminated the problem by using a sand-lined bathtub. Similarly, to test Tropisternus for acoustic orientation, I used a large, sand-lined sink, 15 cm deep and 36 x 66 cm. From the center to the nearest side was 18 cm, which seemed adequate because most species of Cenocorixa, an insect with highly developed hearing organs, did not respond to signals at greater distances than 20 cm.

Two tests were run outdoors, in the sand-lined sink, on a sunny June day at a water temperature of 28°C. An underwater speaker was placed at one end and in the center of the end wall of the sink. Chirps of females were broadcast to males of columbianus in a screen cage until two males were found that were stimulated to give calling chirps in response. These two males were tested individually for five minutes in the sink with a playback of the female chirp broadcast from the speaker at the end. Both individuals chirped in

response to broadcast chirps and swam about in the sink, but did not orient towards the speaker from any position within the tank. When the two males were placed together within the tank, both chirped, responded to each other's chirps and swam frequently, pausing briefly between swims. Phonoresponses were noted when the two beetles were at the opposite extremes of the tank, indicating that under ideal conditions the effective distance of their chirps is at least 60 cm. There was no evidence of orientation by either male from any position within the sink, nor at any distance. When the males passed within 2 cm (visual distance), they turned toward each other, both approached, and one mounted and courted the other.

A similar test was run with l. limbalis, except that a male was paired with a female that had been separated from males for 43 days, but kept with other females. As before, a male was selected on the basis of its phonoresponse to a broadcast female chirp. None of the females available responded to broadcast chirps, so one was chosen arbitrarily. When placed into the sink together, both beetles moved from place to place. The male continued to chirp and occasionally tick in response to broadcast chirps, and the female remained silent. No orientation by the female to the male nor of either beetle to the speaker was observed in the 15 minute period. Once he passed within visual range, however, the male swam in tight circles until he again found the female. The female walked up to the male and ducked her

head under his body, whereupon he mounted, courted, and copulated successfully. This female was re-isolated, and subsequently produced nine egg cases with fertile eggs.

These observations indicate that calling chirps of females of T. columbianus and l. limbalis may stimulate males into random searching behavior, and that directional orientation to acoustic signals probably does not occur in Tropisternus. Orientation seems to depend on close visual contact.

VI. STRIDULATIONS AND THEIR CONTEXTS

Species of Tropisternus stridulate when alone, when handled or otherwise disturbed, and during courtship and copulation. As defined in the introduction, these sounds can be categorized as calling, stress, courtship, copulatory, and aggressive (female rejection) sounds (Alexander, 1967). The calling and courtship sounds can be further subdivided according to sex, and into more specific contexts: alone, responding to acoustic or visual stimuli, approaching another beetle, mounted at the head, and probing. Sounds produced in different contexts may be recognizably distinct to the beetles even though the human ear perceives them as similar, i. e., as buzzes, trills, etc. Although the only appropriate way to decide whether two sounds are distinguishable to the insects is to experimentally obtain differences in some behavioral response of the insects to the sounds, one can determine whether or not measurable characteristics of the sounds are statistically distinct. In this chapter I examine the sounds of four species according to sex and context to see how many of the sounds are statistically similar, and how many distinct. (In Chapter VIII, behavioral response data are presented to show that some species of Tropisternus are able to distinguish between different sounds.)

Statistical comparisons of sample measurements were made in two ways, graphically and by t-tests. In graphical comparisons, samples with non-overlapping 95% confidence intervals are considered significantly different; and samples in which each mean is included within the confidence interval of the other sample are considered not significantly different (Simpson et al., 1960). Samples intermediate between these two cases were compared by Student's t-test. When two or more sounds were found to be similar, their observations were lumped for further comparisons with other sounds.

Tropisternus ellipticus (LeConte)

Tropisternus ellipticus occurs throughout the western United States, Mexico and Central America (Spangler, 1960). In western Oregon this species breeds in temporary pools in the spring and river rock pools in the summer, and overwinters in ponds and lakes.

The stridulations of ellipticus were separated into 14 types according to sex and behavioral context: five male and three female chirps, five types of male buzzes, and one female buzz. Of these, I have no saved recordings of females alone, although observations indicate that females do chirp when alone. A summary of the sound measurements, indicating means, standard errors, sample size, and the 95% confidence intervals for sample means is shown in Table 5.

Table 5. Summary of sound data for Tropisternus ellipticus.

Sound	Duration (seconds)				Pulse rate (pulses/second)			
	\bar{x}	SE	n	95% confidence interval	\bar{x}	SE	n	95% confidence interval
♂ chirp, alone	0.079	0.005	(7)	0.065-0.092 a ¹				
♂ chirp, response	0.072	0.007	(11)	0.056-0.088 a				
♀ chirp, response	0.072	0.003	(27)	0.065-0.079 a				
♂ chirp, stress	0.088	0.004	(74)	0.080-0.106 b				
♀ chirp, stress	0.098	0.004	(74)	0.090-0.106 c				
♀ buzz, rejection	1.070	0.073	(20)	0.918-1.222 d	42.5	1.74	(20)	38.9-46.1 h
♂ buzz, alone	0.937	0.028	(4)	0.847-1.027 e	52.5	2.02	(4)	46.1-58.9 i
♂ buzz, response	0.763	0.033	(33)	0.695-0.831 e	51.2	2.74	(33)	45.6-56.8 i
♂ buzz, at head	0.864	0.044	(52)	0.775-0.953 e	49.2	3.37	(52)	42.4-56.0 i
♂ buzz, probing	0.889	0.099	(13)	0.674-1.103 e	60.7	6.63	(13)	46.3-75.1 j
♂ buzz, copulating	0.868	0.132	(5)	0.503-1.233 e	59.0	3.63	(5)	48.9-69.1 j
					<u>Chirp rate (chirps/second)</u>			
♂ rhythmic chirps, probing	0.065	0.006	(50)	0.053-0.077 f	2.5	0.2	(10)	2.0-3.0 k
♂ rhythmic chirps, copulating	0.092	0.006	(46)	0.079-0.105 g	1.95	0.11	(11)	1.71-2.19 l

¹Different letters in same vertical column indicate significant differences at $\alpha = .05$ level.

When alone, males and females chirped and sometimes males would buzz as well. The same sounds were produced in response to acoustic (calling chirps) or visual (the sight of another beetle) stimuli (Fig. 14). Calling chirps and stress chirps of ellipticus are compared graphically in Fig. 16. The durations of all of these sounds overlap noticeably. The male alone, male response, and female response chirps were not different. However, the male stress chirp was slightly different ($\alpha = .05$) from calling chirps, and the female stress chirp was different from all others ($\alpha = .01$) (Table 5).

Males of T. ellipticus produced buzzes not only in the calling situation but also during courtship at the head and while probing, and at times during copulation. In addition, females sometimes produced buzzes, accompanied by rocking or shaking from side to side, during courting by males (rejection buzz) (Figs. 15 and 16). The durations of buzzes were quite variable but essentially similar in all contexts in males. Female buzzes were of longer duration ($\alpha = .01$). Pulse rates of buzzes were also compared (Table 5). Pulse rates of male probing and copulation buzzes were similar, but distinct from other male buzz rates ($\alpha = .01$). The pulse rate of female rejection buzzes was distinctly slower than that of all male buzzes ($\alpha = .01$) Both parameters of each type of buzz are compared graphically in Fig. 17. Notice the overlap in confidence intervals. Small sample sizes make

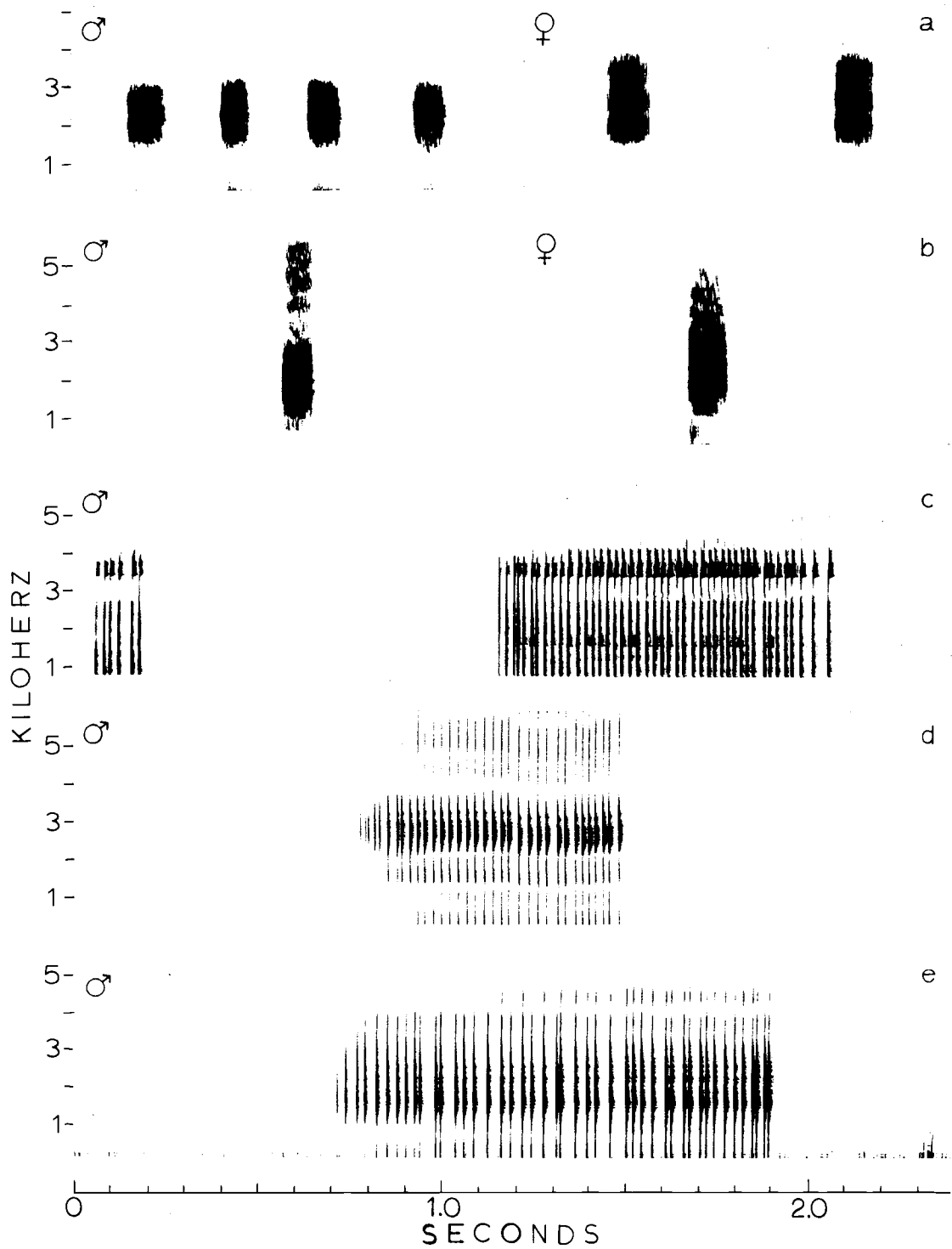


Figure 14. Sonographs of stridulations of *Tropisternus ellipticus*:
 a) stress chirps (27° , 28°C); b) calling chirps (28° , 27°C); c) buzz, alone (22°C); d) buzz, response (27°C);
 e) buzz, at head (28°C).

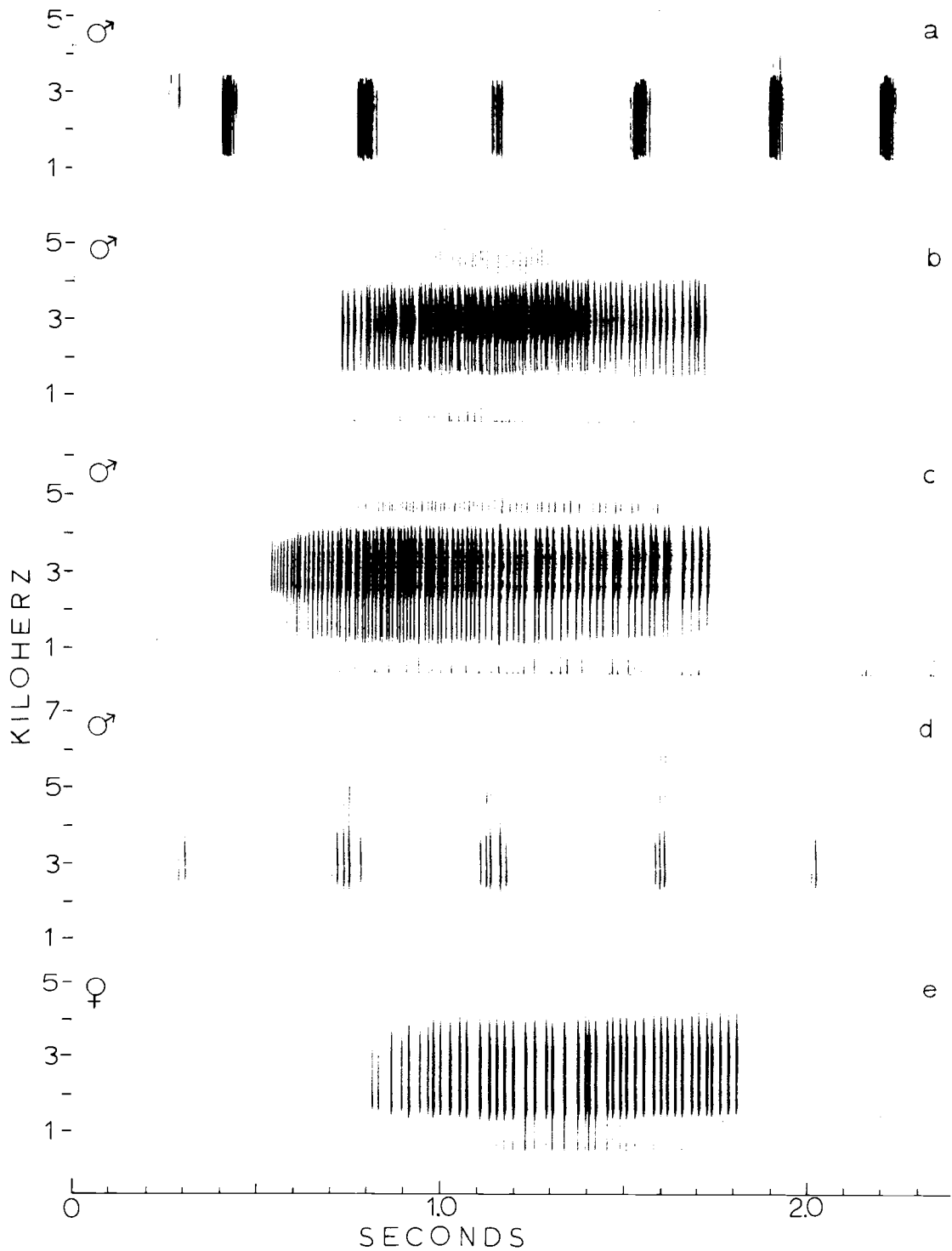


Figure 15. Sonographs of stridulations of *Tropisternus ellipticus*:
 a) probing chirps (24°C); b) probing buzz (27°C);
 c) copulation buzz (29°C); d) copulation chirps (27°C);
 e) rejection buzz (28°C).

