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Size-Related Variation in the Advertisement Call of *Rana clamitans* (Anura: Ranidae), and Its Effect on Conspecific Males

JOHN D. RAMER, THOMAS A. JENSSEN AND CHARLES J. HURST

We tested the hypothesis that the acoustical quality of *Rana clamitans* advertisement calls varies among males and that this variation is associated with differential responses by recipient conspecifics. The single-note advertisement calls of 99 male green frogs were acoustically analyzed. The dominant frequency was negatively correlated ($r = -.69$, $P < .001$) with the callers' snout–vent lengths (SVL). Energy distribution between a call's low (200–450 Hz) and middle (500–1,000 Hz) frequency ranges shifted with a male's SVL. "EIR" scores (low-freq. intensity/mid-freq. intensity ratio) were positively correlated with SVL ($r = .70$, $P < .001$).

Playback experiments broadcasting a call from a small male (0.2 EIR score) and a large male (33 EIR score) to resident males produced a differential response. The $EIR_{0.2}$ call (small male threat) elicited strong agonistic vocalizations by small adult males (SVL $\leq$ 81 mm), while large adult males (SVL > 81 mm) simply decreased their rate of baseline calling. Conversely, the EIR_{33} call (large male threat) elicited agonistic responses from large males and weak responses from smaller males. Large males were much more likely than small males to physically meet with an intruder (i.e., approach the playback speaker), especially if the stimulus was the large male call.

Our behavioral data do not support a traditional view that the frogs' peripheral auditory system is functioning solely in a "lock-and-key" relationship with the large male call characteristics. Subjects responded to the spectral qualities of both call stimuli. In addition, their responses indicate that the green frog advertisement call provides a conspecific male recipient with information with which to assess the size and potential threat of a caller.

FOR many anurans, onset of the breeding season is marked by prominent male vocalization. As reviewed by Littlejohn (1977) and Wells (1977a, b), these calls have been shown to be species typical and to function for certain species as mate attraction signals, as territorial signals and as an agent for reproductive isolating mechanisms. Neurophysiological studies have suggested a mechanism which helps explain how conspecifics differentially respond to their species' vocalizations; the phonoreceptors of some anuran species are "tuned" to the emphasized frequencies in the conspecific call(s) (Capranica, 1976). Neurophysiological evidence also suggests that the signal-receptor system may be doing more than insuring species recognition. The phonoreceptors of some anurans are sufficiently selective to differentiate among a gradation of acoustical (spectral and temporal) qualities (Capranica, 1965, 1966, 1968; Frishkopf et al., 1968). Thus, it seems quite likely that the peripheral auditory nervous system could provide the basis for discrimination between the calls of conspecific males.

The bullfrog (*Rana catesbeiana*) ear, for example, demonstrates the ability to distinguish among conspecific calls. Frishkopf and Goldstein (1963) and Frishkopf et al. (1968) found that the peripheral auditory apparatus contains two distinct sound receptors, the basilar and amphibian papillae. The basilar papilla is innervated by non-inhibitable simple neural units which are tuned to "high" frequencies (1,300–1,500 Hz), whereas the amphibian papilla is tuned to frequencies below 1,000 Hz (Feng et al., 1975). There are actually two subpopulations of fibers which derive from the amphibian papilla: 1) one population is optimally excited by frequencies in the range 200–500 Hz all, of which can be inhibited by energy in the mid-frequency range 500–1,000 Hz and 2) a second population on non-inhibitable fibers which can only be excited by tones in the mid-frequency range. Thus, sound with greater energy in the low-frequency than in the mid-frequency range will excite the low-frequency fibers to a greater degree than the mid-frequency fibers. On the other hand, sounds with greater relative energy in the mid-frequency range will predominantly excite the mid-frequency fibers while suppressing the low-frequency fibers.

Capranica (1965, 1966, 1968) found that the sensitivity of the bullfrog's phonoreceptors to a bimodal spectrum (low- and high-frequency ranges) matched that of the species' advertisement call. He also found a spectral difference between calls of large and small males. While the advertisement call of large males had maximum energy in the low 200–500 Hz and high 1,300–1,500 Hz ranges, the calls of small males contained less energy in the low-frequency range and more energy in the mid 500–1,000 Hz range. Playback of large male calls effectively evoked calling in a laboratory population of conspecific males. However, small male calls failed to elicit calling because much of the energy in their calls was concentrated in the mid-frequency range which can inhibit the low-frequency fibers. Thus, detection of the advertisement call seemed to involve excitation of both low- and high-frequency tuned fibers.

Findings similar to those from studies on the bullfrog were reported for the green frog, *Rana clamitans* (Capranica, 1965; Sachs, 1964). Sachs (1964) reported both complex and simple units. The complex units of the VIIIth nerve were sensitive to frequencies of approximately 200–1,000 Hz, with a 200–450 Hz excitatory low-frequency range and an inhibitory interaction occurring over a 500–1,000 Hz mid-frequency range. The simple units were tuned to a 650–1,500 Hz high-frequency range. Using the species' advertisement call, Capranica (1965) performed playback experiments to laboratory-held green frogs at low to moderate playback levels. He found that both high and low frequencies must be simultaneously presented in the call in order to optimally stimulate calling by the males. Capranica suggested that small male calls contain significant energy in the middle (inhibitory) frequency range such that neural suppression of the low-frequency sensitive fibers resulted in the auditory nerve of neighboring conspecifics.

Our study of the green frog was initiated to: 1) investigate a possible correlation between a caller's body size and his call's "low-frequency amplitude/mid-frequency amplitude" ratio, and 2) investigate whether the agonistic responses of free-ranging males are greater to the playback recording of a larger adult male call than that of a smaller adult male. If the advertisement call of small adult males is not as effective a stimulus as the advertisement call of larger males, then this could have a significant impact upon the ability of smaller adult males to obtain and hold territories.

MATERIALS AND METHODS

The acoustical characteristics of calling male *Rana clamitans* and the response of territorial male green frogs to a playback of their advertisement call were determined from subjects at five pond sites near Blacksburg, Montgomery Co., Virginia during the summers of 1974–1976.

Recording procedure.—Frogs were initially located with the red light of a headlamp, and approached with as little disturbance as possible. The microphone, mounted on a pole and fitted with a 5 m cable, was placed approximately 0.3

m in front of the subject. The observer then retreated several meters and waited for the frog to resume calling. This usually took 5–10 min.

After a frog's calls were recorded, the subject was captured, toe clipped (Martof, 1953), and its snout–vent length measured (Jenssen, 1968). The frog was then released at its capture site.

