

CALLING SITES AND ACOUSTIC PARTITIONING IN SPECIES OF THE *HYLA NANA* AND *RUBICUNDULA* GROUPS (ANURA, HYLIDAE)

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We analysed spatial and acoustic partitioning among four species of *Hyla* belonging to two species-groups: *nana* (*H. nana* and *H. sanborni*) and *rubicundula* (*H. elianae* and *H. jimi*). Field activities were conducted at three permanent ponds, from 1998 through 2001. Four attributes of the calling sites were analysed: perch height, distance of the perch from the edge of the pond, type of perch (vegetation) and the individual's position on the perch. There was extensive overlap in the four calling-site variables analysed. However, we found spatial segregation did occur in calling site height and the distance of perches from pond edges. Bioacoustic analyses revealed behavioural differences among species in calling activity, both time of onset and peak calling in chorus. There was acoustic partitioning among species the fundamental frequency of the advertisement calls, principally as a function of the temporal structure (e.g. note duration, rate of note repetition, duration and rate of repetition of the calling pulses). We propose that differences in physical attributes of calling site and in characteristics of calls allow these species to exist in sympatry.

Key words: acoustic communication, calling site, niche breadth, treefrogs

INTRODUCTION

The study of closely related, sympatric species is of special interest in understanding the factors that influence mate recognition systems and the evolution of reproductive isolation. The observation that some ecologically similar and phylogenetically related species can coexist has typically been explained by reduction of possibilities for interspecific competition (Duellman, 1978; Rossa-Feres & Jim, 2001). For anuran amphibians, calling site occupancy and attributes, location of oviposition site and foraging area have been shown to be fundamentally important for resource partitioning (Crump, 1974; Cardoso *et al.*, 1989; Rossa-Feres & Jim, 2001).

Studies of anuran communities have established that breeding site location and the physical structure of the advertisement call are the most important factors in species segregation within a single locale (Duellman & Pyles, 1983; Cardoso *et al.*, 1989; Cardoso & Vielliard, 1990; Martins & Jim, 2003). Segregation of calling sites by synchronopatric species of anurans has been reported by several workers (Crump, 1974; Hödl, 1977; Duellman & Pyles, 1983; Heyer *et al.*, 1990; Rossa-Feres & Jim, 2001), and may act as a mechanism of reproductive isolation and allow coexistence of several species in the same environment.

Similarly, partitioning of the acoustic space, achieved by differences in the spectral and temporal attributes of male advertisement calls, is of great importance during the breeding season (Hödl, 1977; Duellman & Pyles, 1983; Márquez *et al.*, 1993; Grafe, 1996; Grafe *et al.*, 2000). Differences in species-specific male calls, cou-

pled with female ability to perceive such differences, is the main mechanism of reproductive isolation among sympatric species of anurans (Hödl, 1977). However, closely related species can emit similar calls, and the specificity of the signal must arise from combined spectral and temporal parameters coupled with differences in behaviour (Cardoso & Vielliard, 1990; Martins & Jim, 2003). Thus, in species that call in choruses in the same environment, acoustic interference among species may be reduced by using different frequency bands, as well as through synchronization of the call temporal parameters, thus avoiding overlap between individuals of different species (Littlejohn, 1977).

Our study examined the calling site characteristics and the acoustic characteristics of the advertisement call of four species of *Hyla* belonging to the *nana* group (*H. nana* and *H. sanborni*) and the *rubicundula* group (*H. elianae* and *H. jimi*). These closely allied species share several phenotypic and ecological characteristics: body size, breeding season, types and patterns of calls and strategy of occupying their environment. Therefore they comprise a good system for studying the factors implicated in the coexistence of sympatric species.

METHODS

STUDY SITES

Field activities and recordings were carried out in three permanent ponds in open areas in Botucatu, São Paulo State, Brazil.

Environment I. A large pond, 120 × 50 m, its edges covered predominantly by herbaceous vegetation (Poaceae and Cyperaceae). Location 22°53' S and 48°29' W, altitude 860 m.

Environment II. A large pond, 100 × 70 m, a headwater marsh in *cerrado* (savanna) vegetation. The shoreline vegetation consists of herbaceous plants (Poaceae and Cyperaceae) and bushes (Asteraceae and Melastomataceae). Location 22°57' S and 48°27' W, altitude 810 m.