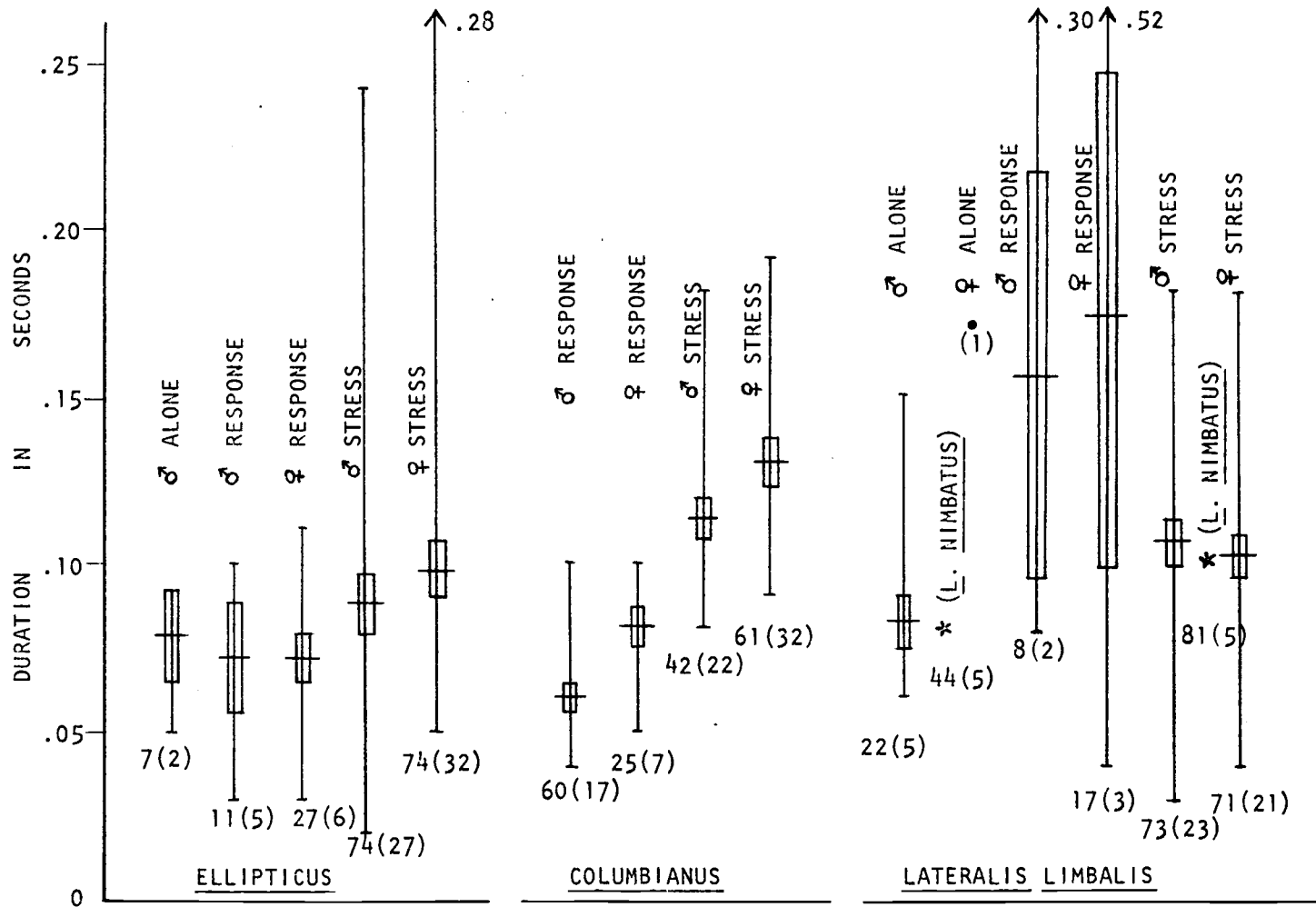


Figure 16. Means, 95% confidence intervals (boxes), and ranges (vertical lines) of chirps of western Oregon species of *Tropisternus*. The first number = number of chirps; bracketed number = number of beetles sampled.

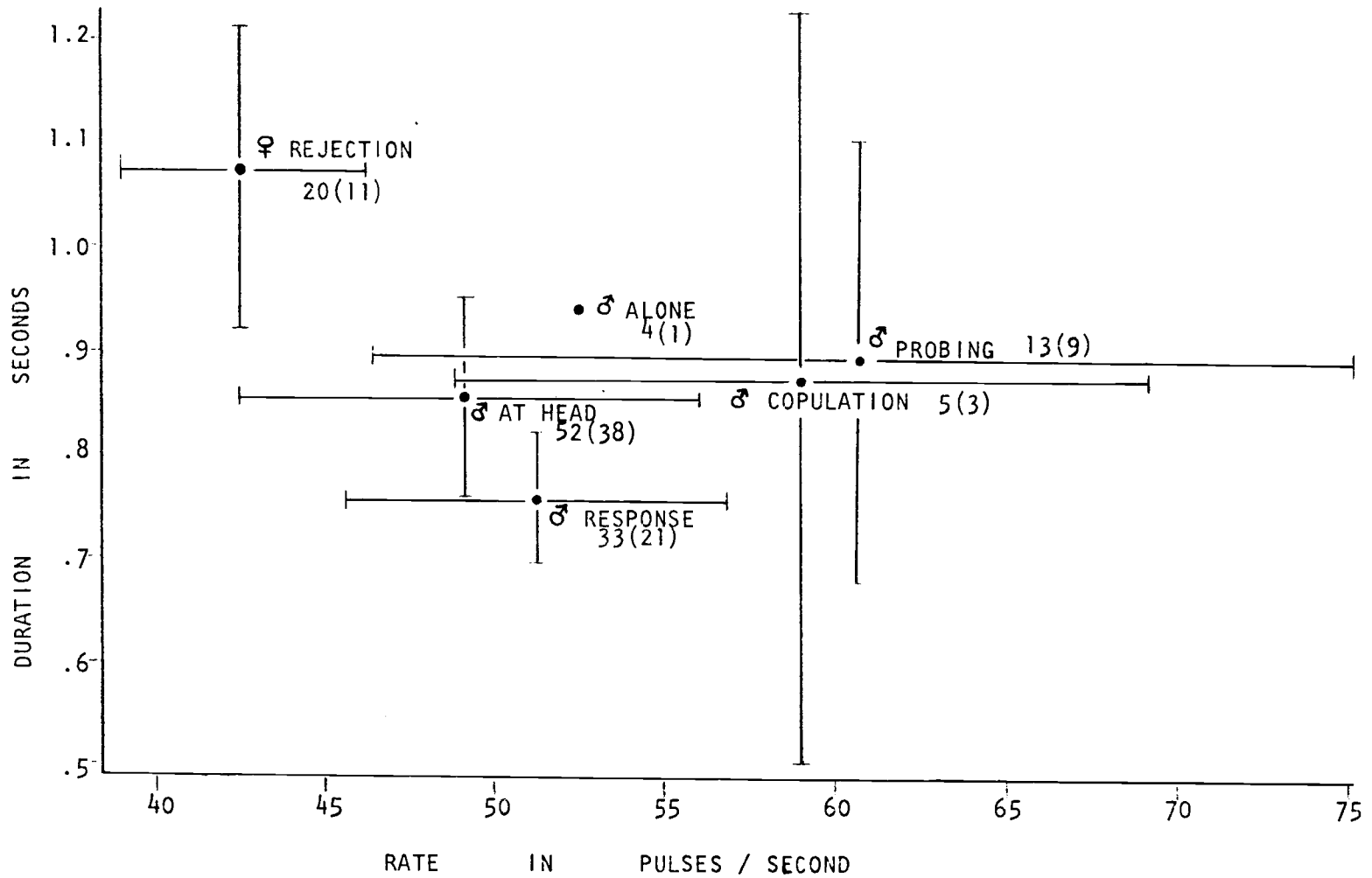


Figure 17. Buzzes of Tropisternus ellipticus, 95% confidence intervals of durations and pulse rates. First number = number of measurements; bracketed number = number of beetles sampled.

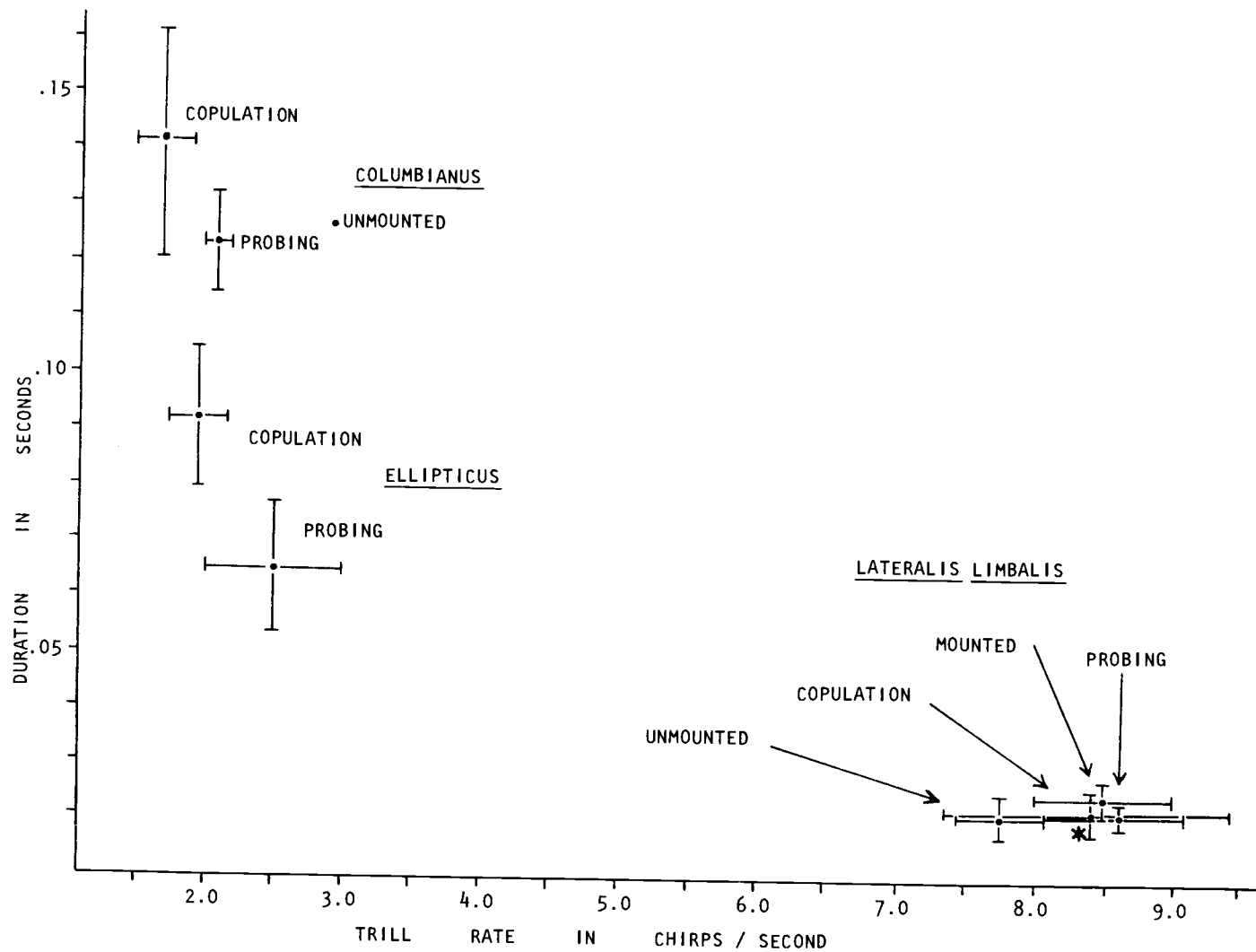


Figure 18. Trills of three western Oregon species of Tropisternus, 95% confidence intervals of durations and trill rates. * = trill, lateralis nimbatus (Mich.)

the pulse rate distinctiveness of probing and copulation buzzes suspect, and it may be that all male buzzes were essentially similar.

Probing and copulation were also occasionally accompanied by rhythmic chirps, or trills, of the males (Fig. 15). These sounds were distinct in duration ($\alpha = .05$), and may be different in the rhythm (chirp rate) ($\alpha = .05$), although the sample size was too small to be sure (Table 5). Both parameters of probing and copulation trills are compared graphically in Fig. 18.

In summary, calling chirps of males and females were similar, but of significantly shorter duration than stress chirps. The buzzes of males were similar in duration, and probably so in pulse rate, in all contexts. Males also occasionally produced slow trills of chirps during probing and copulation, and females had a distinct buzz, emitted during aggressive rejection behavior.