The calls were recorded with a Uher (Model 4000 IC) tape recorder, using a tape speed of 19 cm/s, and a dynamic pressure type microphone (Uher, Model M514). Their respective flat (±4–6 dB) frequency responses were 40 Hz–20 kHz and 40 Hz–14 kHz; both were more than adequate for accurately recording the calls.

The green frog's call is of such short duration that particular care had to be taken when setting the recording level with the VU meter on the recorder. While this meter could not follow the level of the call accurately, its maximum deflection did give some indication of the peak sound level in the call. The recording level was set such that the VU meter needle just reached the zero position at the peak of its swing when the call was recorded. Later, careful examination of the recorded waveforms, using an oscilloscope in the Noise Control Laboratory of the Mechanical Engineering Department, showed no clipped signals using this technique, and all recordings were well above background noise level.

As a further check, field recordings of a calling male were made using the above Uher tape recorder and microphone along with a General Radio (GenRad) Model 1560-P40 microphone and a General Radio (GenRad) Model 1925 Data Recorder. This GenRad recorder is designed for acoustical measurement work and contains step attenuations and a manual reset clip indicator light. The recorder was calibrated before the recording session at five frequencies using a GenRad Type 1562-A calibrator. The GenRad microphone was taped to the pole next to the Uher microphone, with an intervening foam rubber pad to isolate the microphones from vibration.

Simultaneous recordings were made of a frog's calls with both recorders at a pond close to a 110 V source for the GenRad equipment. At the Noise Control Laboratory a particular call recorded by both systems was played back from each system in turn and analyzed. The analysis was made on a Zonic Technical Laboratories (ZTL) Model 5003 Digital Memory System and a Fast Fourier Transform (FFT) analysis system. The pretriggering and sample rate settings were chosen to place the call in the approximate center of the sampled data so the transformation to the frequency domain could be carried out. Careful comparisons of both time histories and the frequency analyses of the calls showed only miniscule differences between the Uher and GenRad systems, confirming the accuracy of the Uher data.

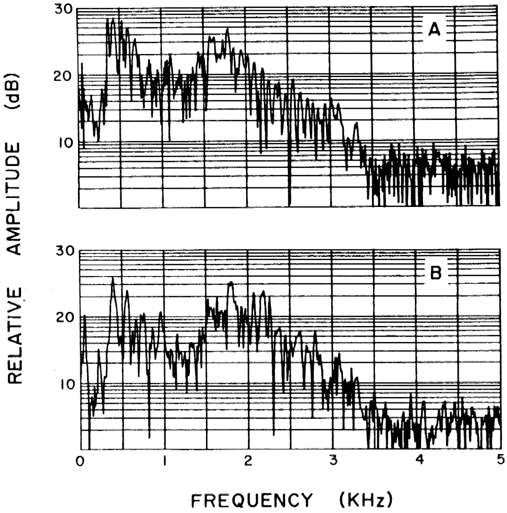

Fig. 1. Frequency analysis output from the ZTL and FFT analysis system of a single-note Type I call A) recorded with the Uher system 0.15 m from a calling male, and B) the same male recorded with the GenRad system from a distance of 3 m.

Because the field recordings using the Uher system were made at a distance of about 0.3 m from each subject, there was concern that near-field effects might have influenced the recorded calls. Of particular concern were sounds ranging from 200–1,500 Hz. At these frequencies the wavelengths are much greater than any dimension of the frog, so that the frog appears to be an equivalent simple spherical point source at reading distances much greater than the "radius" of the frog. At a distance of 0.3 m, the pressure and velocity waves will thus be nearly 90 degrees out of phase; it was important that the recording be done with a microphone which was only sensitive to pressure, and not to pressure gradient or velocity. The Uher microphone design meets this requirement. As a further check for possible near-field effects, simultaneous recordings with both Uher and GenRad microphones and recorders were made

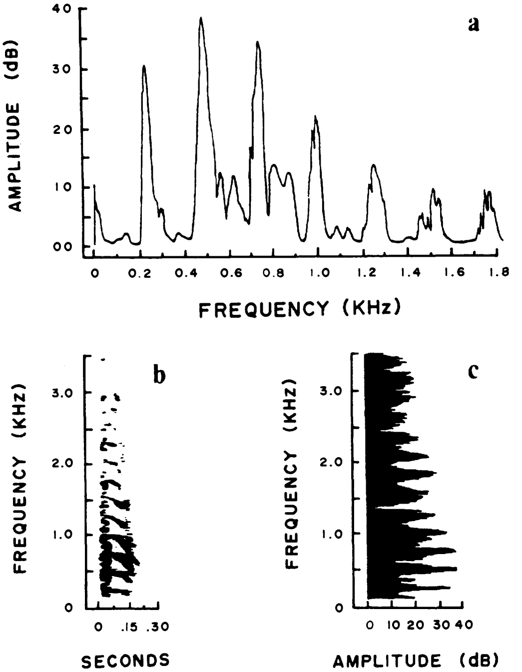

Fig. 2. Single-note Type I call of a small male (SVL 52 mm) as represented by a) a section from a Real Time Analyzer, b) a sonagram from a Kay spectrograph and c) a section from a Kay spectrograph.

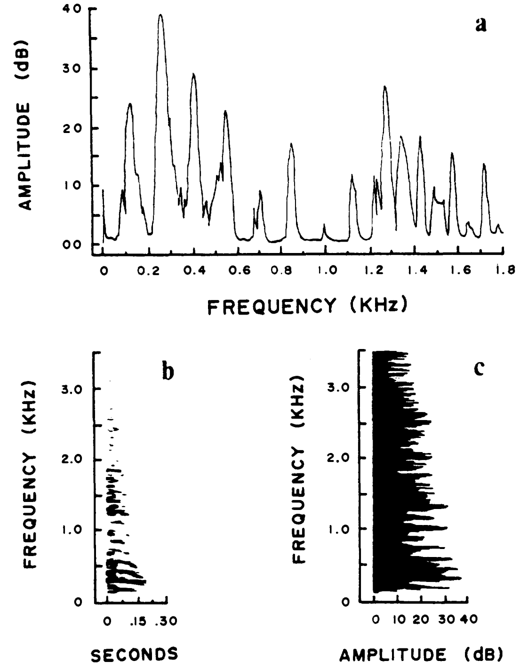

Fig. 3. Single-note Type I call of a large male (SVL 96 mm) as represented by a) a section from a Real Time Analyzer, b) a sonagram from a Kay spectrograph and c) a section from a Kay spectrograph.

at distances of 0.15, 1, 2 and 3 m from a calling frog. These calls were then analyzed using the ZTL and FFT systems. Again, no significant differences were found between calls recorded at different distances. In fact, aside from the inevitable sound level changes associated with different recording distances, many calls were virtually indistinguishable when studied in either the time or the frequency domain. Particular attention was paid to the lower frequencies relative to high frequencies in this distance comparison, since that is the region where the near-field effects would be most evident if present.