Environment III. A small pond, 50 × 20 m, with herbaceous vegetation (Poaceae) along the shore, and dense emergent vegetation (Cyperaceae). Location 22°50' S and 48°25' W, altitude 780 m.

CALLING SITE

Field observations were carried out throughout the breeding season (August through March), during a four-year period from 1998 to 2001. During the breeding season of each year, ponds were visited weekly, beginning at sunset (1700 hrs) and ending when the activity of the species decreased (2400 to 0200 hrs).

To characterize the calling sites, each pond was traversed along its perimeter. For each individual found, the type and height of the perch, the location of the perch in relation to the distance from the pond edge (outer – perches on land outside of the pond margin – and inner – typically calling from emergent or floating mats of vegetation within ponds), and the individual's position (parallel or perpendicular) in relation to the perch were recorded.

The degree of overlap in the variables of the calling sites was calculated using the Morisita–Horn (C_H) similarity index (Krebs, 1989) for the frequency data by category. Multidimensional overlap was determined by considering all variables simultaneously. We considered the species as highly overlapping when the C_H value was above 0.70, as partly overlapping when the C_H was between 0.50 and 0.70, and as non-overlapping when the C_H was lower than 0.50.

The niche breadth for the calling site variables (type and height of the perch, distance from the pond edge and position in relation to the perch) was calculated by Levin's index (Krebs, 1989): $B = Y^2 / \sum N_j^2$, where B = Levin's measure of niche amplitude, Y = total individuals sampled and N_j = number of individuals found using the resource j . We considered as generalists those species for which the values of B were higher than 2.36 for at least two calling site variables (Rossa-Feres & Jim, 2001).

To compare interspecific characteristics of occupation and height of the perch and of the distance of the perch in relation to the pond edge (outer and inner), the non-parametric Kruskal-Wallis test was used, since data deviated significantly from a normal distribution (Kolmogorov-Smirnov test).

BIOACOUSTIC ANALYSIS

The times when calling activity began and when it peaked (chorus) were recorded, noting: (1) individuals during the first 30 minutes after the beginning of calling activity (initial call – between 1800 and 1830 hrs), and (2) individuals calling in chorus, at a mean of two hours

after beginning their calls. To study intra- and interspecific interactions, individuals at the beginning of their calling activity and during chorus calling were compared, and possible differences in both behaviour and spectral and temporal structure were examined.

The calls were recorded under field conditions with a digital (DAT) recorder (Sony TCD-D8) and an analogue cassette recorder (Sony TCM-S64V) coupled to external semidirectional (ME 66) or cardioid (ME 64) Sennheiser microphones. All recorded calls were edited with a sampling rate of 44,100 Hz and 16 bits per sample in the mono pattern. The bioacoustic analyses were performed on a microcomputer using the program CoolEdit 96 (Syntryllium Software Corporation), with a 20,000 Hz sampling frequency. The 256 points option (Fast Fourier Transform, FFT) and, when necessary, the 1024 points option were used, mainly in determining fundamental frequencies.

Six traits of the advertisement calls of the species were quantified: frequency band width, fundamental frequency (=dominant frequency), note duration, note repetition rate, pulse duration and pulse repetition rate. For the analyses and bioacoustic interpretations, the terms used follow Martins & Jim (2003).

The spectral and temporal intra- and interspecific traits of the advertisement call of the four species of *Hyla*, initial calling and chorus calling, were compared statistically using analysis of variance (ANOVA) to test for significant differences in means of pairs of species, and afterwards complemented by the Student-Newman-Keuls test (BioEstat 3.0; Ayres *et al.*, 2003). For analysis of the correlation between the air temperature and rate of call repetition, Spearman's correlation coefficient (r_s) was used, with a 5% significance level (Zar, 1999).

RESULTS

CALLING SITE

Two groups of three or four species occurred in sympatry: in environments I and III, *H. nana*, *H. sanborni* and *H. elianeae*; and in environment II, *H. nana*, *H. sanborni*, *H. elianeae* and *H. jimi*.

Males of *H. nana* called from lower heights, mean height 30 cm (70%, $n=330$; Fig. 1), and occupied perches at the inner edges of the ponds (Fig. 2). This species showed a preference for calling in emergent vegetation (71%, $n=291$; Fig. 3), on leaves and stems, with the body situated parallel to the perch (56.5%, $n=122$).