Both the durations and pulse rates of all buzzes were highly variable, which is not what one would expect of a signal of critical importance in mating behavior. This variability suggests that tactile and chemical cues may be very important in ellipticus. Although the standard errors of stress chirps were as small as those of calling chirps, stress chirps had greater sample sizes. The stress chirps actually had much greater ranges in duration than calling chirps (Fig 16), as one would expect if calling chirps are important signals in the mating sequence but stress chirps are not.

Tropisternus columbianus Brown

Tropisternus columbianus occurs from the west coast of the United States, British Columbia, Canada, and Baja California, Mexico, eastward to the edge of the Great Plains in Michigan. In western Oregon this species is found in permanent ponds and lake edges throughout the year.

The stridulations of T. columbianus were separated into ten types according to sex and behavioral context: three types of chirps for each sex, three types of male trills, and one female buzz. Of these, I have no recordings of males or females alone; however, diel sound activity studies indicate that both sexes chirp when alone. Table 6 provides a summary of the sound measurements, indicating means, standard errors, sample size, and the 95% confidence intervals for sample means.

When alone or when responding to acoustic or visual stimuli, both sexes emitted calling chirps that were of shorter duration than the stress chirps (Fig. 19). Graphical comparison indicates that both the calling chirp and the stress chirp of females were longer than the corresponding chirps in males. None of the four confidence intervals overlap, i. e., there were four distinct chirps (Fig. 16).

Males trilled at times when responding to acoustic or visual stimuli, during courtship when probing, and during copulation (Fig. 19). Durations of probing and copulation trills were similar ($\alpha = .05$)

Table 6. Summary of sound data for Tropisternus columbianus.

Sound	Duration (seconds)				Chirp rate (chirps/second)			
	\bar{x}	SE	n	95% confidence interval	\bar{x}	SE	n	95% confidence interval
♂ chirp, calling	0.060	0.002	(60)	0.057-0.063 a ¹				
♀ chirp, calling	0.081	0.003	(25)	0.075-0.087 b				
♂ chirp, stress	0.113	0.113	(42)	0.107-0.119 c				
♀ chirp, stress	0.130	0.003	(61)	0.123-0.137 d				
♂ trill, probing	0.123	0.004	(46)	0.114-0.132 e	2.12	0.093	(46)	2.03-2.21 f
♂ trill, copulating	0.141	0.010	(14)	0.120-0.162 e	1.71	0.092	(14)	1.51-1.91 g
♂ trill, unmounted	0.127	(one ♂ only)			2.88			
					Pulse rate (pulses/second)			
♀ buzz, rejection	0.688	0.043	(31)	0.600-0.776	43.1	4.8	(19)	33.0-53.2

¹Different letters in same vertical column indicate significant differences at $\alpha = .05$ level.

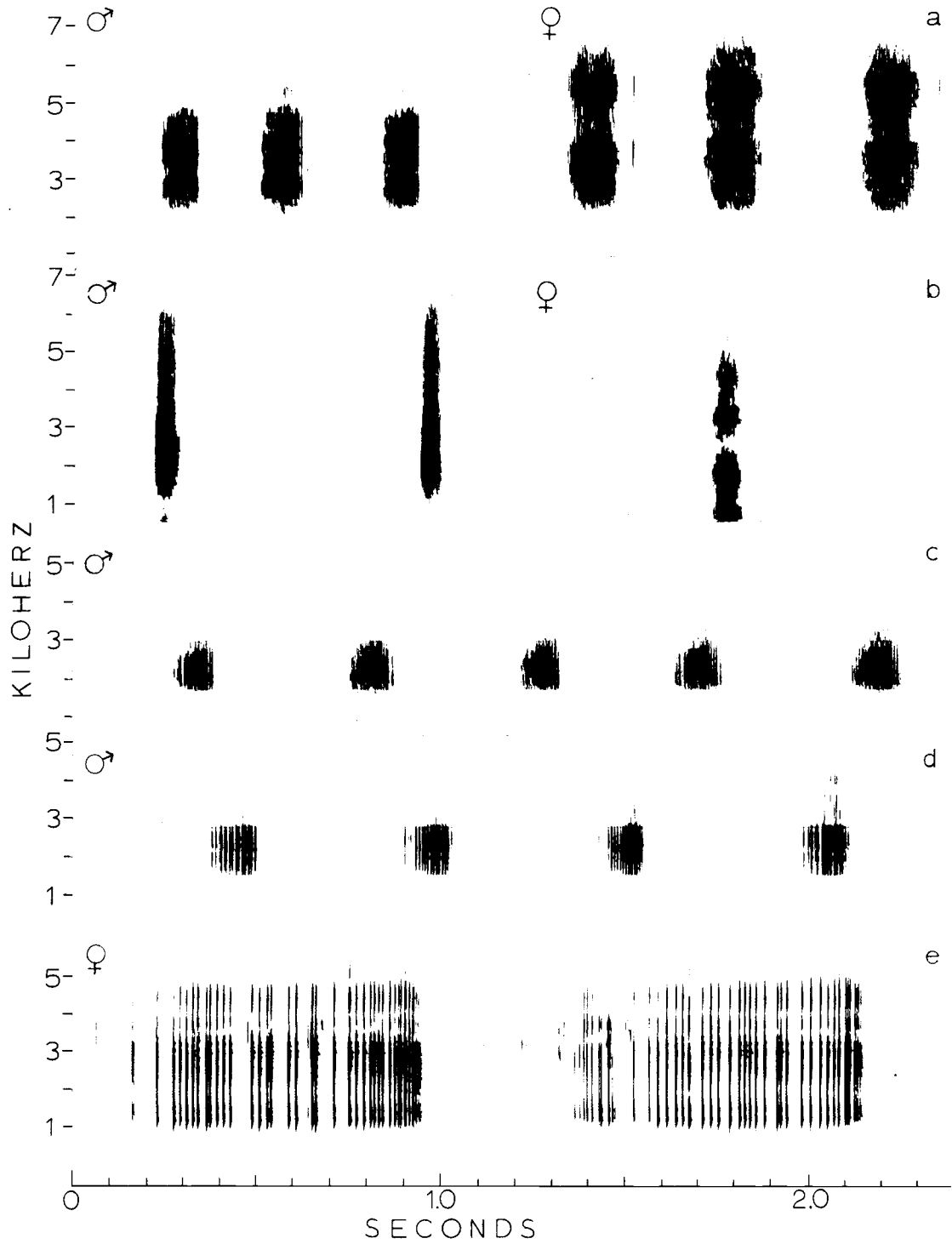


Figure 19. Sonographs of stridulations of *Tropisternus columbianus*: a) stress chirps (26°, 25°C); b) calling chirps (29°, 22°C); c) probing trill (23°C), d) copulation trill (27°C); e) rejection buzz (28°C).

but the rhythm, or chirp rate, of the probing trill was faster than that of the copulation trill ($\alpha = .05$). The trill of an unmounted male was of an even faster rate than these two; but because only one beetle is represented in the data, the rate cannot be compared statistically. The trills are compared graphically in Fig. 18.

Females of columbianus sometimes emitted a rejection buzz similar in pulse structure to that of ellipticus females as they shook and rocked from side to side during male courtship (Fig. 6). This was the only buzz produced by this species.

In summary, male calling, female calling, male stress, and female stress chirps were all distinct in duration, with female chirps being longer than male. Also male copulation trills had a slightly faster rhythm than male probing trills. And the females had a rejection buzz.

Calling chirps had smaller ranges than stress chirps (Fig. 16), as would be expected for signals used in mating behavior.

Tropisternus lateralis limbalis (LeConte)

This is a montane race of T. lateralis that occurs west of Colorado, from British Columbia southward into Central Mexico (Spangler, 1960). In western Oregon, this species overwinters in ponds and breeds in temporary pools or pond edges with a soft mud bottom.

The stridulations of *l. limbalis* were separated into 13 types according to sex and behavioral context: three types of chirps for each sex, five male trills, the male chirp-walk, and male ticking. A summary of the sound measurements, indicating means, standard errors, sample sizes, and the 95% confidence intervals for sample means is shown in Table 7.

When alone or when responding to acoustic or visual stimuli, both sexes emitted calling chirps (Fig. 20). The male chirp given when alone was of shorter duration than the other chirps. I have recordings of only one female alone. Her chirps were about twice the duration of the male alone, but it is possible that they are stress chirps. Sampled response chirps of both sexes were also few in number; however, the ones recorded were longer in duration than male calling chirps when alone. Male stress chirps were similar in duration to female stress chirps and longer than male chirps when alone ($\alpha = .01$). Chirp durations are presented graphically in Fig. 16.

Males produced chirp-trills at times when alone, and trilled when approaching to mount another beetle, when mounted at the head, when probing, and sometimes when copulating (Figs. 20 and 21). The durations of chirps making up trills were similar for all trills. However, the trill rate of unmounted trills was slightly faster ($\alpha = .05$) than the others, which were all similar. Graphical

Table 7. Summary of sound data of Tropisternus lateralis limbalis.

Sound	Duration (seconds)				Chirp rate (chirps/second)			
	\bar{x}	SE	n	95% confidence interval	\bar{x}	SE	n	95% confidence interval
♂ chirp, calling (alone)	0.082	0.004	(22)	0.074-0.090 a ¹				
♀ chirp, calling (alone)	0.170	0.016	(12)	(one ♀ only)				
♂ chirp, calling (response)	0.155	0.025	(8)	0.095-0.215 b				
♀ chirp, calling (response)	0.172	0.034	(17)	0.099-0.244 b				
♂ chirp, stress	0.106	0.003	(73)	0.100-0.112 c				
♀ chirp, stress	0.102	0.003	(71)	0.095-0.108 c				
♂ trill, unmounted	0.022	0.002	(23)	0.019-0.026 d	7.8	0.2	(23)	7.4-8.1 e
♂ trill, mounted	0.025	0.002	(34)	0.022-0.028 d	8.5	0.2	(34)	8.0-9.0 f
♂ trill, probing	0.022	0.001	(29)	0.020-0.024 d	8.6	0.2	(19)	8.1-9.1 f
♂ trill, copulating	0.023	0.002	(14)	0.019-0.027 d	8.4	0.4	(8)	7.4-9.4 f
♂ chirp-trill	0.020	0.006	(23)	0.018-0.022 d	8.1	0.1	(23)	7.9-8.2 g
♂ chirp-walk					3.4	0.1	(6)	3.1-3.7
					<u>Tick rate (ticks/second)</u>			
♂ ticking	0.803	0.038	(39)	0.726-0.881	10.8	0.6	(39)	9.5-12.1

¹Different letters in same vertical column indicate significant differences at $\alpha = .05$ level.

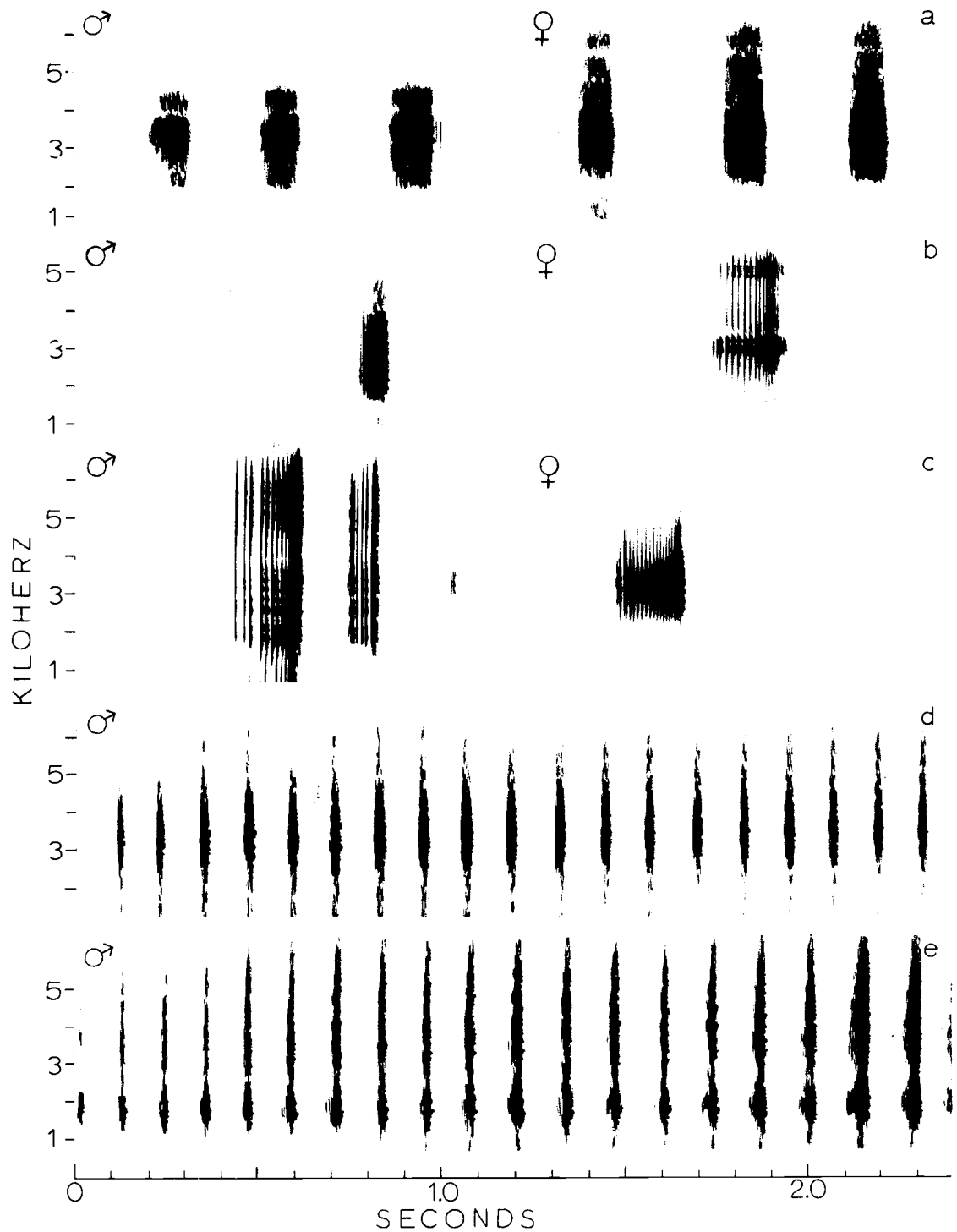


Figure 20. Sonographs of stridulations of *Tropisternus lateralis limbalis*: a) stress chirps (27°C); b) calling chirps, alone (27°C, 21°C); c) calling chirps, response (29°C, 27°C); d) unmounted trill (28°C); e) mounted trill (23°C).