Some appreciation for comparability of recording quality and absence of near-field effects can be obtained by studying Fig. 1. Differences at a few frequencies are within the range of those expected for different calls by the same frog.

Playback procedure.—Male frog responses to recorded calls of different acoustical qualities were tested by broadcasting the call of a small adult male (SVL 52 mm) (Fig. 2) and the call of a large male (SVL 96) (Fig. 3) to subjects in the field. These two playback stimuli were randomly selected from the recorded calls of our smallest (51–55 mm SVL) and largest (96–100 mm SVL) size classes of frogs. Each Uher recorded test call was dubbed onto a separate TDK endless cassette tape via a Wollensack Model 4060 recorder. When played, the call repeated every 30 s, the approximate calling rate of an undisturbed male.

A Poly-Planar speaker (freq. response of 40 Hz–20 kHz) manufactured by Magitran Co. was placed at the water's edge about 3 m from the subject. The subject's vocalizations were recorded for 10 min to establish the frog's baseline behavior. Next, one of the test calls was broadcast for 10 min using the Wollensack recorder (freq. response of 50 Hz–10 kHz). The subject's behavior (e.g., orientation and direction of movement in relationship to the speaker), call types, and frequency of calling were recorded. The male was then captured, toe clipped and SVL measured before releasing at the point of capture.

Again, playback presents another opportu-

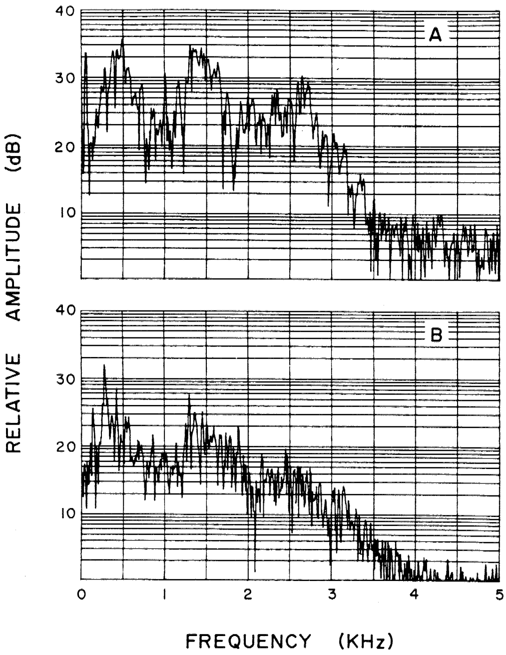

Fig. 4. Frequency analysis output from the ZTL and FFT analysis system of a single-note Type I call A) played back from a dubbed signal on a Wollensack tape deck over a Poly-Planar speaker in an anechoic chamber, with the sound pickup 2.75 m from the speaker, and B) the same call analyzed directly from the Uher tape deck with which the call was originally recorded.

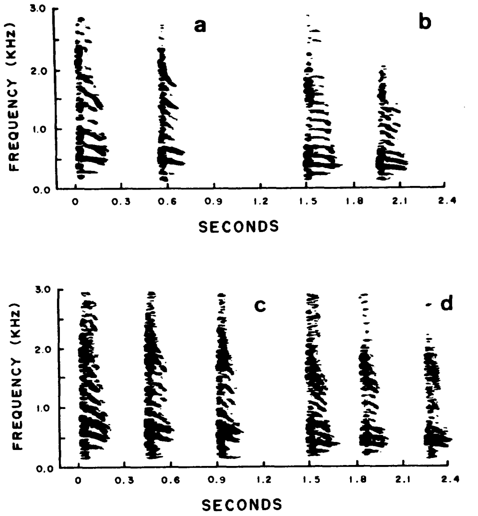

Fig. 5. Sonagrams of the two-note Type I call by a) a small (SVL 67 mm) and b) a large (SVL 94 mm) adult male and the three-note Type I call by c) a small (SVL 67 mm) and d) large (SVL 86 mm) adult male.

nity for sound distortion. The Wollensack recorder has continuously variable volume and tone controls and a more limited frequency range than the Uher recorder. A Radio Shack (Realistic Model 33-1028) sound level meter, used for setting playback volume level, has no peak hold capability. To compensate for these limitations, the following procedure was followed. The tone control was centered, marked and never changed. Next the volume level was set at a sound level subjectively equivalent to that of a calling frog using the Radio Shack sound meter. Playback at this setting in the field caused the Radio Shack meter to "bounce" to about 75 dB when positioned 3 m from the speaker. The volume control knob was then marked and maintained at this level for all playback sessions. As a check, the Wollensack recorder and Poly-Planar speaker were taken into an anechoic chamber in the Noise Control Laboratory. In this environment a maximum 75 dB value was obtained on the Radio Shack meter at 2.75 m from the speaker. To evaluate this reading, a Bruel and Kjaer Model 2301 precision sound level meter with peak holding capability registered 89 dB at 2.75 m from the speaker. This latter reading is consistent with the peak levels noted when recording green frog calls with the GenRad recorder at a comparable distance. Thus, the subjectively chosen playback level approximated what was actually produced by a calling frog.

The next test evaluated how reliably the playback experiment reproduced the originally recorded sound. Short duration sounds, such as the green frog call, place a severe demand on a speaker. We made a frequency analysis comparison of a playback call captured in the anechoic chamber with the same call taken directly from the Uher recorder (Fig. 4). While some differences were evident, the overall shape of the frequency response curve was clearly preserved (Fig. 4).

Sound distortion from the potential irregularity of the acoustical environment in the field was negligible since the subjects were situated at the edges of the ponds which were relatively free of obstructions that would influence the lower frequencies. Furthermore, the distances involved (<3 m) meant that atmospheric diffraction and absorption effects were negligible in the frequency ranges of interest. We con-

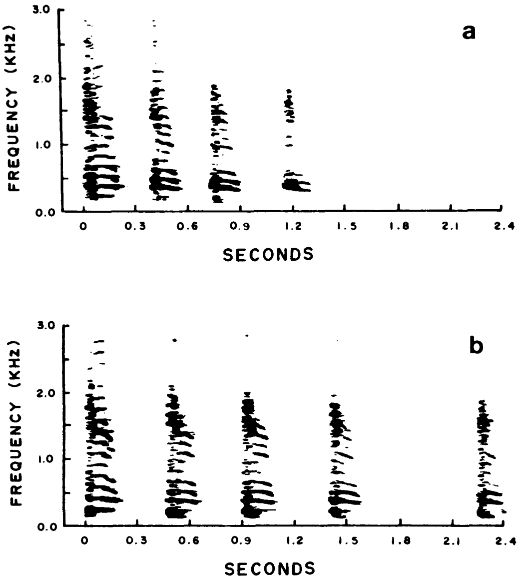

Fig. 6. Sonagrams of the a) four-note and b) five-note Type I calls.