Most *H. sanborni* were calling at perch heights between 20 and 60 cm (73%, $n=116$). The males perched on leaves of herbaceous and emergent vegetation, with the body perpendicular to the perch (69.47%, $n=66$) and on the edge (Figs. 1–3).

Mean perch height differed significantly between the species of the *nana* group (Kruskal-Wallis, $H=77.0$; $P<0.05$): males of *H. nana* called at a mean height of 27.04 ± 15.89 cm ($n=471$), and *H. sanborni* at 43.77 ± 18.83 cm ($n=130$).

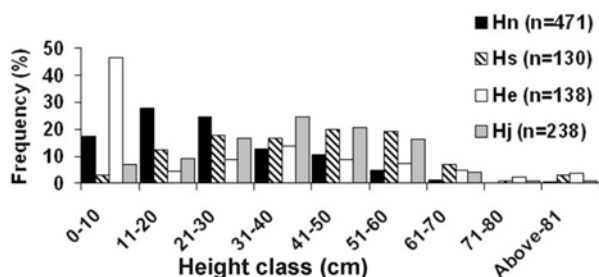


FIG.1. Frequency distribution of the perch heights of *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo.

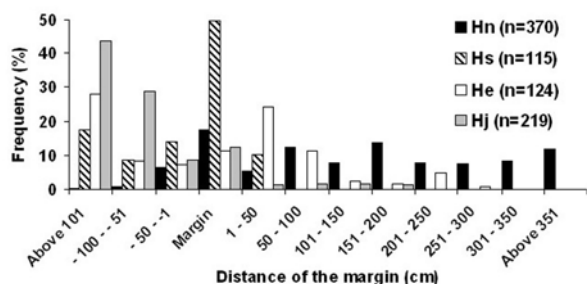


FIG. 2. Distribution of frequencies of occupation of the calling site in relation to distance from the outer and inner edges of the pond, among *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo.

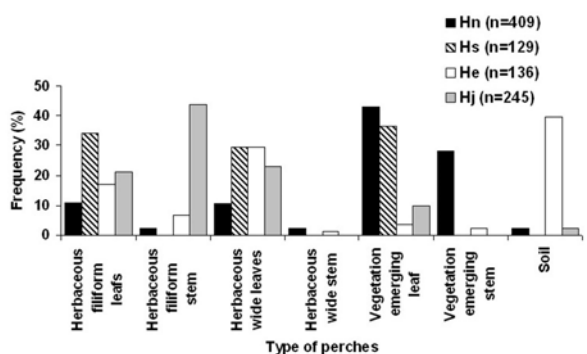


FIG. 3. Relative frequency of the types of perch used as the calling site among *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo.

TABLE 1. Niche amplitude, calculated by Levin's index, for the four variables of the calling sites of *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo.

| Variables | Hn | Hs | He | Hj |
|---|------|------|------|------|
| Perch height | 5.05 | 6.30 | 3.84 | 5.81 |
| Type of perch | 3.96 | 2.98 | 3.58 | 3.35 |
| Distance of the perch from the edge of the pond | 9.57 | 3.18 | 5.60 | 3.34 |
| Position on the perch | 1.97 | 1.74 | 2.00 | 1.57 |

Males of *H. elianeae* preferentially called from lower heights, mean height 10 cm (46.4%, $n=64$; Fig. 1). There was no apparent preference for distance of calling sites from the outer and inner edges of the pond (Fig. 2). The individuals were observed on the ground, among the vegetation (39.7%, $n=54$; Fig. 3). When they were perching, the males called from leaves of herbaceous vegetation, with the body parallel or perpendicular to the perch (50%, $n=32$).

Individuals of *H. jimi* mostly called from heights between 20 and 50 cm (77.7%, $n=185$; Fig. 1). There was a preference for calling sites around the pond (Fig. 2), on the thin stems of grasses or sedges (44%, $n=107$; Fig. 3), with the body perpendicular to the perch (76%, $n=102$).

The mean perch heights of *H. elianeae* and *H. jimi* differed significantly (Kruskal-Wallis, $H=32.54$; $P<0.05$). The preferred calling height of *H. elianeae* was 27.11 ± 31.22 cm ($n=138$), and of *H. jimi*, 40.47 ± 17.33 cm ($n=238$).

