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The Advertisement Call of *Hyla intermedia* Boulenger, 1882 in Comparison to that of *Hyla arborea arborea* (Linnaeus, 1758) (Anura: Hylidae)

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Abstract. Advertisement calls recorded within the range of distribution of *Hyla intermedia* at temperatures between 8.7°C and 20.1°C were analysed. The calls consist of pulse groups comprising 8.55 pulses on average. The durations of the pulse groups and of the intervals between them are negatively correlated with air temperature. Comparison with the advertisement calls of *H. a. arborea* reveals only slight differences. The pulse-group duration is the same in both species, but in *H. intermedia* the intervals are somewhat shorter and the number of pulses per group is slightly lower than in *H. a. arborea*.

Key words. Advertisement calls, comparative call analysis, *Hyla intermedia*, *H. arborea*.

1. INTRODUCTION

On the basis of electrophoretic data regarding genetic variability, NASCETTI et al. (1995) established the tree-frog species *Hyla intermedia*, the range of which encompasses continental Italy and Sicily. The tree frogs inhabiting this region differ in nine out of 28 protein loci from *H. a. arborea*, collected in Switzerland and Germany. Their morphological differences are limited to a single characteristic: in *H. intermedia* the tympanic membrane is larger than in *H. a. arborea*.

No analysis of the advertisement call seems to have yet been published, although this call is a reliable distinguishing feature of tree frogs. Many studies have demonstrated the species specificity of the advertisement calls, and in more than a few cases bioacoustic results have revealed kinship relations that were otherwise unclear, giving rise to new systematic classifications or even resulting in the discovery of new species.

MERTENS & WERMUTH (1960) regarded all the tree frogs in Europe as belonging to the six subspecies of *H. arborea*. Comparative analyses of the advertisement calls of all subspecies then showed that the one in the western Mediterranean region differs considerably from *H. arborea* and placed beyond doubt the species status of *H. meridionalis* (SCHNEIDER 1966, 1967, 1968, 1974, 1977, 1978). The tree frog in the Middle East that had previously also been regarded as a subspecies of *H. arborea* was likewise shown by bioacoustic results to be a distinct species, *H. savignyi* (SCHNEIDER & NEVO 1972). Subsequent discrimination experiments confirmed the independence of *H. meridionalis* and *H. savignyi*, because the females of these two species can reliably distinguish the advertisement calls of their conspecific males from those of *H. a. arborea* (SCHNEIDER 1982; SCHNEIDER et al. 1984; SCHNEIDER & Institut für den Wissenschaftlichen Film 1985). According to the results of behavioural experiments with male *H. savignyi*, they are also capable of such discrimination, inasmuch as they are incited to begin calling more rapidly by conspecific advertisement calls than by those of *H. a. arborea* (BRZOSKA et al. 1982).

Hybrids appear to be rare among tree frogs. OLIVEIRA et al. (1991) discovered a natural hybrid of *H. arborea* and *H. meridionalis*, identified by its intermediate advertisement call.

Once *H. intermedia* had been established, it seemed desirable to investigate the advertisement call of this species as well, so that information would be available about the structure of this important characteristic. A comparison with the call of *H. a. arborea* was also of interest, in view of the fact that Italy and Sicily had previously been considered part of the range of *H. a. arborea*.

2. MATERIALS AND METHODS

Tape recordings of tree-frog advertisement calls were obtained at two localities in Italy: (1) in swampy terrain ca. 1 km west of Colfiorito, Umbria (43°01'46" N, 12°52'42" E, 764 m altitude) on April 30, 2002 at 8.7-10.5°C air temperature (2 males), and (2) at drainage ditches in Padule di Fucceˊchio ca. 800 m southeast of Anchione, Tuscany (43°48̇'52" N, 10°45'53" E, 17 m alt.) from May 1 to 3, 2002 at 14.9-15.5°C air temperature (8 males) and from May 5 to 6, 2003 at 16.0-20.1°C air temperature (16 males). The location data were obtained with a Magellan GPS 2000 XL. The calls were recorded with a Stellavox SP 8 tape recorder and the condenser microphone Sennheiser MKH 816 T (tape speed 9.5 cm/s). The recordings were analyzed in the form of oscillograms (Tektronix 502 A os-