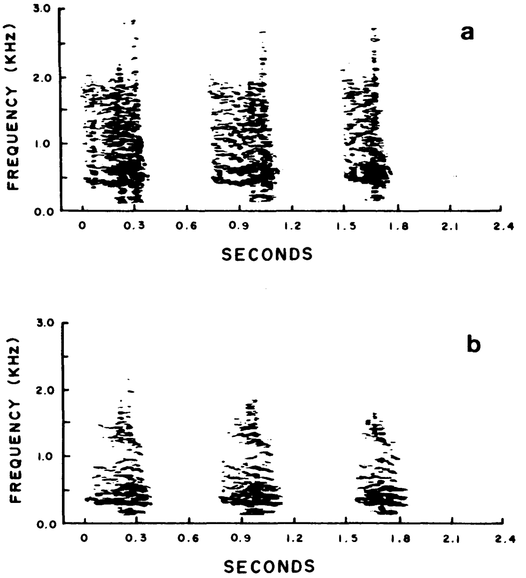

Fig. 7. Sonagrams of the Type II call by a) a small (SVL 63 mm) and b) a large (SVL 92 mm) adult male.

clude that the sound presented to the frogs was a good representation of a real call, both in magnitude and frequency distribution.

Analysis procedure.—The taped vocalizations captured with the Uher system were analyzed with a Kay Elemetric Sound Spectrograph (Model 7029-A). To calculate call duration, a "sonagram" was made which portrays time on the x-axis and frequency on the y-axis. To calculate the relationship of amplitude (dB) and frequency (Hz) within a call, a "section" was made at approximately the call's midpoint. In a section, the sound within a small increment of time is partitioned into its constituent frequencies (displayed on the y-axis) and their corresponding amplitudes (displayed along the x-axis). All audiospectrograms were produced with a narrow band filter and a frequency setting of 40 Hz–4 kHz.

Sonagrams and sections were made for calls from 99 males. The sections were used to locate the frequency with the greatest amplitude within each of three frequency ranges (200–450 Hz, 400–1,000 Hz and 650–1,5000 Hz). For each range, the most prominent frequency component and its amplitude were measured with a gridded plastic overlay.

The calls of 61 of the 99 frogs were also analyzed with a Real Time Analyzer (Model SD 330 A, Spectral Dynamics Corporation) that was available for only a limited time. Similar to a section, the RTA stores the frequency amplitude data generated during a small time increment at the beginning of the call. A digital readout of the frequencies and their amplitude is provided as well as a graphic output plotted by a Hewlett Packard x-y plotter (Model 7004B).

RESULTS

Call repertoire.—Wells (1978) presented five call types for the vocal repertoire of *R. clamitans* (Types I–V). We worked with only the two most frequently heard calls, the Type I or "advertisement call" (Wells, 1978:1052) and the Type II or "high intensity advertisement call" (Wells, 1978:1053), also labeled "territorial call" (Littlejohn, 1977:266). We classified the two call types into three categories: 1): single-note call which sounds like the plucked bass string of a banjo (Figs. 2b, 3b); 2) multiple-note Type I calls, the above sound repeated in a two-, three-, four- or five-note call in which each successive note is of a decreasing amplitude (Figs. 5, 6); and 3) Type II calls, a sound similar to Type I, but having less distinct harmonics and a more prolonged note duration (Fig. 7).

TABLE 1. DESCRIPTIVE STATISTICS FOR THE MOST INTENSE FREQUENCY (F) AND ITS AMPLITUDE (L) IN THE LOW RANGE (200–450 Hz), MID-RANGE (500–1,000 Hz), AND HIGH RANGE (650–1,500 Hz); THE DOMINANT FREQUENCY; AND THE EIR SCORES FROM SINGLE-NOTE TYPE I CALLS OF *Rana clamitans*. A refers to data from 61 males analyzed with the Real Time Analyzer, B to the same data analyzed with a Kay spectrograph, and C to data from 99 males analyzed with a Kay spectrograph. Also presented are the correlation coefficients (*r*) for comparisons of the SVL with each acoustical property.

		Low range		Mid range		High range				
Sample	Statistics	F_1 (Hz)	L_1 (dB)	F_2 (Hz)	L_2 (dB)	F_3 (Hz)	L_3 (dB)	Domin. freq. (Hz)	$L_1 - L_2$ (dB)	EIR score
A	$\bar{x}$	346	36.0	610	31.2	1,082	28.8	420	4.8	10.31
	SE	8.3	0.8	12.4	0.7	42.6	0.6	15.1	1.16	1.74
	95% C.L.	363–330	37.7–34.3	635–585	32.5–29.9	1,168–997	29.9–27.7	450–389	7.14–2.48	13.79–6.83
	r	+.002	+.592	−.119	−.491	+.604	−.397	−.694	+.702	+.701
B	$\bar{x}$	389	36.1	565	34.4	748	29.3	428	1.64	2.03
	SE	6.7	0.3	8.4	0.3	13.8	0.6	13.7	0.53	0.21
	95% C.L.	412–376	36.7–35.4	582–549	35.1–33.8	776–721	30.4–28.1	456–401	2.69–0.59	2.45–1.61
	r	−.579	+.555	−.353	−.564	+.358	−.623	−.756	+.686	+.687
C	$\bar{x}$	383	35.5	563	33.8	748	28.8	424	1.74	2.63
	SE	5.2	0.4	6.7	0.3	10.7	0.4	11.9	0.52	0.37
	95% C.L.	393–373	36.3–34.8	576–550	34.4–33.2	769–727	29.6–27.9	447–400	2.78–0.70	3.37–1.89
	r	−.568	+.421	−.283	−.459	+.273	−.538	−.638	+.577	+.539

TABLE 2. ACOUSTICAL ANALYSIS OF THE SINGLE-NOTE TYPE I CALL FOR 10 SIZE CLASSES OF *Rana clamitans* (SAME DATA SET AS IN TABLE 1). Mean values are given for all variables.