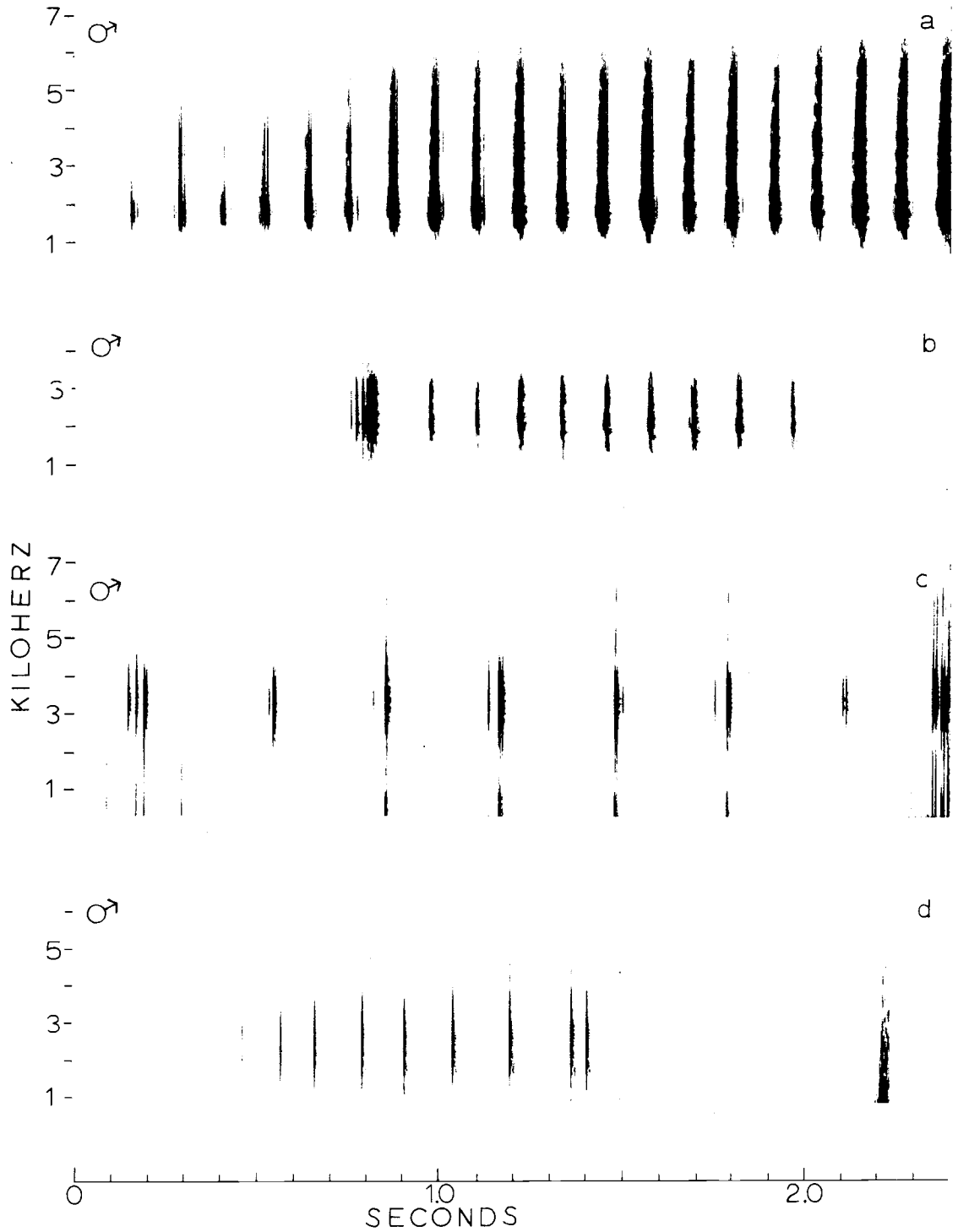


Figure 21. Sonographs of stridulations of *Tropisternus lateralis limbalis*: a) copulation trill (22°C); b) chirp-trill (28°C); c) chirp-walk (28°C); d) ticking (23°C).

presentation (Fig. 18) shows how closely similar trills were in all of these contexts, forming a much tighter group than trills in other species.

Males produced ticking sounds at times when alone, and particularly after being stimulated by chirping or by visual contact with another beetle (Fig. 21). Usually the immediate response to such stimulation was to chirp, but in less than a minute a male would switch to ticking if he was not further stimulated. Ticking phrases contained about eight ticks, ranging between 5 and 13 ticks.

After a beetle (or model) passed by a sexually responsive male, often he began walking stiff-legged and jerkily and produced a series of small sounds I have termed the chirp-walk (Fig. 21). Chirp-walking males invariably attempted to mount any beetle-like object coming into visual range. Chirp-walk sounds are probably a by-product of the state of excitement of the male as he searches for a female, with no signal value.

In summary, calling chirps of males were of shorter duration than male and female stress chirps. All male trills, whether in the context of calling, courtship, or copulation, were similar, although unmounted trills were slightly faster in rate. In addition, males chirp-walked when sexually stimulated, and ticked in calling situations.

As in other species, the range of durations was less in the calling chirp of a male alone than in other chirps.

Tropisternus lateralis nimbatus (Say)

This race of T. lateralis occurs from Canada and the United States east of the Rocky Mountains southward to Panama and the West Indies (Spangler, 1960). The population studied was at Ann Arbor, Michigan. This population breeds in pond edges and temporary pools.

The acoustic signals of T. l. nimbatus have already been described (Ryker, 1972), and were very similar to the male calling chirps, ticks, and trills, and the stress chirps of both sexes of l. limbalis (Figs. 16 and 18, l. nimbatus denoted by *). Additional behavioral observations have added information about contexts in which each signal occurs, as well as three more signals--male chirp-trills, female rejection rattles, and chirp-walks by both sexes (Fig. 22). No attempt was made to quantify measurements of sounds of this species, which was used mainly in studies of signal discrimination.

When alone, males produced calling chirps and, occasionally, ticking sounds, which were previously reported to occur only as responses to the presence of a female. Females produced calling chirps when alone.

Response chirps of males and females to either acoustic or visual stimuli were often of longer duration than chirps given by

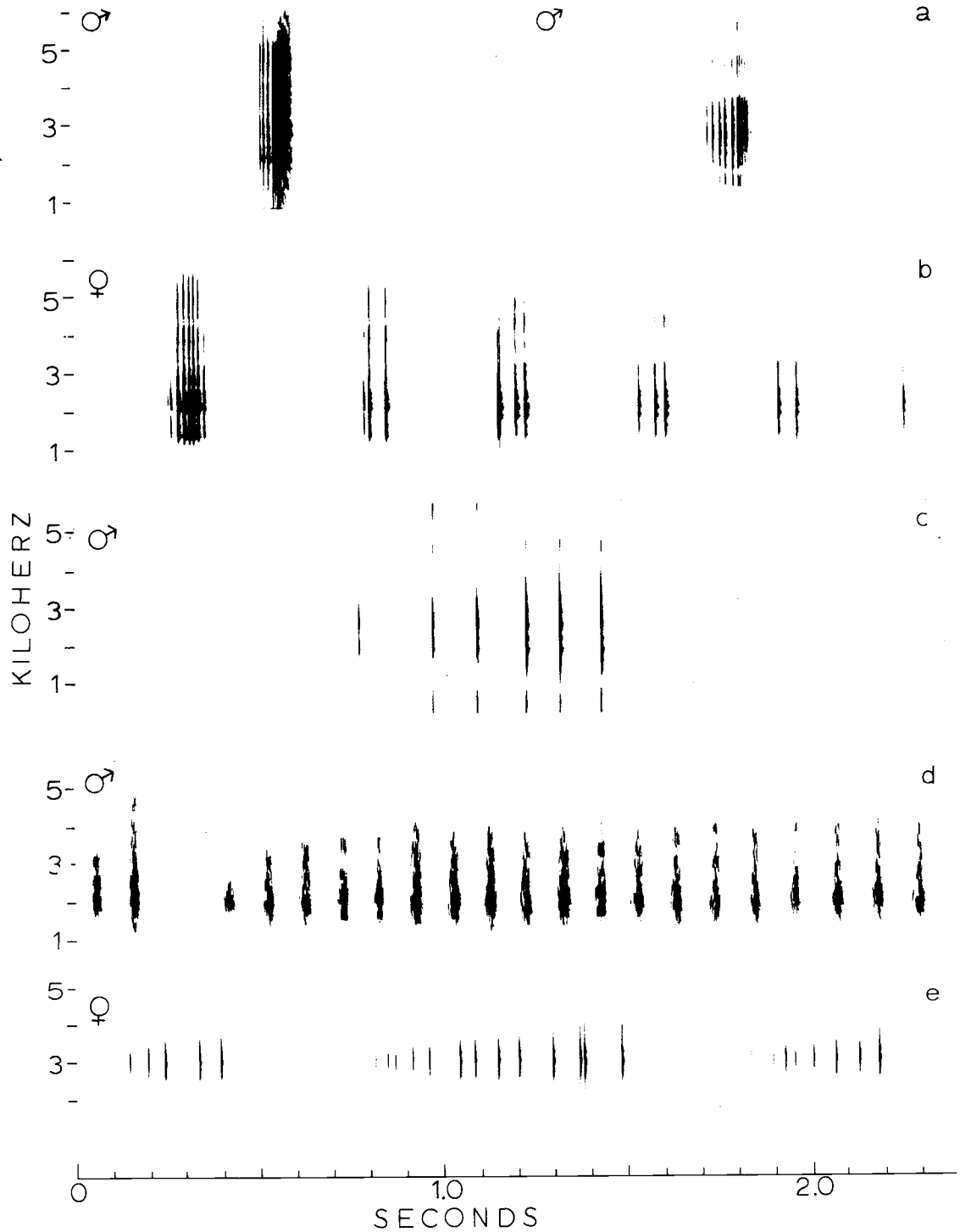


Figure 22. Sonographs of stridulations of *Tropisternus lateralis nimbatus*. a) Calling chirps, alone (left, 26°C), response (right, 26°C); b) response chirp + chirp-walk (27°C); c) ticking (27°C); d) mounted trill (26°C); e) rejection rattle (27°C).

unstimulated beetles (Fig. 22). Sexually responsive beetles of both sexes emitted chirp-walk sounds for a short time following stimulation. Within a minute, males often switched to ticking, although they sometimes continued to give calling chirps.

Males trilled at times when approaching another beetle in preparation to mounting, usually when mounted at the head and during probing, and, rarely, during copulation.

Females sometimes emitted a rejection rattle (= buzz) when a male was mounted on her and courting; this was accompanied by vigorous shaking and rocking from side to side.

Tropisternus natator (D'Orchymont)

This species occurs mainly in the United States east of Colorado. The population studied here was at Ann Arbor, Michigan. It breeds in pond edges and temporary pools.

The stridulations of T. natator were separable into ten types according to sex and behavioral context: three types of chirps for each sex, and four male buzzes. However, because this species was previously studied (Ryker, 1972) and used here mainly in experimental studies of signal discrimination, the sounds were not measured separately for each context. Calling chirps of beetles when alone were not distinguishable in sonographs from response chirps, but both contexts of calling chirps are strikingly different ($\alpha = .01$) from stress chirps (Fig. 23) (Table 8).

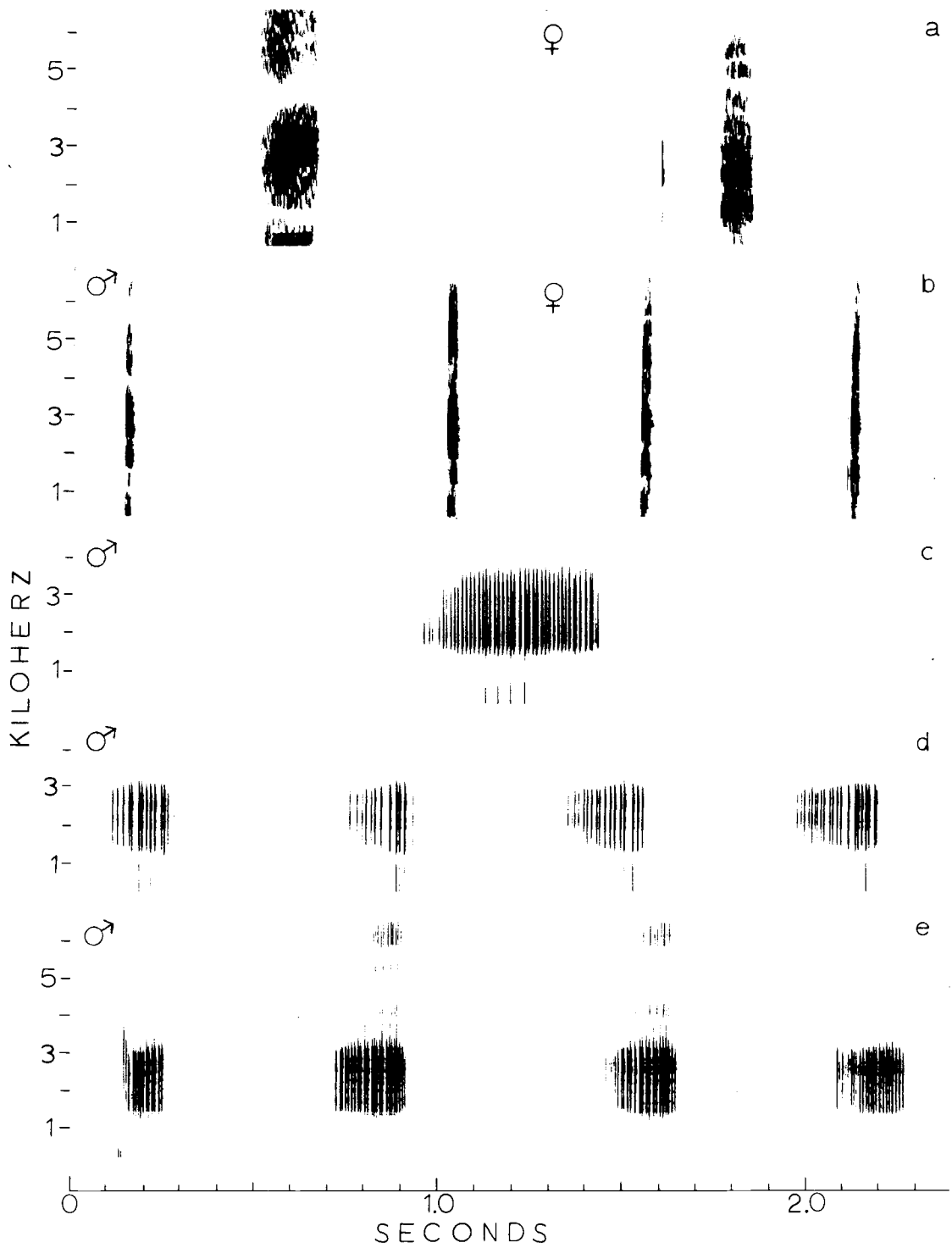


Figure 23. Sonographs of stridulations of *Tropisternus natator*:
 a) stress chirps (28°C); b) calling chirps (21°C, 27°C);
 c) response buzz (27°C); d) mounted buzz (25.5°C);
 e) copulation buzz (27°C).