The mean height of calling perches did not differ significantly between *H. sanborni* and *H. jimi* (Kruskal-Wallis $H=1.53$, $P>0.05$), or between *H. nana* and *H. elianeae* (Kruskal-Wallis $H=1.03$, $P>0.05$). The mean distance of the occupied perch from the inner pond edge was significantly different between *H. nana* and the other species, and between *H. sanborni* and *H. elianeae* (Kruskal-Wallis $H=160.9$, $P<0.05$). In regard to the mean distance of the perches from the outer pond edge, there was no significant difference (Kruskal-Wallis $H=0.48$, $P>0.05$) between *H. elianeae* and *H. jimi*. The other species differed significantly (Kruskal-Wallis $H=128.0$, $P<0.05$) when they occupied sites outside the pond.

The four species were generalists in relation to the occupation of the calling site (Table 1). There was extensive overlap in height of calling site and position on the perch (Table 2). Among the species of the *nana* group, the degree of overlap was high only in relation to perch type; for the species of the *rubicundula* group, there was overlap in occupation of the perches in relation to the distance from the pond edge. The multidimensional analysis of the calling sites demonstrated that only two pairs of species showed high overlap (Table 2).

For each of the calling site variables, the grouping analyses resulted in different patterns of similarities among the species (Fig. 4a-d). The greatest similarity in the multidimensional analysis of calling sites was observed for *H. sanborni* and *H. jimi* (Fig. 4e).

BIOACOUSTICS

The advertisement calls of these four species of *Hyla* are composed of simple notes, pulsed and emitted in consecutive series. The males of these species call frequently in large choruses, forming reproductive aggregations around the ponds. The spectral and temporal characteristics of the advertisement calls of *H. nana*, *H. sanborni*, *H. elianeae* and *H. jimi* are presented in Table 3.

TABLE 2. Niche overlap, calculated by the Morisita–Horn similarity index (C_H) for four calling site variables among *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo.

| Species | Perch height | Type of perch | Distance of the perch from the edge of the pond | Position on the perch | Multidimensional overlap |
|---------|--------------|---------------|---|-----------------------|--------------------------|
| Hn/Hs | 0.766 | 0.721 | 0.492 | 0.876 | 0.711 |
| Hn/He | 0.634 | 0.274 | 0.433 | 0.992 | 0.486 |
| Hn/Hj | 0.761 | 0.343 | 0.184 | 0.814 | 0.505 |
| Hs/He | 0.444 | 0.520 | 0.599 | 0.929 | 0.526 |
| Hs/Hj | 0.970 | 0.553 | 0.574 | 0.993 | 0.737 |
| He/Hj | 0.532 | 0.509 | 0.725 | 0.880 | 0.579 |