SVL class	Sample size	Body wt. (g)	Low range		Mid range		High range		Domin. freq. (Hz)	L_1–L_2 (dB)	EIR score	Call duration (s)
			F_1 (Hz)	L_1 (dB)	F_2 (Hz)	L_2 (dB)	F_3 (Hz)	L_3 (dB)				
51–55	1	11	249	14.9	505	22.1	753	18.6	505	−7.2	0.19	0.21
56–60	3	17	294	2.9	675	13.6	704	13.6	600	−10.7	0.09	0.17
61–65	5	25	348	7.3	658	17.2	714	16.7	658	−9.9	0.29	0.18
66–70	5	31	383	15.1	692	11.8	872	9.3	469	+3.3	4.53	0.20
71–75	8	40	329	16.1	549	14.6	933	9.8	428	+1.5	4.30	0.20
76–80	4	46	404	16.2	628	12.4	992	6.0	490	+3.8	7.01	0.20
81–85	22	57	361	21.0	574	11.7	1,262	8.5	361	+9.3	12.61	0.20
86–90	7	64	325	18.1	648	10.5	1,126	8.8	347	+7.6	10.45	0.21
91–95	4	66	314	18.5	624	6.4	1,406	9.7	354	+12.1	23.38	0.20
96–100	2	80	300	22.8	600	6.2	1,260	9.5	300	+16.6	48.84	0.20

Single-note Type I calls.—The data were originally analyzed from Kay audiospectrograms, and later a portion of the calls was re-examined with a Real Time Analyzer (RTA). The two techniques, as compared in Table 1A, B, generated similar results. Some comparisons of corresponding acoustical properties were statistically significant as evidenced by their nonoverlapping 95% confidence limits, but both data sets yielded a similar biological interpretation. Even though the Kay audiospectrogram data were less variable (see SE values, Table 1), we felt that the RTA data, which appear as a digital readout, were slightly more objective, being independent of any hand measurements. Therefore, the subsequent data presentation is primarily based on the RTA results in Table 1A, 2.

The frequency-amplitude relationship of single-note Type I calls by 99 male green frogs was examined by separating the calls, as suggested by the neurophysiological study of Sachs (1964), into three frequency ranges; 200–450 Hz (low); 500–1,000 Hz (middle); and 650–1,500 Hz (high).

To evaluate Sachs' data that energy received in the middle range tends to inhibit low-frequency complex fibers (see Introduction), the amplitude was measured (in dB) for the most intense frequency (L_1) in the low-frequency range and the most intense frequency (L_2) in the mid-frequency range for each frog's calls. L_2 was subtracted from L_1 to compare the relative energy in the excitatory low-frequency range with that in the inhibitory mid-frequency range for each call. To express this difference as a ratio, the logarithmic decibel scale was converted to an intensity ratio:

$$10^{\mathrm{exp}}\frac{L_1 - L_2}{10} = I_1/I_2 = \text{EIR score}$$

where I_1 is the intensity (W/m^2) of L_1 and I_2 is the intensity of L_2. This ratio is the Excitatory-Inhibitory Ratio, we term the EIR score. The calls most likely to be heard by conspecifics should be those in which $L_1 - L_2 > 0$ or where EIR > 1; calls with these acoustical qualities are produced by the larger adult males (Fig. 8).

A positive and statistically significant correlation ($r = .70$, $P < .001$) resulted when comparing snout–vent lengths of males with the EIR scores of their calls (Table 1). Fig. 8 and the EIR scores of Table 2 also show that calling males fall into three classes: 1) "inhibitory" callers (SVL 51–65 mm, EIR < 1), 2) "transitional" callers (SVL 66–80 mm, EIR $\approx$ 1–7) and 3) "excitatory" callers (SVL 81–100 mm, EIR > 10). Specifically, as adult frogs become larger, the amplitudes in the low-frequency range increases while the amplitudes decrease in the middle range. Other features of call quality covaried with the caller's snout–vent length (Tables 1,2). The frequency of the strongest or most prominent component in the high range increased with body size ($r = .60$, $P < .001$), while its relative amplitude decreased ($r = -.40$, $P < .001$), being 500–700 Hz in small adult males (SVL 51–60 mm) and grading to 300 Hz in the largest males (SVL 96–100 mm) (Fig. 9). Ambient temperature did not seem to have an appreciable effect on the calls. The dominant frequencies for 22 males of the most numerous

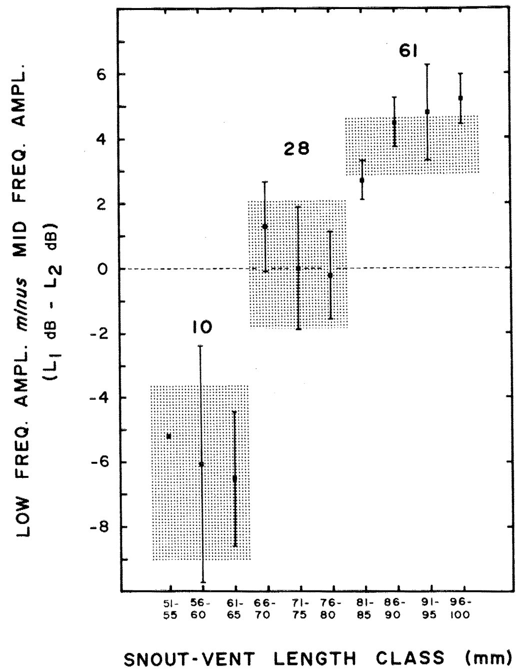

Fig. 8. Caller's body size in relationship to the amplitude distribution in the lower and middle frequency ranges of a male's single-note Type I call. Each small square and its intersecting vertical line represents the $\bar{x} \pm$ SE for a size class. The 10 size classes are pooled into inhibitory, transitory, and excitatory call quality groups; the shaded areas give the 95% confidence limits for the three groups, with sample sizes indicated over each shaded area.

size class (SVL 81–85 mm) were correlated with air temperature (subjects were emerged or entirely out of the water) with no significant results ($r = .32$, $P > .10$). Furthermore, the call duration was almost constant for all males, having approximately a 0.2 ± SE .003 s duration.

Multiple-note Type I and Type II calls.—The multiple-note Type I calls of 50 males were recorded and analyzed. With the exception of duration, there were essentially no differences between the measured acoustical properties of the single-note calls and the notes comprising the multiple-note calls (Table 3). It was the inter-note durations which showed the most variation.

The Type II calls are quite different from Type I calls in sound quality (Figs. 2, 3, 5–7). In comparison to Type I calls, the Type II calls were more intense, had a longer duration, began more softly and ended with greater amplitude.