Table 8. Summary of sound data for Tropisternus natator.

Sound	Duration (seconds)				Pulse rate (pulses/second)			
	\bar{x}	SE	n	95% confidence interval	\bar{x}	SE	n	95% confidence interval
♂ chirp, calling	0.020	0.005	(45)	0.018-0.022 a ¹				
♀ chirp, calling	0.026	0.002	(13)	0.022-0.030 b				
♂ chirp, stress	0.151		(38)	Data combined with Ryker (1972) data-- variance not available				
♀ chirp, stress	0.181		(32)					
♂ buzz, response	0.430	0.029	(23)	0.365-0.491 c	111.0	7.6	(23)	95.2-126.8 e
♂ buzz, rhythmic (mounted)	0.172	0.009	(50)	0.154-0.191 d	123.6	5.3	(50)	112.9-134.3 e
					<u>Buzz rate (buzzes/second)</u>			
					1.62	0.04	(35)	1.54-1.70

¹Different letters in same vertical column indicate significant differences at $\alpha = .01$ level.

Buzzes of males mounted at the head, probing, or copulating were repeated rhythmically and of longer duration than buzzes of unmounted males (Fig. 23), but pulse rates were similar ($\alpha = .05$) (Table 8).

Females gave calling chirps when alone and at times when approaching a male. They were not heard to give rejection sounds.

When alone, males generally emitted calling chirps, but occasionally buzzed. They mostly gave calling chirps in response to calling chirps (sometimes buzzes), but usually switched to buzzes when approaching to mount another beetle. Rhythmic buzzes were given during courtship at the head and while probing, and during copulation.

Signal Distinctiveness between Species

The calling chirps of the three western Oregon species of Tropisternus are quite similar in duration-- .060 sec in T. columbianus, .079 sec in ellipticus, and .082 sec in l. limbalis. Although the chirp of columbianus is statistically shorter, the other two are not different.

In courtship behavior, however, ellipticus is the only species of the three to have a buzz, and the fast trill of l. limbalis is also distinctive. Males of columbianus are silent at the head and always give a slow trill of rhythmic chirps during probing. This trill is

similar in chirp rate to that sometimes given by probing males of ellipticus, but the duration of each component chirp is twice as long in columbianus as in ellipticus.

It seems, therefore, that courtship sounds have more signal specificity than calling sounds. Buzzes of ellipticus and ticking sounds of l. limbalis produced in calling situations do not make the calling signals of the three species distinctive because these calling signals are often omitted. In fact, the mating sequence frequently begins with mounting by the male upon contact with a female, with no prior signals.

Signal Distinctiveness within Species

Six distinct signals are produced by T. ellipticus: calling chirps, stress chirps, male buzzes, probing slow trills, copulation slow trills, and the female rejection buzz.

Seven distinct signals are produced by columbianus: male calling chirps, female calling chirps, male stress chirps, female stress chirps, male probing trills, copulation trills, and the female rejection buzz.

Four distinct signals are produced by l. limbalis: calling chirps, stress chirps, male trills, and male ticking.

Five distinct signals are produced by l. nimbatus: the four signals mentioned for l. limbalis above plus the female rejection rattle.

Four distinct signals are produced by natator: calling chirps, stress chirps, unmounted male buzzes, and mounted rhythmic male buzzes.

VII. SIGNAL CONTEXT VARIABILITY

Species of Tropisternus do not have distinct acoustic signals for each context in which sounds are used; the use of signals varies in two ways. A species may use a measurably identical sound in two or more contexts, and in a particular context a species may use more than one sound.

Context-specificity of Sounds

Table 9 summarizes observations on signals used in mating sequences by five species of Tropisternus, showing how frequently each type of signal was used in each of five contexts: in acoustic contact with the female, having made visual contact with the female, mounted and positioned above the head of the female, mounted and probing, and copulating.

In all five species, calling chirps were only produced in calling situations and never during courtship or copulation. If a female moved during copulation, males would give stress chirps (not shown in table), but not chirps measurably similar to calling chirps.

Except for T. columbianus, males used the courtship sound in more than one context. For example, ellipticus used its buzz in the calling situation, while mounted at the head, while probing, and even during copulation. The two subspecies of lateralis (limbalis and

Table 9. Signals and signal contexts for males of five species of *Tropisternus*.¹

Calling		Courtship		Copulation
Acoustic contact	Visual contact	At head	Probing	
<u>ellipticus</u>				
96 SILENT	54	15	69	50
2 CHIRP	6			
2 BUZZ	44	85	17	20
(n = 54)	(n = 54)	(n = 52)	17 s-TRILLA (n = 52)	30 s-TRILLb (n = 20)
<u>columbianus</u>				
60 SILENT	76	96		25
40 CHIRP	24			
(n = 25)	(n = 25)	4 s-TRILL (n = 25)	100 (n = 25)	75 (n = 4)
<u>l. limbalis</u>				
80 SILENT	66	34	15	67
13 CHIRP	7			
7 TICK	3			
(n = 30)	27 f-TRILL (n = 30)	66 (n = 29)	85 (n = 26)	33 (n = 6)
<u>l. nimbatius</u>				
25 SILENT	24	7	54	89
44 CHIRP	29			
31 TICK				
(n = 16)	35 f-TRILL 12 c-WALK (n = 17)	93 (n = 14)	46 (n = 13)	11 (n = 9)
<u>natator</u>				
35 SILENT				
41 CHIRP	13			
24 l-BUZZ	87 l-BUZZ	100 r-BUZZ	100 r-BUZZ	100 r-BUZZ
(n = 17)	(n = 15)	(n = 15)	(n = 15)	(n = 5)

¹ Numbers are percentages. s-TRILL = slow trill; f-TRILL = fast trill; l-BUZZ = long buzz; r-BUZZ = rhythmic buzz; c-WALK = chirp walk.

nimbatus) used the fast trill in the same four contexts. And natator used its rhythmic buzz in courtship at the head and while probing, and during copulation.

Tickling sounds produced by l. nimbatus and l. limbalis are specific to the calling situation, and are only used when the males are alone or after they have contacted the female but are unable to see her.

In summary, calling sounds are restricted to the calling situation in Tropisternus; however, sounds used in courtship may also be produced in calling and copulation contexts.

Signal-specificity of Contexts

All species of Tropisternus are not equally variable in their use of signals. Some species are more stereotyped and almost always are either silent in a particular context, or give a particular sound. Other species may in a particular context be silent, give one, or give some other sound.

Sounds given during copulation can have no recognition or sexual stimulation function, and are likely to be more variable in their use. The two subspecies of T. lateralis, for example, were often silent, but sometimes trilled, during copulation (Table 9). And ellipticus was silent half the time, and either buzzed or slow-trilled the other times.

On the other hand, natator gave rhythmic buzzes five out of five times.

Behavior in calling situations was quite variable, with males of a species either being silent or producing from one to four different sounds. For example, males of l. nimbatus were either silent, chirped, ticked, fast-trilled, or chirp-walked before the male found and mounted a female. The Oregon species, ellipticus, columbianus, and l. limbalis, were more frequently silent before mounting than were the two Michigan species, natator and l. nimbatus. The least variable species was columbianus, which always gave calling chirps if it stridulated.

T. ellipticus was the most variable species in courtship sounds; males were usually silent, but sometimes buzzed or gave slow trills. In fact, the same individuals varied in courtship behavior. One male was observed in five mating sequences: during probing he was silent twice, buzzed twice, and slow-trilled once; and during copulation he was silent twice, buzzed once, and slow-trilled once.

T. columbianus and natator were very consistent in their courtship behavior: columbianus was almost always silent at the head, and always slow-trilled during probing; and natator always gave rhythmic buzzes both at the head and during probing.

Although courtship in the two subspecies of lateralis involves the same sound, fast trills, it was produced more often at the head than

during probing in nimbatus, and more often during probing in limbalis.

In summary, species of Tropisternus may produce several sounds in calling situations, and usually have a single courtship sound. The two Michigan species produced calling sounds before mounting more often than the Oregon species. T. ellipticus was the most variable species in courtship sounds, and columbianus and natator were the least variable.

VIII. EXPERIMENTS IN ACOUSTICAL SIGNAL RECOGNITION

Four species of Tropisternus were tested with a patterned series of experiments to determine what kinds of stimuli were discriminated as signals, and particularly, which sounds might function as calling sounds. Tape recorded stridulations were broadcast to beetles via an underwater speaker, and models of beetles were presented both separately and in combination with other stimuli. In some tests, the entire experimental design was repeated with either a caged, silenced male or a caged, silenced female present but not visually apparent in the test chamber. Responses to chemical and visual stimuli are discussed in an earlier chapter, but the entire experimental design will be considered here intact. Two types of responses to stimuli were recorded: the number of stridulations by the tested beetle during five minutes and the reaction of the beetle to three presentations of a model (plastic-coated, dead beetle, glued to the tip of a slender glass rod). General activity, such as feeding, cleaning, walking, swimming, and resting was also noted. These data will be discussed by species and by sex.

Beetles were tested singly in a small, plexiglas chamber. Recorded stridulations were broadcast underwater through a small speaker at intensities similar to those produced by live beetles, as monitored by the VU meter of a second tape recorder, which was also

used to tape record some of the responses. Sounds broadcast to a beetle were repeated at varying intervals, usually every 5-10 seconds. Test beetles were placed into the test chamber and allowed to acclimate to the new surroundings for one or more hours before the test series was begun. Tests were never initiated or resumed unless the test beetle was feeding and showed no signs of stress. Each individual beetle was given the entire series of tests within several hours in the same day to avoid day to day changes in behavior.

Tropisternus natator

Both males and females of this species were tested in July, 1974, using three stridulations (of natator) as stimuli--stress chirps, calling chirps, and the male courtship buzz. Samples of these broadcast stridulations were re-recorded and are illustrated by sonographs in Fig. 24. Table 10 summarizes the results of the tests. Preceding the tests with broadcast sounds, a control was run using visual stimuli (the model) only (Series 1); and in each of the following series of tests, acoustic signal presentation was preceded by a five minute silent control period. Responses during the period immediately following model presentations are recorded as -, 0, +, B, and C, denoting escape response, no response, approach response, buzz response, and chirp response, respectively. The last column (Sex. Resp.) refers to the results of an opportunity for the test beetle to

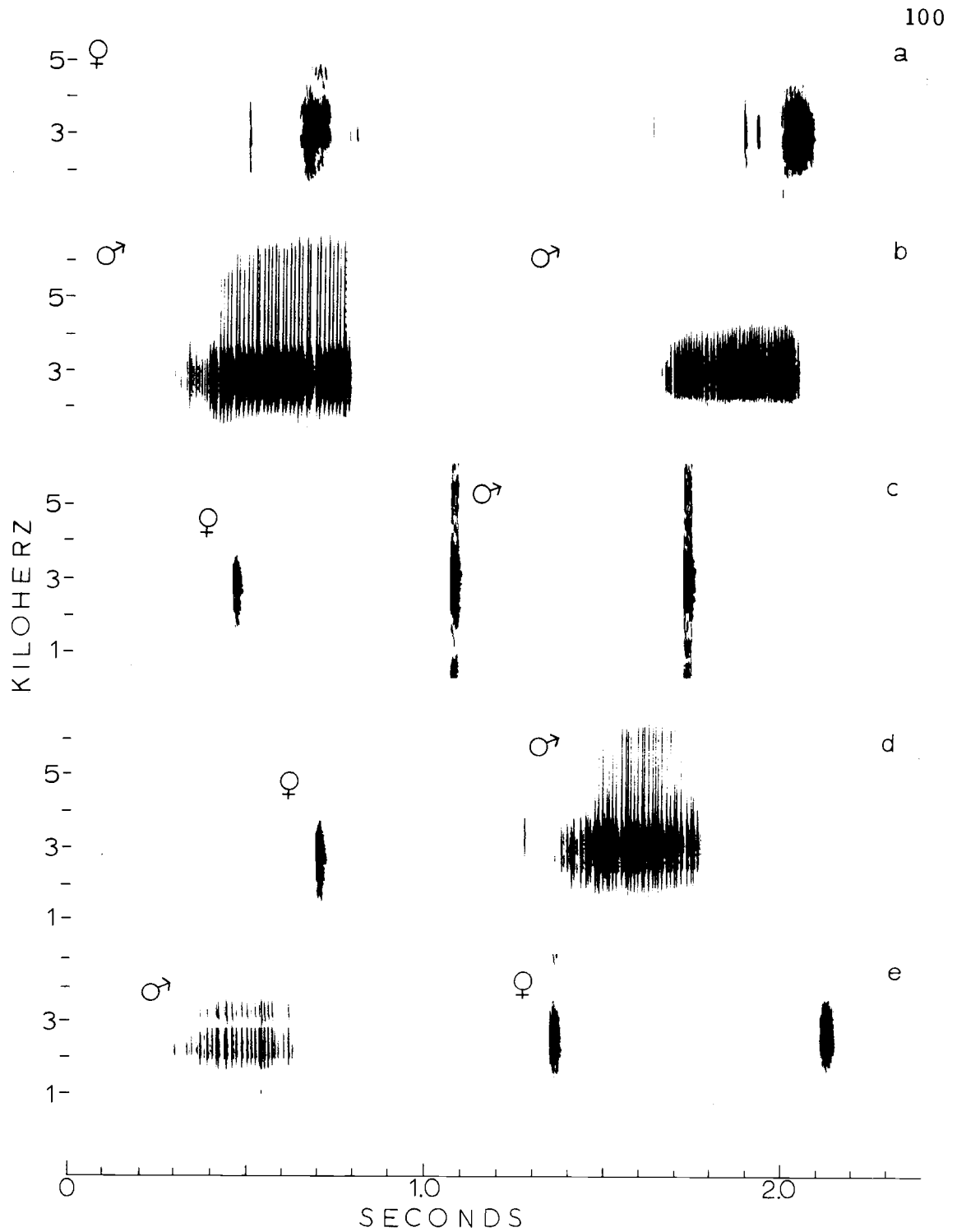


Figure 24. Sonographs of stridulations of *Tropisternus natator*: tape recorded broadcast signals and responses: a) broadcast ♀ stress chirps; b) buzz (left) and broadcast buzz (right); c) broadcast ♀ calling chirp and two ♂ chirp responses; d) broadcast ♀ calling chirp and ♂ buzz response; e) ♀ chirps in response to ♂ buzz.