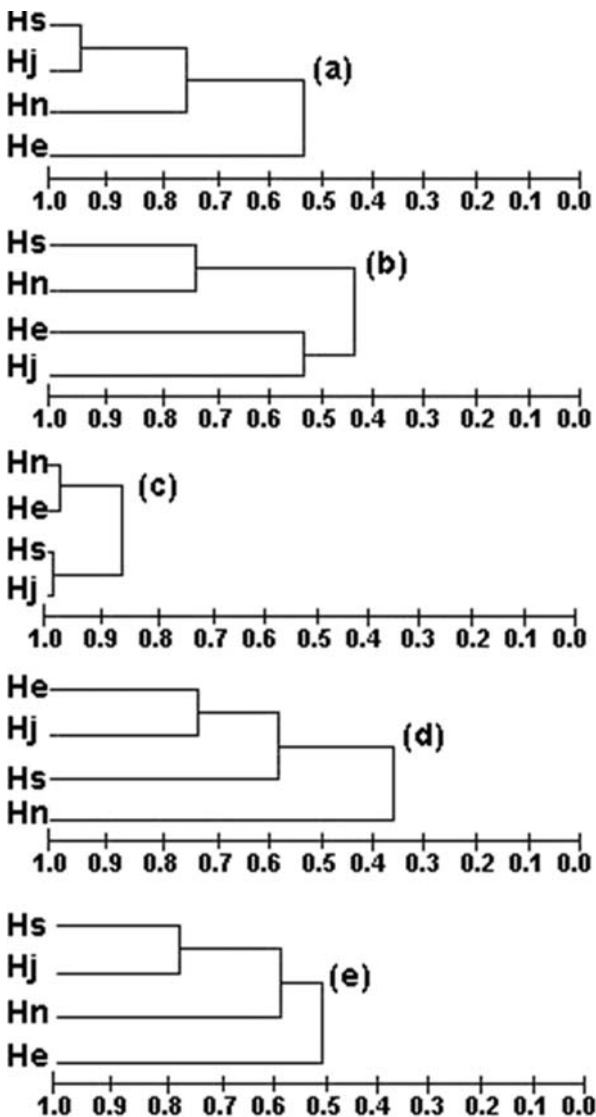


FIG. 4. Similarity of the dimensions of the calling site among *Hyla nana* (Hn), *Hyla sanborni* (Hs), *Hyla elianeae* (He) and *Hyla jimi* (Hj), near Botucatu, São Paulo. (a) Height, (b) type of perch, (c) position on the perch, (d) distance of the perch from the pond edge, and (e) similarity of the four variables together.

The four species of *Hyla* have calls with close frequency bands, except for *H. sanborni*, which has calls with higher frequencies. The species of the *nana* group have bands with wider mean frequencies, between 2,950 and 5,950 Hz, whereas the species of the *rubicundula* group have narrower frequency bands, between 2,400 and 4,900 Hz. In both species groups, the notes of the advertisement calls have a fundamental frequency with energy concentration above 3,000 Hz. All the advertisement calls have sound characteristics suited for short or medium distances.

The frequencies band width of the advertisement calls of the four species overlapped. The smallest degree of overlap was recorded for *H. sanborni* and *H. elianeae* (Fig. 5). For the species of the *nana* group and the *rubicundula* group, the fundamental frequency was shown to aid in acoustic partitioning among the species (Fig. 5). The fundamental frequency (=dominant frequency), the band where most of the energy of the notes is concentrated, was shown to be one of the factors that aided in partitioning the acoustic space. In spite of the low degree of overlap observed between *H. nana* and *H. jimi* (Fig. 5), there was a significant difference (ANOVA, $F=11.5$, $P<0.01$) between the fundamental frequencies of all the species.

The temporal parameters of the advertisement call were important factors which that the species in partitioning acoustic space. Without phonotaxis experiments we really do not know how these species partition acoustic space, at least insofar as what is important to what conspecific and heterospecific males and females might actually respond to. The intraspecific characteristics of the advertisement call during the beginning of calling activity and chorus activity revealed significant differences (ANOVA, $P<0.01$) in note duration (Fig. 6a), pulse duration, rate of note repetition (Fig. 6b), and rate of pulse repetition in *H. nana*, *H. sanborni*, *H. jimi* and *H. elianeae*.

In the interspecific analyses of the emissions of the advertisement call at the beginning of calling, there was no significant difference between the rate of repetition of the notes of *H. sanborni* and *H. elianeae* (ANOVA, $F=0.82$, $P>0.01$), or of *H. sanborni* and *H. jimi* (ANOVA, $F=1.66$, $P>0.01$). Note duration, rate of

TABLE 3. Characteristics of the six advertisement call variables for the four species of *Hyla* studied near Botucatu, São Paulo. The temporal bioacoustic parameters of the advertisement call are presented for songs at the beginning of calling activity and during the chorus. The data represent the mean±one standard deviation and (range).

| | | <i>Hyla nana</i> (n=73) | <i>Hyla sanborni</i> (n=58) | <i>Hyla elianeae</i> (n=51) | <i>Hyla jimi</i> (n=49) |
|----------------------------------|--------------|-----------------------------|--------------------------------|--------------------------------|------------------------------|
| Frequency bands (Hz) | | 2950–4850±50 (2600–5500) | 3860–5950±300 (3600–6450) | 2400–4380±140 (2350–4470) | 3000–4900±200 (2900–5100) |
| Fundamental frequency (Hz) | | 3985±129 | 5165±136 | 3396±157 | 4069±149 |
| Note duration (ms) | Initial call | 44.4±9.2 (26–61) | 38.2±9.8 (26–65) | 16.6±2.6 (10–23) | 34.1±6.4 (26–47) |
| | Chorus | 20.5±2.3 (16–26) | 30.8±5.5 (20–43) | 18.4±2.9 (12–26) | 52.1±9.2 (37–72) |
| Pulse duration (ms) | Initial call | 2.54±0.61 (2–3) | 4.55±0.86 (3–6) | 3.48±0.84 (2–6) | 5.2±1.07 (3–6) |
| | Chorus | 2.7±0.65 (2–4) | 4.02±0.94 (3–6) | 4.89±0.82 (2–6) | 6±1.1 (3–6) |
| Note repetition rate (notes/sec) | Initial call | 1.14±0.31 (0.77–2.01) | 1.41±0.44 (0.44–2.08) | 1.32±0.3 (0.72–1.88) | 1.6±0.3 (0.92–1.98) |
| | Chorus | 4.73±0.87 (2.75–6.51) | 3.67±0.7 (2.25–4.76) | 3.12±0.45 (2.6–4.02) | 2.64±0.51 (2.1–4.93) |
| Pulse repetition rate (ms) | Initial call | 3.98±0.85 (3–5) | 6.84±0.69 (6–8) | 4.52±0.71 (3–6) | 11.37±0.96 (9–12) |
| | Chorus | 4.06±0.66 (3–5) | 7.54±0.49 (6–9) | 4.89±0.82 (3–6) | 15.57±1.23 (12–18) |