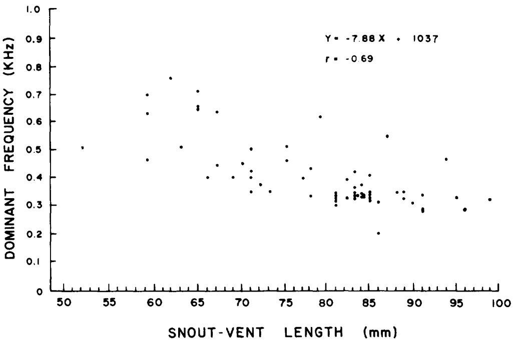

Fig. 9. Dominant frequency in the single-note Type I calls of 61 male green frogs in relationship to the callers' snout–vent lengths.

Male playback experiment.—The small-male and large-male single note test calls had a dominant frequency of 505 Hz and 288 Hz, respectively, and EIR scores of 0.19 and 33.1, respectively (Figs. 2, 3). These test calls are subsequently referred to as the "EIR$_{0.2}$" call and the "EIR$_{33}$" call.

The vocal responses of the subjects were evaluated by comparing the call types and their frequency of occurrence during a 10 min baseline period with the vocal behavior produced during a 10 min test period when the test call was broadcast from a distance of 3.0 m from the subjects. Vocal responses consisted of no significant change from baseline behavior, or increasing or decreasing numbers of single-note Type I, multiple-note Type I and Type II calls during the test period. In addition, approaches by the subjects toward the speaker during the test period were also scored.

Baseline data show that single-note Type I calls predominated over the multiple-note Type I calls and especially over the Type II calls (Fig. 10A). The large male frogs of the sample (SVL > 81 mm) produced more calls than the smaller adult males (SVL ≤ 81 mm). Large frogs performed 35% more single-note calls and almost 500% more multiple-note calls than the smaller males (Fig. 10A).

When the test calls were broadcast, there was a dramatic change in the vocal behavior of the subjects (Fig. 10B, Tables 4, 5). Type II calls by small and large males increased 1,000% and

TABLE 3. DESCRIPTIVE STATISTICS ($\bar{x}$ ± SE) FOR ACOUSTICAL PROPERTIES OF TYPE I AND II CALLS OF *Rana clamitans.*

Call type	Notes/call	Sample size	Call duration (s)	Avg. note duration (s)	Avg. inter-note duration (s)	Dominant freq. (Hz)
I	single-note	99	0.21 ± 0.003	0.21 ± 0.003	not applicable	424 ± 12
I	multiple-note					
	2	38	0.69 ± 0.002	0.21 ± 0.004	0.25 ± 0.01	453 ± 20
	3	8	1.04 ± 0.10	0.19 ± 0.01	0.23 ± 0.04	443 ± 39
	4	3	1.14 ± 0.10	0.18 ± 0.01	0.14 ± 0.03	401 ± 65
	5	1	2.42	0.20	0.35	326
II	4.7 ± 0.4	20	2.96 ± 0.18	0.31 ± 0.02	0.46 ± 0.06	401 ± 27

750% over the baseline levels, and small males produced 350% more multiple-note calls than during their baseline period. Both of the test calls produced statistically significant changes in the vocal behavior of the recipient males (Table 6). This verifies that the males could hear a call with a low EIR score, and they were responding to the $EIR_{0.2}$ call as if it were a conspecific male.

Large and small adult males responded differently during playback. To the $EIR_{0.2}$ call, large males performed significantly fewer single-note calls than during baseline observations, and smaller males produced significantly more multiple-note Type I and Type II calls (Table 6). To the EIR_{33} call, small males again responded with more multiple-note calls than during their baseline period, while larger males gave significantly more Type II calls (Table 6). This difference is size-related responses includes the number of males who approached the speaker. The $EIR_{0.2}$ and EIR_{33} calls elicited about the same number of approaches by small males (23% and 20%, respectively). However, 55% and 90% of the large males approached the $EIR_{0.2}$ and EIR_{33} sound source, respectively. This suggests that large males are much more likely than small males to physically meet with an intruder, especially if the intruder is a large male (i.e., more important competitor).

Fighting behavior.—Physical encounters observed in the field followed a similar pattern which can be generalized by the following scenario. An intruder enters the area of a known resident and moves within 3–4 m of the resident. Both males perform single-note calls, progressing to multiple-note calls. As one male shifts to multiple-note calls, the other male follows in kind. The intruder moves toward the resident who responds by orienting toward the intruder. The resident then rapidly advances toward the intruder, making loud splashing noises. After a brief period, the resident begins Type II calling while the intruder continues to give single-note and multiple-note Type I calls. The resident again moves rapidly in a splashing manner to (Though not noted in our observations, a Type III call has been recorded at this point in the encounter [Wells, 1978].) within several centimeters of the intruder. Both frogs give Type II calls. (See also Wells [1978] for an additional vocalization within this context.) Next they jump at each other, bounce off, re-orient and lunge again. They clasp forearms in a bipedal wrestling stance and grapple while emitting gutteral noises. When the males break their holds, it is usually either the smaller or the nonresident frog which swims several meters away and remains silent. The winner resumes Type I calling.

DISCUSSION

The results of our study unequivocally support past inferences (Capranica, 1965; Oldham, 1967) that call frequency in green frogs is related to the body size of the caller. As in some other anuran species (Blair, 1955a, b, 1958; Bogert, 1958; McAlister, 1961), it was found that the dominant frequency of the green frog advertisement call was negatively correlated with the caller's snout–vent length. Also correlated with caller's size was a shift in the energy distribution among the call's low- (200–450 Hz) and middle- (500–1,000 Hz) frequency ranges, such that EIR scores were positively correlated with snout–vent length.