Table 10. Number of stridulatory responses/5 minutes by males and females of Tropisternus natator in four series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded stress chirps followed by model presentation and then a silent period. Series 3: response to recorded calling chirps of the opposite sex followed by model presentation and then a silent period. Series 4: response to recorded male buzzes followed by model presentation and then a silent period. Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, c = chirp response, and b = buzz response. Each series was begun with a control period (No. stim.). Responses during the period immediately following model presentations (After model) were also counted. Last column (Sex. resp.) indicates whether males courted and females copulated when paired with the opposite sex immediately following the test series.

Table 10.

Beetle no.	No. stridulations per 5-minute period															Sex resp.
	Series 1			Series 2				Series 3				Series 4				
	No stim.	Model	After model	No stim.	Stress chirps	Model	After model	No stim.	Calling chirps	Model	After model	No stim.	Courting buzz	Model	After model	
♂ 1	0	+00 b	0	0	0	+++ bbb	0	0	60	+++ bbb	15	2	34	+++ bbb	5	Yes
♂ 3	1	0++	2	0	1	+++	6	4	115	+++ ccc	15	0	5	0+0	0	Yes
♂ 4	31	+++ bbb	2	1	2	+++ bc	42	26	152	+++ bbb	27	0	0	0+0	0	Yes
♂ 5	0	+++ bbb	0	0	0	+++ bbb	0	0	53	+++ bbb	0	0	6	+++ bbb	7	Yes
♂ 6	21	0++ bb	4	8	16	+++ bb	6	6	151	+++ bbb	24	5	1	000 bb	1	Yes
♂ 7	0	+++ bbb	0	0	0	+++ bbb	2	0	23	+++ bbb	0	0	0	+++ bbb	0	Yes
Σ	53		8	9	19		56	36	554		81	7	43		13	
♀ 2	0	000	0	0	0	+0+ c	0	0	0	+++ cc	0	0	0	+00 c	0	Yes
♀ 3	11	+-	5	2	0	+++	0	1	12	+++ cc	0	0	0	+++ ccc	1	No
♀ 4	1	+0 c	0	0	0	+00	0	5	0	+00	0	0	0	+++	0	Yes
♀ 5	0	+++	0	0	0	+++	0	0	1	+++	0	0	0	+++	0	Yes
♀ 6	0	+++	0	0	0	+++	0	0	0	+++	0	0	0	+++	0	No
♀ 7	0	---	0	1	2	+++ ccc	0	0	0	+++	0	0	2	-+0	0	No
Σ	12		5	3	2		0	6	13		0	0	2		1	

court or be mated. Sexually responsive beetles are indicated by "yes," and unresponsive ones by "no."

The results of the test series with males of T. natator are clearcut. As measured by stridulatory responses, the males discriminated tape recorded broadcasts of calling chirps from stress chirps and male courtship buzzes, increasing their chirping or buzzing from an average of six stridulations to 92 stridulations per five minute period when stimulated by calling chirps. As shown by the Wilcoxon signed ranks, matched pairs test, the response to calling chirps is greater ($\alpha = .05$) than to its control, than to model presentation, and than to stress chirps or to courtship buzzes. Responses to all stimuli other than calling chirps are not significantly different from controls.

None of the females tested showed similarly marked responses to any of the acoustic stimuli, even though three of the six females were sexually responsive and copulated following the tests. However, females sometimes chirped when approaching the beetle model. Although females showed no response to the male courtship buzz in these tests, one female was tape recorded responding to both male chirps and buzzes (Fig. 24e) when both beetles were in the same chamber to test for sexual receptivity.

With the exception of one male, the majority of male responses to calling chirps were also chirps. However, the majority of the

responses to visual stimuli were (in addition to approaching) buzzes. Once a female is contacted, even if only briefly, males usually switched to buzzes. Both chirp- and buzz-responses of males to the broadcast female calling chirp are shown with the broadcast signal in Fig. 24c and d.

As males increased in their rate of chirping, often responding by chirping just after a broadcast chirp, they also decreased the time spent feeding and increased the time spent walking and swimming. Males chirping or buzzing in response to calling chirps always (Table 10, Series 3, Model column) stridulated and approached the model, often swimming directly to the mounted position, rather than walking up to the model.

These data establish, at least for T. natator, several points more firmly than casual observation has in the past:

1. A species of Tropisternus can detect sounds transmitted through water, even though no auditory organs for sound reception have been found.
2. Males are able to discriminate calling chirps from stress chirps and male courtship buzzes.
3. Calling chirps release several behaviors in males--stridulation (chirping or buzzing), increased walking or swimming, and an increased likelihood of approaching and mounting another beetle when encountered.

Tropisternus lateralis nimbatus

Males and females of this species were tested in July, 1974, with two acoustic stimuli--calling chirps and male ticking. The procedure for testing was similar to that of T. natator, except that individual males were run through the series of tests two or three times, on different days.

Six males were tested for a total of 16 replications (Table 11). The controls (No Stimulus) in each series were tested against each other and are not different ($\alpha = .05$), so there is no measurable carryover effect from one series to another. The increased chirp response with visual stimuli (models) was discussed previously.

Broadcasts of ticking received the smallest chirp response (ns, $\alpha = .05$), and calling chirps received the greatest response, greater than the responses to all other stimuli ($\alpha = .01$). Responses to calling chirps were chirps in 15 of 16 males, and the other male responded with both ticks and chirps. Notice (Table 11) that individual males respond differently on different days.

Like natator, males of T. nimbatus that responded to broadcast chirps decreased the time spent feeding, increased the time spent walking or swimming, and always approached the models. Once a female was contacted, even if only briefly, males often switched to ticking stridulations. The stimulus to begin ticking may be either visual, chemical, or both.

Table 11. Number of stridulatory responses/5 minutes by males of Tropisternus lateralis nimbatus in three series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded female chirps (Chirp stim.) followed by model presentation and then a silent period. Series 3: response to recorded ticks (Tick stim.) followed by model presentation and then a silent period. Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, and c = chirp response. Each series was begun with a control period (No stim.). Responses during the period immediately following model presentation (After model) were also counted. Last column (Sex. resp.) indicates whether male courted a female immediately following the test series.

Table 11.

Male no.	No. of stridulations per 5-minute period												Sex resp.
	Series 1			Series 2				Series 3					
	No stim.	+ Model	After model	No stim.	Chirp stim.	+ Model	After model	No stim.	Tick stim.	+ Model	After model		
1	7	--0	4	1	160	+++ ccc	10	0	0	+++ c	0	-	
	0	+++ cc	0	0	4	+++	0	0	2	+++	0	Yes	
	10	--0	4	5	143	+++	20	21	14	+++	8	Yes	
2	55	+0+	64	38	91	+++ ccc	98	0	2	+++ ccc	25	-	
	0	--0	0	0	0	+++	0	0	0	+00	0	Yes	
	17	+++ ccc	19	7	39	+++ ccc	53	2	8	+++ ccc	27	Yes	
3	24	--	64	69	296	+++ c c	65	61	38	+++ cc	54	-	
	0	0+0	0	0	0	000	0	0	0	000	0	Yes	
	0	+00	3	0	0	+00	0	0	0	000	0	No	
4	44	+++	94	56	128	+++ ccc	52	39	46	+++ ccc	41	-	
	7	---	12	39	273	+++ ccc	78	28	25	+++ ccc	34	Yes	
	15	+++ ccc	53	23	450	+++ ccc	91	17	14	+++ cc	30	Yes	
5	0	000	0	5	4	+++	0	0	0	000	0	-	
	0	000	2	0	0	+00	0	0	0	000	0	Yes	
6	1	+++ ccc	10	44	90	+++ ccc	81	49	23	+++ ccc	57	-	
	24	000	30	12	112	+++ ccc	40	20	21	+++ ccc	39	Yes	
Σ	204		359	299	1790		588	237	193		315		

Females tested did not show similarly marked responses to either chirp or tick broadcasts, with the exception of female No. 1 (Fig. 25c) (Table 12). Surprisingly, female No. 1 was not responsive to mating attempts by a male, even though she gave phonoresponses to calling chirps. Other females, which were sexually responsive and copulated with males, did not give phonoresponses except to visual stimulation from models (Fig. 25a). On rare occasions, females have been observed to respond to male chirping and ticking by chirping and swimming actively (Ryker, 1972), and a threshold response that depends upon sexual receptivity may be involved.

The data for males provide additional support for the three conclusions about acoustic communication in Tropisternus mentioned for data from T. natator males.

Tropisternus lateralis limbalis

Males of T. l. limbalis were collected August 9, 1974, maintained under continuous light, and tested until August 24. As with l. nimbatus, two stridulations were broadcast, ticks and calling chirps (Fig. 25d, e) (Table 13). Test series with both acoustic stimuli were duplicated so that they were run once with a caged, silenced male present (Series 2 and 3), and again with a caged, silenced female present (Series 4 and 5). Visual and chemical stimuli were discussed previously.

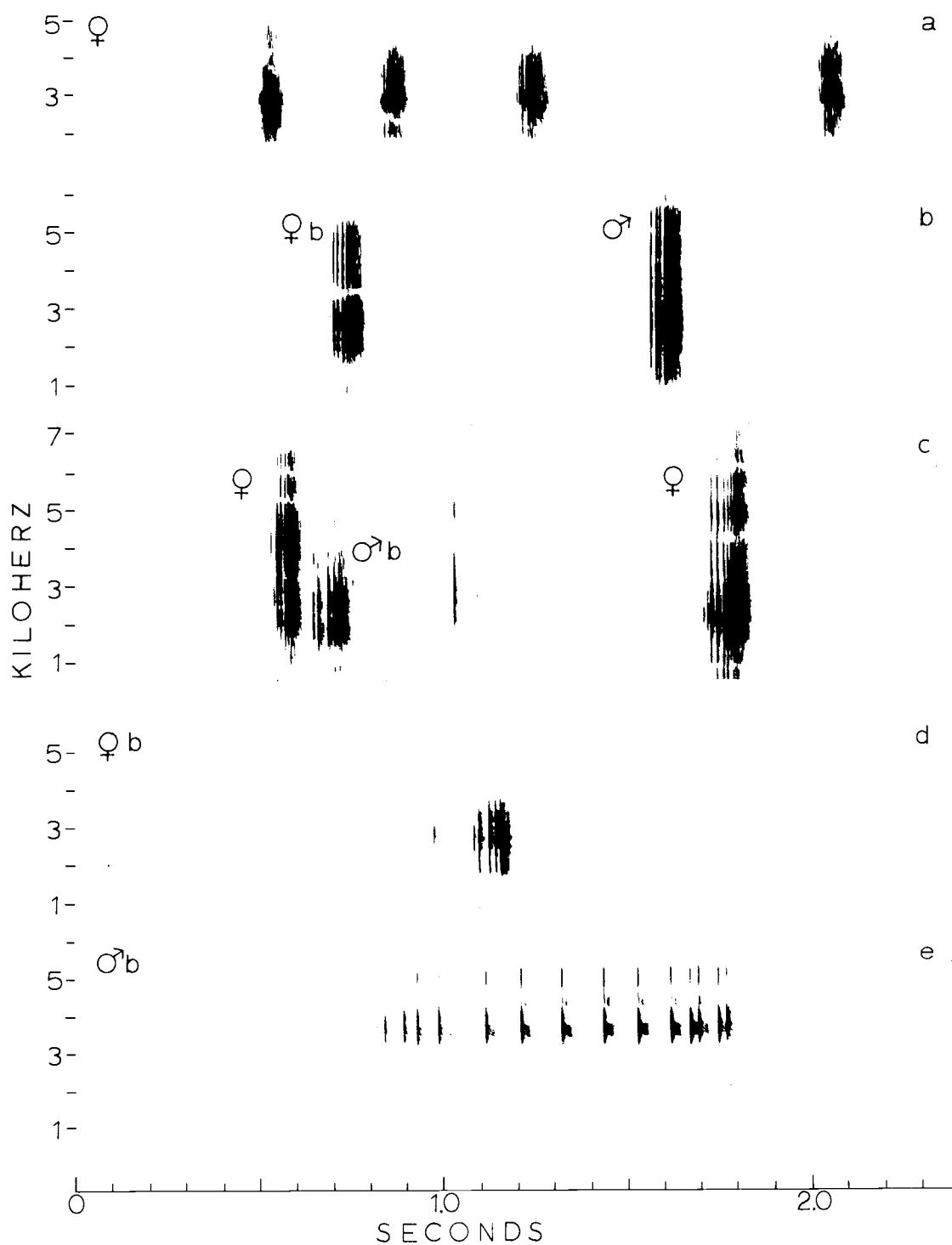


Figure 25. Sonographs of stridulations of Tropisternus lateralis - tape recorded broadcast signals and responses; l. nimbatu: a) calling chirps as ♀ approaches model; b) broadcast chirp (b) and response; c) broadcast chirp and two responses; l. limbalis: d) broadcast ♀ chirp; e) broadcast ticking.