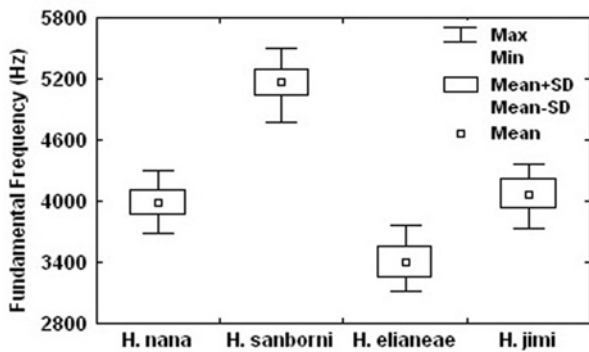


FIG. 5. Fundamental frequencies of the advertisement calls of *Hyla nana*, *H. sanborni*, *H. elianeae* and *H. jimi*, near Botucatu, São Paulo.

pulse repetition and pulse duration differed significantly among the species during the beginning of calling activity (ANOVA, $P < 0.01$).

During chorus calling, the interspecific analyses revealed significant differences among the four species (ANOVA, $P < 0.01$) in all the temporal variables of the advertisement call.

There was a positive correlation between air temperature and the rate of note repetition during the chorus for *H. nana* ($r_s = 0.62$; $P < 0.001$; $n = 73$), *H. sanborni* ($r_s = 0.78$; $P < 0.001$; $n = 58$) and *H. jimi* ($r_s = 0.80$; $P < 0.001$; $n = 49$). For *H. elianeae* there was no correlation between the rate of note repetition and air temperature ($r_s = 0.29$; $P > 0.01$; $n = 51$). There was no correlation for any of the four species between rate of note repetition and air temperature during the beginning of calling activity ($P > 0.05$).

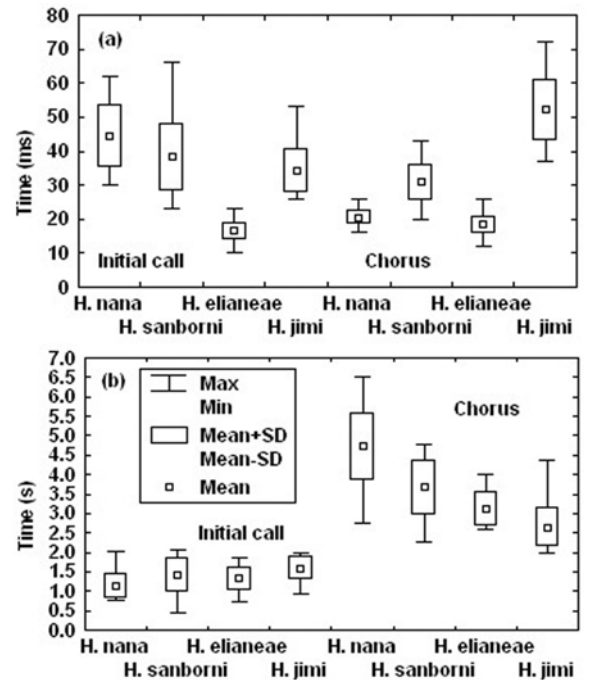


FIG. 6. (a) Note duration (ms) and (b) note repetition rate (s) of the advertisement call of *Hyla nana*, *H. sanborni*, *H. elianeae* and *H. jimi* (Hj), near Botucatu, São Paulo, at the beginning of calling activity and during calling in chorus.