From the early neurophysiological evidence of Sachs (1964) and Capranica (1965), it might be expected that the calls of small males (e.g.,

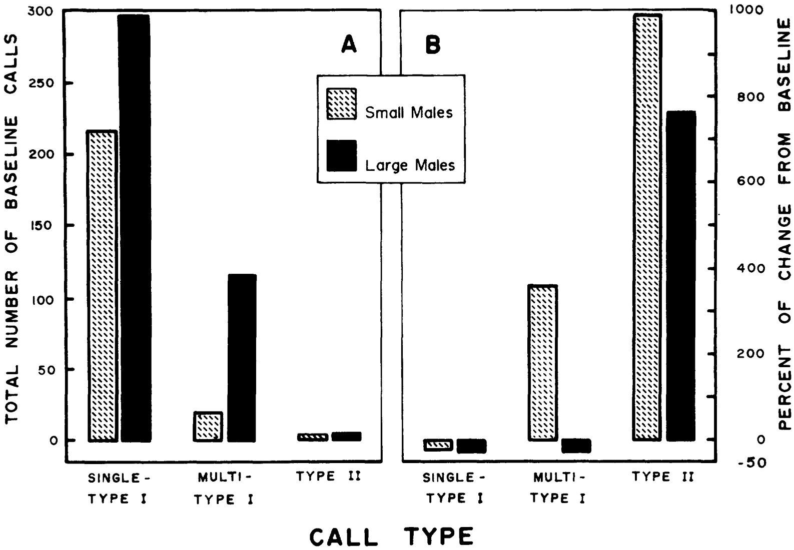

Fig. 10. A) Frequency of occurrence of call types by small (SVL ≤ 81 mm) and large (SVL > 81 mm) male green frogs during baseline observations. B) Percent increase or decrease for each call type from its corresponding baseline levels during the playback portion of the experiments.

EIR$_{0.2}$ call) would have sufficient energy in the mid-frequency range to block the call's perception by conspecifics due to inhibition of the complex low-frequency fibers of the auditory nerve. However, the EIR$_{0.2}$ call caused highly significant changes in recipient behavior. Subjects (both large and small males) responded to the small-male stimulus as if a conspecific male were vocalizing within their territories.

Our behavioral data do not support a traditional view that the male auditory system is functioning solely in a "lock-and-key" relationship with the large male call characteristics. More recent neurophysiological data from ranids have shown that the amphibian papilla also contains non-inhibitable fibers tuned to the middle frequencies of the call; in fact, these constitute a third type of auditory fiber in the VIIIth nerve (Capranica, 1976). All three fiber types (inhibitable low-frequency, non-inhibitable mid-frequency, and non-inhibitable high-frequency fibers) of the two auditory papillae project to a thalamic auditory center. Therefore, the call stimulus is not simply processed by a receptor filter. The sensory output from the ear seems to receive additional processing in the central nervous system (CNS), where suspected ratio detectors (R. R. Capranica, pers. comm.) are responsive to mid- and high-frequency as well as low- and mid-frequency energy combinations (Capranica, 1978). This signal analysis capability at the receptor and CNS level suggests that

TABLE 4. DESCRIPTIVE STATISTICS OF VOCAL BEHAVIOR BY MALE GREEN FROGS OF TWO SIZE CLASSES DURING BASELINE AND DURING PLAYBACK OF A SINGLE-NOTE TYPE I CALL BY A SMALL MALE (SVL 52 MM).

		Single-note calls		Multiple-note calls		Type II calls	
Subject size	Statistics	Baseline	Playback	Baseline	Playback	Baseline	Playback
≤81 mm	$\bar{x}$	5.1	4.2	0.4	2.0	0.1	1.0
(N = 20)	SE	1.6	1.0	0.3	1.0	0.1	0.5
	95% C.L.	8.4–1.7	6.2–2.4	1.2–0.5	5.2–1.2	0.2–0.1	2.6–0.6
>81 mm	$\bar{x}$	8.2	5.5	1.5	2.2	0.2	0.5
(N = 19)	SE	1.2	1.0	1.0	1.0	0.1	0.2
	95% C.L.	10.7–5.6	7.4–3.5	3.1–0.1	4.4–0.1	0.4–0.1	1.6–0.6

Table 5. Descriptive Statistics of Vocal Behavior by Male Green Frogs of Two Size Classes During Baseline and During Playback of a Single-note Type I Call by a Large Male (SVL 96 mm).

		Single-note calls		Multiple-note calls		Type II calls	
Subject size	Statistics	Baseline	Playback	Baseline	Playback	Baseline	Playback
≤81 mm	$\bar{x}$	7.9	8.2	0.9	2.3	0.1	0.6
(N = 15)	SE	1.4	2.1	0.6	1.5	0.1	0.5
	95% C.L.	10.9–5.0	12.6–3.8	2.1–0.3	5.5–1.0	0.4–0.2	1.6–0.4
>81 mm	$\bar{x}$	7.8	8.8	5.0	3.5	0.2	1.5
(N = 18)	SE	1.4	1.3	1.8	1.3	0.1	0.4
	95% C.L.	10.9–5.0	11.5–6.1	9.0–1.1	6.2–0.9	0.4–0.1	2.4–0.6

a conspecific recipient could easily differentiate between a small and large caller, and may even be able to discriminate between calls of a neighbor and a strange intruder.

The playback experiments suggested a response sequence to the territorial behavior of the male green frog (single-note Type I call → multiple-note Type I call → Type II call → approach → physical contact). The single-note call serves to establish the location of a calling male for neighboring conspecifics (advertisement call). The multiple-note Type I call is probably a more agonistic form of the single-note advertisement call, and may be used during non-directed calling by frogs with stable territories. The Type II call appears to be a strong agonistic signal to encroaching males. The "approach" is not only an escalating aggressive behavior, but it is frequently conducted with exaggerated splashing. Considering that this noise could attract predators such as turtles (e.g., *Chelydra serpentina*), splashing probably has a positive value by being an intimidating factor emphasizing the resident's approach. If none of the above behaviors discourage an intruding male, the wrestling contest establishes who stays.

The extent to which the above sequence is used is related to the body size of the resident male and the acoustical characteristics of the intruder's call. When responding to the EIR$_{0.2}$ test call (i.e., simulation of a small encroaching male), small males (SVL ≤ 81 mm), and especially large males (SVL > 81 mm), decreased their baseline rate of Type I calling. The small

Table 6. Wilcoxon Matched-pairs Signed-rank Tests (Siegel, 1956) Comparing the Number of Times Each Call Type was Given During the Baseline Period by Each Male with the Number of Times It Was Performed by the Subject During the 3 min Playback Period. Arrows within the parentheses give the direction of change (increased or decreased over baseline level).

Playback stimulus	Subjects (sample size)	Single-note Type I call	Multiple-note Type I call	Type II call
Small male call (EIR$_{0.2}$)	All males (N = 39)	$P = 0.03$* (<)	$P = 0.01$** (>)	$P = 0.003$** (>)
	Small males (N = 20)	$P = 0.29$ (<)	$P = 0.01$** (>)	$P = 0.01$** (>)
	Large males (N = 19)	$P = 0.01$** (<)	$P = 0.18$ (>)	$P = 0.09$ (>)
Large male call (EIR$_{33}$)	All males (N = 33)	$P = 0.32$ (>)	$P = 0.13$ (<)	$P = 0.005$** (>)
	Small males (N = 15)	$P = 0.46$ (>)	$P = 0.05$* (>)	$P = 0.23$ (>)
	Large males (N = 18)	$P = 0.26$ (>)	$P = 0.35$ (<)	$P = 0.01$** (>)

* Statistically significant.