Table 12. Number of stridulatory responses /5 minutes by females of Tropisternus lateralis nimbatus in three series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded male chirps (Chirp stim.) followed by model presentation and then a silent period. Series 3: response to recorded ticks (Ticking stim.) followed by model presentation and then a silent period. Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, and c = chirp response. Each series was begun with a control period (No stim.). Responses during the period immediately following model presentation (After model) were also counted. Last column (Sex. resp.) indicates whether female copulated with a male immediately following the test series.

Table 12.

Female no.	No. of stridulations per 5-minute period												Sex resp.
	Series 1			Series 2				Series 3					
	No stim.	Model	After model	No stim.	Chirp stim.	Model	After model	No stim.	Ticking stim.	Model	After model		
1	0	+++	0	0	39	+++ ccc	0	0	0	+++ c	0	No	
2	12	+++ c	1	3	1	+++	1	0	0	+++ c	0	Yes	
3	0	+++ ccc	0	0	0	+++ ccc	0	0	0	+++ c	4	Yes	
4	0	000	5	0	0	+-- c	0	0	0	+++ c	0	No	
5	0	---	0	0	0	00+ c	0	0	1	000	0	Yes	
6	0	000	0	0	0	000	0	0	0	000	0	Yes	
Σ	12		6	3	40		1	0	1		4		

Table 13. Number of stridulatory responses/5 minutes by males of Tropisternus lateralis limbalis in five series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded male ticks (+ Tick stim.) in the presence of caged silenced male, preceded by silent period and followed by model presentation and then another period of male ticks (After model). Series 3: the same as Series 2, except caged, silenced female present. Series 4: response to recorded female calling chirp (+ Chirp stim.) in the presence of caged, silenced male, preceded by silent period and followed by model presentation and then another period of calling chirps (After model). Series 5: same as Series 4, except caged, silenced female present. Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, and C = chirp response. Each series was begun with a control period (No stim.). Last column (Sex. resp.) indicates whether male courted when paired with a female immediately following the test series.

Table 13.

Male no.	No. of stridulations per 5-minute period												Sex resp.
	Series 1			Series 2				Series 3					
	No stim.	+ Model	After model	Silent ♂	+ Tick stim.	+ Model	After model	Silent ♀	+ Tick stim.	+ Model	After model		
1	0	000	0	0	1	+0+ c c	0	4	20	+++ ccc	3		
2	0	+++	0	1	4	+++	0	4	17	+++	0		
3	6	+++ cc	1	4	1	+++	0	27	22	+++ ccc	25		
4	6	+++ ccc	47	0	0	+00	0	0	0	+++	0		
5	7	00-	26	20	5	+++	7	13	15	+++	0		
6	0	+++	0	0	0	+++	0	0	0	+++	0		
7	0	+++	0	0	0	+++ cc	0	0	0	+++ ccc	6		
Σ	19		64	25	11		7	48	74		34		
				Series 4				Series 5					
				Silent ♂	+ Chirp stim.	+ Model	After Model	Silent ♀	+ Chirp stim.	+ Model	After model		
1				1	0	+++ ccc	1	1	7	+++ ccc	23	Yes	
2				0	0	+++	0	26	2	+++ cc	7	Yes	
3				5	10	+++	1	54	35	+++ cc	8	Yes	
4				2	0	+++	3	6	0	+++	0	Yes	
5				23	40	+++	1	6	0	+++	0	Yes	
6				0	0	+++	0	0	0	+++ cc	0	Yes	
7				2	0	++0	0	0	0	00+	0	Yes	
Σ				33	50		6	93	44		38		

None of the responses to any stimulus are statistically different from the controls or other stimuli. Regardless of the fact that the Michigan subspecies, l. nimbatus, markedly responded to similar calling chirps in the same experimental setup three weeks earlier, males of l. limbalis did not. However, males of l. limbalis courted females vigorously (three successful copulations) when paired with them following the tests. Also, males responded to models similarly to l. nimbatus, often chirping while approaching.

Tropisternus ellipticus

Males of T. ellipticus were collected August 3, 1974, maintained under continuous light conditions, and tested until September 12. Female calling chirps and male courtship buzzes (Fig. 26) were broadcast (Table 14). The two stridulation series were run once with a silenced, caged male present (Series 2 and 3) and duplicated with a silenced, caged female present (Series 4 and 5). Visual and chemical stimuli were discussed previously.

Even without statistical analysis, the lack of response to acoustical stimuli (whether or not a silenced female was present) is apparent. Three of the five males tested were sexually responsive, but were not stimulated to stridulate by broadcast sounds. However, males did respond to models by approaching either silently, with chirping, or with buzzing.

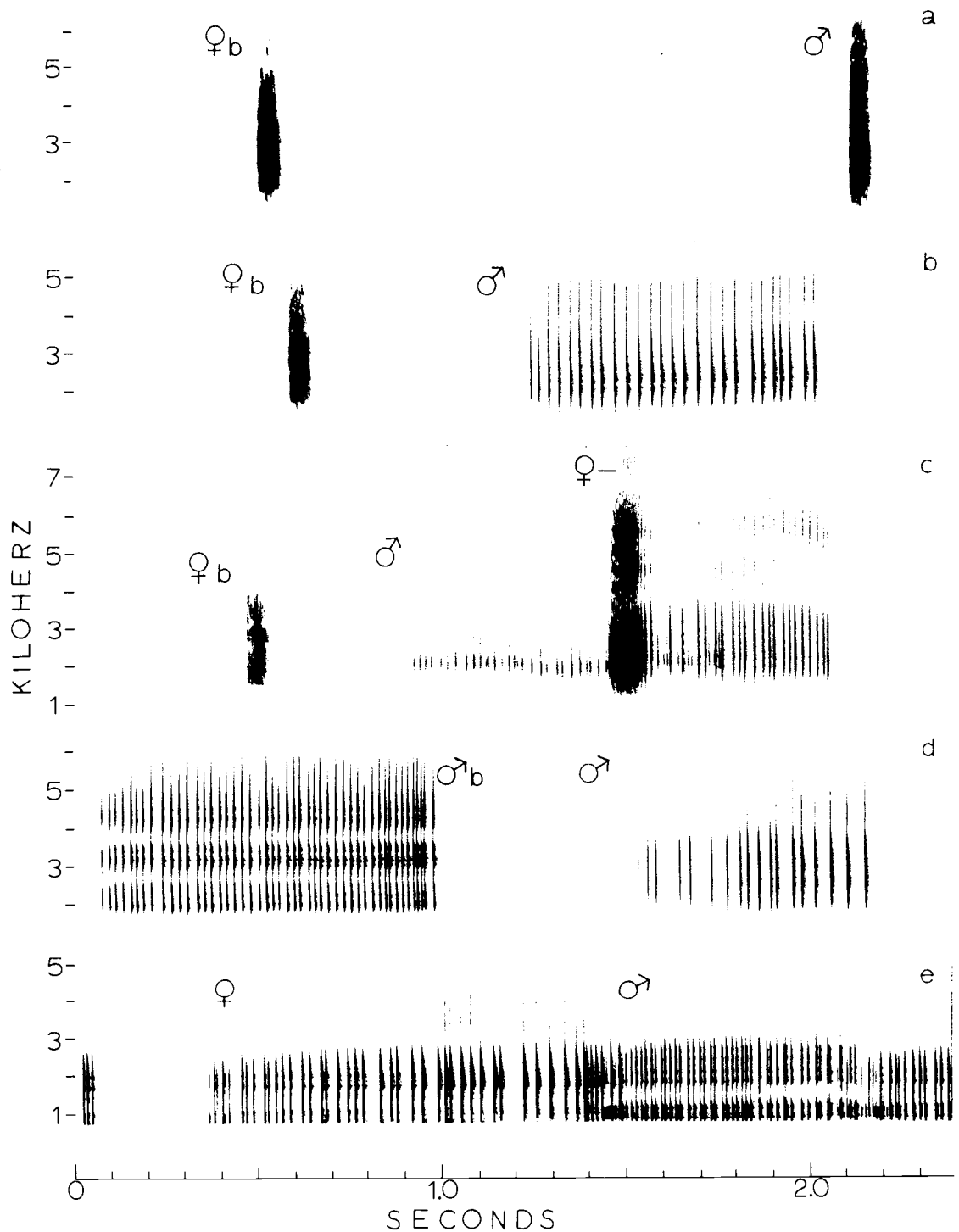


Figure 26. Sonographs of stridulations of *Tropisternus ellipticus* - tape recorded broadcast signals and responses: a) broadcast calling chirp (b) and response chirp; b) broadcast calling chirp and buzz response; c) broadcast calling chirp and responses of male and female; d) broadcast buzz and buzz response; e) interaction of courting male and rejecting female, face to face.

Table 14. Number of stridulatory responses/5 minutes by males of Tropisternus ellipticus in five series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded female calling chirps (+ Chirp stim.) in the presence of caged, silenced male, preceded by silent period and followed by model presentation and then another period of calling chirps (After model). Series 3: the same as Series 2, except caged, silenced female present. Series 4: response to recorded male buzz (+ Buzz stim.) in the presence of caged, silenced male, preceded by silent period and followed by model presentation and then another period of male buzzes (After model). Series 5: the same as Series 4, except caged, silenced female present. Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, b = buzz response, and c = chirp response. Each series was begun with a control period (No stim.). Last column (Sex. resp.) indicates whether male courted when paired with a female immediately following the test series.

Table 14.

Male no.	No. of stridulations per 5-minute period												Sex resp.
	Series 1			Series 2				Series 3					
	No stim.	Model	After model	Silent ♂	+ Chirp stim.	+ Model	After model	Silent ♀	+ Chirp stim.	+ Model	After model		
1	0	+++ bbb	0	0	0	+++ cc	0	13	6	+++ bbb	1		
2	0	+++ c	0	0	0	++0	0	0	0	+++ cc	0		
3	0	000	0	0	0	++0	0	0	0	++0	0		
4	0	+++	0	1	0	+++	1	1	0	++0	0		
5	0	+++ cc	8	0	0	+++	0	0	0	+++ c	5		
Σ	0		8	1	0		1	14	6		6		
				Series 4				Series 5					
				Silent ♂	+ Buzz stim.	+ Model	After model	Silent ♀	+ Buzz stim.	+ Model	After model		
1				2	1	+++ cb	2	0	0	++0	0	Yes	
2				0	0	+++ c	0	0	0	000	0	Yes	
3				0	0	+++	0	1	0	++0	0	No	
4				0	0	+++	1	2	0	+++	0	Yes	
5				0	0	+++	0	0	0	++0	0	No	
Σ				2	1		3	3	0		0		

Numbers of females were not tested, but a wide variability in response to acoustic stimuli is shown by tests of two females, both of which copulated in response to male courtship after the test series (Table 15). One of the females responded by chirping, especially in response to male calling chirps; but the other female was completely silent in all stimulus situations, and did not stop feeding until mounted by the male. The female that gave phonoresponses also interrupted her feeding to walk and swim frequently, particularly during broadcasts of calling chirps.

Two virgin females of ellipticus were obtained by rearing. They eclosed from pupal chambers September 15, 1974, were kept under continuous light, were acclimated to the testing chamber for two weeks, and were tested between November 9 and December 14. The first female escaped and was lost during the fifth test series, but the second was paired with a similarly reared male on December 21. She produced three cases of fertile eggs during the next three weeks. These two females, then, were probably physiologically ready for mating during the testing period.

On three of nine occasions the virgin females gave chirp responses when stimulated with broadcast male calling chirps, but were silent in six other tests, even though sexually responsive (Table 16). That the females were receptive to mating was shown by their permitting copulation when paired with males. Copulations were

Table 15. Number of stridulatory responses/5 minutes by two sexually responsive females of *Tropisternus ellipticus* in three series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Female no.	No. of stridulations per 5-minute period												Sex resp.
	Series 1			Series 2				Series 3					
	No stim.	Model	After model	No stim.	Buzz stim.	Model	After model	No stim.	Chirp stim.	Model	After model		
1	0	000	7	3	8	+0+ c	0	18	39	000	32	Yes	
2	0	-00	0	0	0	+++	0	0	0	+++	0	Yes	

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period. Series 2: response to recorded male buzzes (Buzz stim.) followed by model presentation and then another period of male buzzes (After model). Series 3: response to recorded male calling chirps (Chirp stim.) followed by model presentation and then another period of calling chirps (After model). Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, and c = chirp response. Each series was begun with a control period (No stim.). Last column (Sex. resp.) indicates whether female copulated with a male immediately following the test series.

Table 16. Number of stridulatory responses/5 minutes by reared, virgin females of Tropisternus ellipticus in three series of stimulus situations run consecutively, with a 10 minute calming period interposed (double vertical lines) between each series.

Series 1: response to presentation of a model without acoustic stimulation, followed by a silent period.

Series 2: response to recorded male calling chirp (Chirp stim.) followed by model presentation and then another period of calling chirps (After model). Series 3: response to recorded male buzz (Buzz stim.) followed by model presentation and then another period of buzzes (After model). Columns headed "Model" indicate responses to three presentations of a beetle model: plus (+) = approach, zero (0) = no response, minus (-) = escape response, and c = chirp response. Each series was begun with a control period (No stim.). Last column (Sex. resp.) indicates whether female copulated with a male immediately following the test series.

Table 16.