DISCUSSION

CALLING SITE

Habitat type can be an evolutionarily conservative characteristic among closely related species, so that they tend to share similar life history attributes in the same habitat, but in slightly different locations; i.e. they are spatially separated (Heyer *et al.*, 1990). According

to MacNally (1985), spatial segregation may occur not only through occupying different habitats, but through differences in the behaviour of the species.

The data obtained in the present study for the occupation of calling sites by species of the *nana* group are similar to those presented by other workers in different regions (Barrio, 1962; Bernarde & Anjos, 1999; Bernarde & Kokubum, 1999; Rossa-Feres & Jim, 2001; Bertoluci & Rodrigues, 2002). Ecological information about the species of the *rubicundula* group is scarce (Jim, 2002; Martins & Jim, 2004).

In spite of the overlap recorded for at least one of the calling site variables, there were differences between the species groups in how they occupied their calling sites. The species of the *nana* group differed in height and distance from the pond edge. In the areas where they occurred in sympatry, males of *H. sanborni* tended to call from a higher position than did males of *H. nana*. Distance from the pond edge was the calling-site variable which made possible the greatest spatial segregation between the two species. The males of *H. nana* called predominantly towards the middle of the pond, whereas *H. sanborni* was recorded further out, near the edge.

In the *rubicundula* species group, the segregation occurred in relation to the height and type of the perch. Males of *H. jimi* called from a higher position than did males of *H. elianeae*. The differences in occupation of the perch were closely related to height. Most males of *H. elianeae* were observed on the ground, while males of *H. jimi* predominantly perched on sedges.

Comparing the species of the *nana* and *rubicundula* groups, we observed that there was great similarity between *H. sanborni* and *H. jimi* in the occupation of the calling site, showing the greatest degree of overlap. The mean perch height was similar in the two species. *Hyla sanborni* occupied the region near the pond edge, and *H. jimi* established itself further out from the pond edge.

The species which showed the greatest degree of segregation in the occupation of the calling site was *H. elianeae*. This species has wide behavioural plasticity. In environment II, where *H. elianeae* coexists with *H. sanborni*, *H. nana* and *H. jimi*, the males of *H. elianeae* call on the ground, while the other species call from perches. In environments I and III, where it coexists with *H. nana* and *H. sanborni*, individuals of *H. elianeae* call from perches; although they differ from *H. nana* and from *H. sanborni* in that they use perches located farther from the pond edge.

Ptacek (1992), analysing the use of calling sites in *H. chrysoscelis* and *H. versicolor*, observed that the most important difference between their calling sites was perch height, and that this partitioning of calling site according to perch height may be important in preventing mismatings between these two species. Given (1990) found little evidence for differences in microhabitat use by two species of *Rana*; however, he recorded a differential use in the location of the calling site in relation to distance from the pond edge between the species.

The ecological similarities among the species studied here may be related to adaptive convergence, or may be a consequence of the high degree of relatedness. Zimmerman & Simberloff (1996) argued that phylogenetically close species share morphological and behavioural characteristics, because of the brief time since their speciation event. From this viewpoint, the observed similarity in occupation of the calling site among the species analysed near Botucatu can be interpreted as an interaction between the availability of environmental resources and the limitations imposed by the evolutionary history of the taxonomic groups.

Rossa-Feres & Jim (2001), studying a community of anurans in a temporary environment, observed that in regard to one behavioural variable in calling site use – position on the perch – overlap was greater between closely related species. In the present study, this variable showed the greatest overlap. Nevertheless, this high degree of overlap was not observed for the most closely related species, but rather between a species of the *nana* group and one of the *rubicundula* group (*H. sanborni* and *H. jimi*). An important aspect to be considered is that although the species studied are closely related to each other within the groups, the two groups are also closely related, as opposed to the situation studied by Rossa-Feres & Jim (2001), who did not analyse two closely related species groups.

The most important variables for the coexistence of the species studied were height, type of perch and distance of the calling site from the pond edge. Analysing the distribution of frequencies along the gradients of the resources analysed, we found that the species occupied a wide range of gradients. Within this range, however, each species showed a specific preference for the use of available resources (calling site variables), which differed among the species. These differences were often subtle, resulting in close ecological similarity between two species, such as between *H. sanborni* and *H. jimi*.