** Highly statistically significant.

males showed a highly significant shift to multiple-note Type I and Type II calls, while the larger males tended to be less vocal. The small males, in response to the $EIR_{0.2}$ call, showed an obvious increase in agonistic vocalizations (ritualized aggression) which was not shared by the large males. However, large males were responding to the $EIR_{0.2}$ call as a potential conspecific threat because 55% of the large males approached the speaker as compared with only 23% of the smaller male sample. When presented with the $EIR_{0.2}$ call, large males either remained in place or sought out the "intruder" with little use of the more aggressive vocalizations.

When the stimulus was the EIR_{33} test call (i.e., simulating a large encroaching male), small males only increased the number of multiple-note Type I calls. They did not use the more aggressive vocalizations to the same extent when responding to the EIR_{33} call as they did to the $EIR_{0.2}$ call. The large males, however, showed a much greater frequency of aggressive behavior toward the EIR_{33} call than the smaller subjects, produced a highly significant increase in Type II calls, and almost always approached the speaker.

In summary, small males demonstrated more agonistic behavior to the small-male test call and large males were more aggressive in response to the large-male test call. We conclude that the acoustical quality of the Type I call contains more information than just the species identification; it provides a means by which a conspecific can estimate the size of the caller. Our data indicate that territorial males tend to alter their aggressive behavior in response to an intruder's call characteristics.

OVERVIEW

Recent ethoecological studies (Emlen, 1968, 1976; Howard, 1978a, b; Ryan, 1980a; Wells, 1977a, b, c, 1978; Wiewandt, 1969) have begun to document a relatively complex social organization for the ecologically similar green frog and the bullfrog. The picture which is emerging is that these prolonged breeders are polygynous. Through intense agonistic interactions, males defend calling and oviposition sites. The females, as they approach the moment of oviposition, move singly among the calling males, actively choose their prospective mates, and initiate the amplexus sequence. The larger of the males are the more frequently solicited. These males also tend to occupy the "high quality" oviposition sites (Wells, 1977c) in which early embryo mortality is lowest (Howard, 1978a).

Call characteristics can serve a communicatory function at two places within the above social scheme. The first is during male-male encounters for territories. Selection should favor the recognition of signals which would predict the strength and size of a potential antagonist, and cannot be varied by deceptive conspecifics (Dawkins and Krebs, 1978). This selective pressure should be enhanced during the prolonged breeding season, apparently a period of physiological stress. During the three month breeding season, adult males eat proportionately less and have proportionately lighter livers than either adult females or juveniles (Jenssen, 1972). They are also known to lose weight during this period (Wells, 1978). Thus, ritualized behavior, which would circumvent the energy expenditure and possible injury associated with physical contests, should increase the fitness of one or both interacting males.

Our data and the above interpretation closely fit an animal conflict model based on individual selection (Parker, 1974). Parker theorized that the relative strengths of the combatants are mutually estimated during the ritualized portion of an aggressive encounter (process of assessment). Wells (1978) suggested that assessment by green frog males could take place during the physical contact (i.e., wrestling) phase of the agonistic encounter. However, as our data indicate, this assessment process begins sooner with the broadcast of the initial acoustical signals. A similar mechanism of assessment during conventional displaying has been identified in *Bufo bufo* (Davies and Halliday, 1978).

Parker's model predicts that the signals used for assessment should serve as a probabilistic indicator of the winner should the encounter escalate to injurious attack. When the combatants are closely matched (as determined by assessment), conventional displaying should lead to physical contact and possible injury. If the opponents are unevenly matched, the higher ranking individual should usually be prepared to escalate, while the lower ranking individual should withdraw before or during initial escalation. We have demonstrated a mechanism for assessment in the acoustical characteristics of male green frog calls. Furthermore, the male responses observed during the playback experiments corroborate the predictions of the Parker model.

The second social context in which call characteristics can have a communicatory function involves mate selection. The same acoustical properties which convey the size of a potential antagonist in male-male interactions should also signal a caller's relative body size to gravid females. Acoustical characteristics, such as that measured by a caller's EIR score, should give a female her first approximation about a male's potential or relative fitness (also a process of assessment); such a system has recently been documented for the neotropical frog, *Physalaemus pustulosus* (Ryan, 1980b). In green frogs a large EIR score would indicate a large and long-lived male. Longevity could indicate a favorable genotype. Larger males probably also hold a more favorable oviposition site. We hypothesize that females should be attracted to those males with large EIR scores to their calls, and then, as Wells (1977a) suggested, make their final mate selection based on quality of the male's territory.

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Unilateral Testicular Pigmentation in the Mexican Lizard *Sceloporus variabilis*

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The Mexican lizard *Sceloporus variabilis* exhibits unilateral testicular pigmentation; the left testis being black and the right white. This phenomenon is present in sexually mature males of all four subspecies. A correlation exists between testicular pigmentation and reproductive activity, with the disappearance of pigmentation in quiescent testes. Testicular pigmentation is less prevalent at higher elevations. Histological examination indicates that melanophores are only in the outer covering or tunica of the testis. An apparent difference exists in the rate of testicular recrudescence, as evidenced by a more advanced stage of spermatogenesis in the black versus the white testis of individual males. Additionally, black testes exhibit both 1) a higher temperature and 2) a more rapid uptake of heat than do white nonpigmented testes. This information suggests that unilateral testicular pigmentation may play a role in the thermal physiology of the testis; it does not appear to function as a protective barrier to mutagenic solar radiation.

INTERNAL pigmentation, especially of the dorsal peritoneum, has been reported in lizards (Porter, 1967). Melanophores are the most common pigment cell type found in internal organs of mammals, reptiles and amphibians (Bagnara and Hadley, 1973). Two possible adaptations have been proposed for the presence of internal pigmentation. The first hypothesis suggests that internal pigmentation functions in thermoregulation of ectotherms (Atsatt, 1939; Hunsaker and Johnson, 1959). Solar radiation passing through the body wall is absorbed by melanin, and thus internal pigmentation may facilitate thermoregulation by reducing heat loss. Secondly, internal melanin deposits may reduce absorption of damaging solar radiation (Klauber, 1939; Porter, 1967). Solar radiation passing through unpigmented body walls could damage organs and kill or mutate gametes (Waring, 1963). Porter (1967) demonstrated that the pigmented peritoneum in lizards screens out mutagenic solar radiation. He also observed that internal pigmentation functioned insignificantly in thermoregulation.