Rep. no.	Female no.	No. of stridulations per 5-minute period											Sex resp.	
		Series 1			Series 2				Series 3					
		No stim.	Model	After model	No stim.	Chirp stim.	Model	After model	No stim.	Buzz stim.	Model	After model		
1	1	0	000	0	0	0	++0	0	0	0	+++	0	Yes	
2	1	0	000	0	0	44	+++ ccc	52	0	0	000	0	-	
3	1	0	000	0	0	0	000	0	0	0	000	0	-	
4	1	0	---	0	0	0	+++ cc	0	0	0	+++	0	Yes	
5	1	0	+++ ccc	0	0	62	+++ ccc	70	(escaped)			-	-	-
6	2	0	+++ c	1	0	0	+++ ccc	0	1	7	+++	0	Yes	
7	2	0	+++	1	0	0	+00	0	0	0	+++	0	-	
8	2	0	+++ cc	0	0	21	+++ cc	0	0	1	000	0	Yes	
9	2	0	000	0	1	0	++0	0	0	0	000	0	No	
Σ		0		2	1	127		122	1	8		0		

interrupted and the spermatophores collected to be sure that the virgins were not inseminated. Responses to buzz broadcasts were not as marked as those to calling chirps.

In summary, tests with virgin females of T. ellipticus indicate that sexually responsive females sometimes give phonoresponses to male calling chirps, but often do not. The threshold of responsiveness to calling chirps seems to vary, but the nature of the variation and whether it is keyed to a biological clock within the beetles is unknown.

Silenced Males

Because of the lack of response to acoustic signals in males and the erratic response in females of T. ellipticus, I wondered how essential to mating the acoustic signals of the males were. So a number of males were silenced and paired with females to observe their mating success.

Of the 26 pairings observed, six resulted in copulation, of which three were silenced males and three were males for which the silencing enamel had been removed to again permit stridulation. In three cases females refused silenced males and then copulated with normal males. Nevertheless, three of the females copulated with silenced males, indicating that in this species calling and courtship sounds are not necessary preliminaries to mating. I have also observed a completely silent mating by a normal male. He mounted

the female, performed palpus-touching behavior with about three side to side shifts, moved back to probe and was immediately accepted by the female. The female had been separated from males for about 45 days, and was probably highly sexually responsive.

Tests with silenced males of other species have not been made.

Female Rejection Buzzes

Female rejection shaking occurred in all species of Tropisternus I have studied. Female rejection buzzes have been heard in T. mixtus (Ryker, 1972), ellipticus, columbianus, and l. nimbatus. I will summarize the observations and experiments with rejection behavior for ellipticus, in which species it has most frequently been observed.

In the initial contact with a male, a female usually did not begin rejection buzzes until the male mounted her and was courting. However, after that contact females buzzed in response to two stimuli--the sight of the male and the sound of his courtship buzz (Fig. 26e). Responses to these two stimuli were also shown experimentally with broadcasts of stridulations and the use of models. The responses varied, however. Some females buzzed immediately whenever a buzz was broadcast. Others buzzed when presented with a model. One female, tested after she rejected a courting male with shaking and buzzing, would only buzz when simultaneously stimulated by broadcast

buzzes, a model, and the touch of a glass rod. Substituting broadcast calling chirps for broadcast buzzes was not sufficient to stimulate her to buzz.

Rejection buzzing was observed May 31, 1974 in a jar containing only females, which were walking about on the submerged vegetation just below the surface of the water. The females had been in the jar five days, and apparently some of the females were sexually responsive and some were not. Some of the females were continually approaching and touching the others; and other females were almost constantly shaking, buzzing, and moving away from the approaching females.

This variety of observations indicates that as an unreceptive female is stimulated by contact with another beetle, usually a male, her threshold for the rejection buzzing response lowers until merely the sight of the male or the sound of buzzing (the male courtship sound) will cause her to buzz.

Summary of Acoustical Signal Experiment Results

Sexually responsive males of T. natator and I. nimbatus gave phonoresponses preferentially to broadcasts of calling chirps, discriminating these from other conspecific stridulations.

Once a female was contacted, even if only briefly, males of I. nimbatus switch to their second calling sound (ticking), and males of natator switch to their courtship buzz.

Calling chirps release a "searching" behavior pattern in natator and l. nimbatus, consisting of a phonoreponse, increased walking and swimming, and an increased likelihood of approaching and mounting another beetle when encountered. In these two species, acoustic signals termed calling chirps on the basis of context (Ryker, 1972) do seem to have a calling function.

Sexually responsive males of ellipticus and l. limbalis did not give phonoreponses to conspecific calling or courtship sound broadcasts.

Females of all species tested were usually silent and showed no phonoreponse to broadcast stridulations, even when sexually responsive. On rare occasions, however, females of l. nimbatus and ellipticus have given phonoreponses to calling chirps.

Test pairings of females of ellipticus with silenced males indicated that acoustic signals are not necessary preliminaries to mating in this species.

Sexually unresponsive females of several species of Tropister-nus respond to courtship with shaking and rejection buzzes. In ellipticus, after initial contact with a male, the female's threshold for release of this response lowers until either visual or acoustic stimuli can release it.

IX. SIGNIFICANCE OF DIFFERENCES IN COMMUNICATION BETWEEN SPECIES

Although each species of Tropisternus studied has a comparable complement of stress, calling, and courtship sounds, both the amount of stereotypy in the use of the sounds and the responsiveness to broadcasts of the sounds varied greatly between species. Differences in dispersal and selection of breeding sites by different species may be important in explaining differences in stereotypy and responsiveness to acoustic signals.

The species and subspecies of Tropisternus I studied are from populations in two geographic areas. T. natator and lateralis nimbatus were from Ann Arbor in southern Michigan; and lateralis limbalis, columbianus, and ellipticus were from the Willamette Valley of western Oregon. Four species, including the two I studied, apparently breed in the same pools and pond edges in southern Michigan. However, the three species in western Oregon may not breed in the same habitats, and therefore may not suffer the same amount of mating interference. Certainly ellipticus breeds in a different habitat from the other two species--it disperses into temporary rock pools along rivers to breed. Also, observational evidence indicates that lateralis limbalis may disperse from ponds into flooded grassy swales or ditches with soft, muddy bottoms to breed. T. columbianus apparently remains in ponds to breed.

A number of differences in the use of acoustic signals by different species were apparent. Data from mating observations and signal-response experiments showed that the two Michigan species studied, T. natator and l. nimbatus, stridulated much more frequently in calling situations (alone and unstimulated) than did the Oregon species. Also, males of natator and l. nimbatus responded markedly to broadcast calling chirps by stridulating and "searching," whereas l. limbalis and ellipticus showed little response. It is particularly striking that the two subspecies of lateralis have similar chirps, ticks, and trills, and the Michigan subspecies gives phonoresponses to calling chirps but the Oregon subspecies does not.

T. ellipticus has by far the most variable acoustic behavior of the species studied. Males are sometimes silent and sometimes stridulate in every step of the mating sequence, and can even court and copulate completely silently. Although the six other species in which courtship has been observed have but a single courtship sound, ellipticus males are sometimes silent, sometimes buzz, and sometimes slow trill during both probing and copulation. Also, both males and sexually responsive, virgin females fail to stridulate in response to broadcast calling chirps, or respond only erratically. This species also has the most ecologically distinct breeding habitat of the seven species.

T. columbianus has very consistent courtship behavior. The male is silent when mounted and at the head, and slow trills while probing. The response of this species to broadcast calling chirps was not studied experimentally. So the functional importance of its calling chirps is not known. If both columbianus and l. limbalis frequently breed in the same pond edges, a response to calling chirps might be expected. However, since the calling chirps of these two species are of similar duration, reproductive isolation may be maintained by differences in mounted courtship behavior and (possibly) chemical cues.

The possibility remains that the lack of response to calling chirps observed in T. ellipticus and l. limbalis is the result of subtle differences in experimental conditions, or to age or seasonal differences in the beetles tested. The Michigan tests were done in July, and the western Oregon tests in August and September. Several things make this possibility unlikely. Preliminary tests on Oregon species were run in June (not included in this thesis), and the lack of response was so marked that I then decided to run similar signal-response tests on representative Michigan species. So the tests run later on ellipticus and l. limbalis were designed to closely replicate those done on Michigan species in an attempt to verify or refute differences I had noted in June. Also, males of the Oregon species readily courted and females copulated in the late summer tests, another

indication that they were in the proper physiological state for mating behavior.

In summary, it seems that although the western Oregon populations of T. ellipticus and I. limbalis have a normal complement of calling and courtship stridulations, they are poorly stereotyped in ellipticus and show little or no calling function in either species. These qualities of their acoustic signals may represent a degeneration of acoustic communication as a result of dispersal to breeding sites in different microhabitats. Southern Michigan populations of natator show greater stereotypy of courtship signals than ellipticus; and populations of natator and I. nimbatus have a strong phonoresponse and searching response to calling sounds. These qualities of the acoustic signals (of the Michigan species) may be related to mating interference from other species that share the same breeding sites and times.

X. DISCUSSION AND CONCLUSIONS

This study had two overall objectives: 1) to describe and compare the acoustic, tactile, visual, and chemical signals of the western Oregon species of Tropisternus and to evaluate the relative importance of acoustic signals in this genus; and 2) to attempt to ascertain what life functions, or adaptive value, can be attributed to various acoustic signals.

The communicative system of Tropisternus has a strong acoustical component, with various species having from four to seven distinct sounds. Sounds are involved in stress reactions, calling, courtship, copulation and aggressive rejection behavior. However, other signaling modes are also important; and at least in T. ellipticus, mating can occur in the absence of acoustic signals. Mating behavior often begins when a male is visually stimulated by a nearby female, without the preliminary of calling signals. Once initiated, male courtship involves three to four stereotyped tactile signals-- palpus - touching, hindleg - sweeping, foretarsi - touching, and probing. Chemical signals in the form of waterborne odors or direct tasting of other beetles with the palpi may be important; however, the evidence is inconclusive. Regardless of the contributing role of visual, tactile, and chemical signals, the diversity of acoustic signals and their use in several contexts is remarkable.

In a preliminary study (Ryker, 1972), I described stress, calling, and courtship sounds as part of the communicative behavior of four sympatric species of Tropisternus. The functional designations for stridulations in Tropisternus were based on the behavioral contexts in which the various signals were observed but were not supported with experimental evidence. Also, the implied functional interpretation depended upon several unstated assumptions: that the beetles could hear the sounds, that they could discriminate one (apparently different) sound from another, and that the sounds were produced and interpreted by the beetles in stereotyped ways. This study does not elucidate functions for every stridulation in every species studied, but some functions are eliminated as possibilities, others are shown to vary between species, and the validity of some assumptions is analyzed.

A field study showed that individuals of T. ellipticus do not aggregate, have territorial behavior, nor home ranges. Apparently, wandering is the rule in the aquatic environment, and dispersal between aquatic sites is frequent. These data rule out territorial signaling as a signal function, at least in ellipticus.

Aggressive behavior by females towards courting males has been observed many times in all species studied. Females of several species have a characteristic buzz that accompanies other rejection behavior like shaking and striking the male's palpi with her forelegs.

Females of ellipticus that have rejected a male can be stimulated to buzz by the sight of another beetle or the sound of the male courtship buzz. Also, recently-rejected males either avoid buzzing females or court only very briefly.

Study of the responses of T. natator and I. nimbatus to broadcasts of stridulations supported the idea that their "calling" chirps have a calling function, i. e., act to bring together a male and female for mating. Males responded to the broadcast calling chirps by emitting calling chirps, by walking or swimming instead of feeding, and by approaching and mounting any beetle encountered. These data also validate the assumptions that the beetles can hear the sounds and can discriminate between sounds. Males of natator, for example, discriminated calling chirps from stress chirps and courtship buzzes.

A similar study of the responses of T. ellipticus and I. limbalis to broadcast stridulations gave different results. Males of these two species did not respond to broadcasts of female calling chirps even though they were sexually responsive to females and approached models of beetles. Although males and females have been observed on rare occasions to exchange calling chirps in these two species, I was unable to demonstrate a calling function for this signal experimentally. Apparently, either the sound no longer has a function, or the critical stimuli or timing of its release are very different from the two Michigan species.

Females of studied species of Tropisternus only rarely responded to broadcast calling chirps of males, even when sexually responsive to courtship. Apparently, the threshold for release of phonoresponses and searching behavior varies from one time to another, but the nature and timing of this variation is not known.

In general, males of Tropisternus may produce two or three different sounds in the calling situation, but have only one courtship sound. T. natator and columbianus are very stereotyped in their courtship signals--natator always buzzes in each step of the mating sequence, and columbianus is silent when positioned at the head of the female and trills while probing. In contrast, ellipticus is highly variable in its courtship signals, and clearly illustrates the danger of assuming stereotypy in the behavior of insects. Males of ellipticus may be silent, buzz, or give slow trills during both palpus-touching and probing. Lack of signal stereotypy, which is more pronounced in ellipticus than in any other species, may result from the trait of dispersing to breeding sites that are not shared with other species of Tropisternus. Maintenance of reproductive isolation by (breeding) habitat selection may have allowed the signaling system to degenerate by relaxing the mating interference from other species.

In 1. nimbatus and natator, species for which calling chirps seem to have a calling function, the calling chirps of males and females are very similar. Also, males can be stimulated to chirp

by broadcasting either male or female (conspecific) calling chirps. For males to get excited and begin chirping and searching every time they hear chirps of other males would seem very inefficient, a waste of time and energy. However, both of these species have a shut-off valve for this contingency built into their behavior patterns. After a certain period of time elapses following the initial stimulation, males of l. nimbatus switch from calling chirps to ticking sounds; and males of natator switch from calling chirps to buzzes. The second sound used in the calling context by each of the two species does not stimulate phonoresponses in males. So when one of the males switches to the second calling sound, the other male will lose interest and return to feeding.

Thus, the results of this study support the idea that at least some stridulations by species of Tropisternus have communicative value, i. e., are more than incidental embellishments of their behavior. They can accurately be referred to as signals. Indeed, Tropisternus is one of the only genera of Coleoptera for which acoustic calling signals have been demonstrated.

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