BIOACOUSTICS

The wide variety and abundance of some sympatric related species of tropical anurans have attracted attention for studies of interspecies coexistence and their interactions in partitioning acoustic space (Duellman, 1967; Hödl, 1977; Duellman & Pyles, 1983; Cardoso & Vielliard, 1990; Márquez *et al.*, 1993; Schwartz & Wells, 1983, 1984). When many individuals are signalling within an actively partitioned space, the details of the acoustic interactions can be complex. Additional complexities are imposed by changes in the conditions of social interactions and the presence of noises that interfere with the signals. The acoustic space in a community of anurans is characterized precisely by these complexities (Schwartz, 2001).

Calling is the main isolation mechanism among species that occupy the same type of environment. Nevertheless, sympatric species can have similar calls, and the specificity of the signal must be established by combined spectral and temporal parameters (Hödl, 1977).

Littlejohn (1977) suggested mechanisms through which acoustic interference can be reduced: (1) use of different frequency bands by different species; (2) spatial segregation, each species using specific calling sites within the environment; (3) temporal acoustic segregation, synchronization and different temporal structures of the notes, avoiding overlap; and (4) different patterns of specific codes.

For the species of *Hyla* analysed in this study, frequency band width was not a parameter that promoted acoustic isolation, because of the overlap in the frequency range. The fundamental frequency differs significantly among the species, indicating acoustic partitioning and representing different channels of sound communication among them.

Cardoso (1981) observed that the dominant frequency of the song was different in most of the species that he studied, indicating acoustic partitioning among species in the region where the highest energy of the song was concentrated.

According to Littlejohn (1977), the frequent occurrence of superposition of the same frequency band in different anuran species is a result of selection process and ecological pressures, which act on the species and become evident in morphological characteristics such as body size. This relationship between body size and the frequency band of the calls was confirmed by Duellman (1967). Blair (1964) observed that body size and the size of the vocal apparatus affect the frequency used by anurans. Márquez *et al.* (1993) assumed that similar-appearing species have common anatomical and physical factors, and that such factors would determine the close similarity in the use of acoustic and spatial resources. For the populations of the species studied here, body size did not differ very much, all of them being small (15–26 mm), and using similar frequency bands (Martins & Jim, 2003, 2004).

Communication is a dynamic process, in which a signal emitted by one individual can influence the behaviour of another (Schwartz, 2001). Frequently, individuals calling in large chorus groups change the rhythm of their calls in response to the calls of other, nearby males, in order to preserve the integrity of their signals and reduce acoustic interference, in addition to aiding in location of males by females and in maintaining spacing between conspecific individuals (Wells, 1977, 1988; Passmore & Telford, 1981; Schwartz, 1987).

In the present study, the species of the *nana* group showed a wider frequency band width than did the species of the *rubicundula* group. The use of narrow frequency bands, as observed for *H. elianeae* and for *H. jimi*, can increase communication efficiency by minimizing interference from the environment (Straughan, 1973), and by this means can reduce competitiveness among the species. According to Cardoso (1981), sound communication in open areas may favour those species that have calls in wide frequency bands, as recorded in the present study for *H. nana* and *H. sanborni*, because

this greater frequency spread allows for more opportunity for adaptation, if there is competition for communication channels.

The temporal structure of the calls of the species studied was shown to be a strong mechanism for effecting partitioning of acoustic space, allowing coexistence in similar habitats during the same activity periods. The variables of pulse duration, rate of pulse repetition, note duration and rate of note repetition differed significantly among the species, mainly during chorus activity, when the competition for acoustic space is greatest in the aggregations of individuals.

The overlap in temporal parameters observed among the species occurred at different moments of calling activity. The rhythm of note emission by individuals in chorus was an important factor in partitioning the acoustic space, and can be considered as yet another parameter of species recognition.

Hödl (1977), studying an anuran community in Amazonia, noted that the songs of four species in which the frequency spectra overlapped were strongly differentiated in temporal structure. Cardoso & Vielliard (1990) observed that in five synchronopatric species of hylids that emitted simultaneous sound signals on the same frequency band, acoustic space was partitioned by means of differentiation of the temporal structures.

The partitioning of spatial and acoustic occupation among the species studied cannot be explained simply through isolated analysis of a single variable, but rather by the interaction among all the variables present. These combinations of variations in the characteristics of the different environments occupied allow for many possibilities for adjustments in the use of spatial and bioacoustic resources by the species (Jim, 2002), as observed in the present study. Nevertheless, even if two species show overlap in one or more dimensions of their niches, there will always be some specific aspect that makes possible coexistence between sympatric, phylogenetically related species.

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