cilloscope, Toennies Recordine camera; recording speed 250 mm/s). The spectrograms were prepared with the program BatSounds. For the mathematical treatment of the data GraphPad Prism, Version 2.0, and Statgraphics, Version 4.0 were used.

Until recently a call in the case of tree frogs was considered to be a single pulse group. This was appropriate to the situation in *H. meridionalis*, because in this species both the calls and the intervals between them are very long, so that each call represents an individual sound event. However, in tree-frog species with short pulse groups and intervals a call consists of a series of pulse groups. This terminology is used here, as was already the case in the first studies of the advertisement call of *H. a. arborea* (SCHNEIDER 1966, 1967). To obtain the values for the pulse groups, intervals and pulses per group, in about the middle of a call three pulse groups and the associated intervals were measured, the number of pulses per pulse group determined and the means of these were used for the calculations.

3. RESULTS

3.1. Calling behaviour

At the two sites Colfiorito and Anchione, the tree frogs began producing their advertisement calls (Fig. 1) at about 19:30 CET, when twilight was well advanced. Initially the pauses between calls were relatively long, 5-40 s. As it grew darker the calling was more persistent, with inter-call intervals shorter than 5 s. At Colfiorito the air temperature when calling began was only slightly above the lower threshold for calling. One male, uninfluenced by the other males' calling, separated its calls by pauses of very different lengths, ranging from 1.3 to 35.5 s. Because the air temperature here fell rapidly, the local tree frogs stopped calling at about 21:30. In Anchione at the beginning of May, 2002 rainy weather prevailed, with air temperature that was unusually low for the time of year and showed little change during the evening. Despite this favourable temperature, the tree frogs fell silent towards midnight. In contrast, the beginning of May in 2003 was characterized by a stable high-pressure zone in Tuscany, with daytime temperature maxima between 32 and 34.5°C. The tree frogs were strongly stimulated, so that males spending the day in bushes or on trees near the water were already giving a few calls before noon. Sustained calling, however, did not begin until the evening. The actively calling frogs were small; for example, two males had an SVL of 33 and 36 mm.

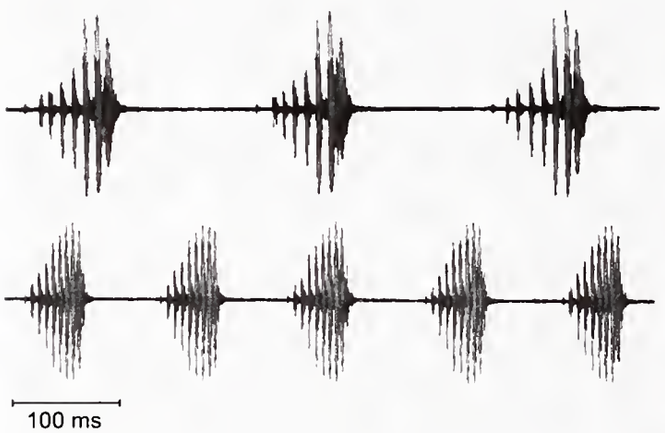


Fig. 1. Oscillograms showing sections of two advertisement calls, recorded at the air temperatures 8.7°C (above) and 19.8°C (below). During the same period of time three pulse groups are produced at the lower temperature and five at the higher one.

For calling the males approached the water. Occasionally the first calls came from the shrubbery fringing the body of water, as the frogs were on the way to their calling sites. Many males chose sites near the water's edge, sitting on the land or in the shallow part of the water, whereas others settled on mats of algae so that they made little contact with the water. By choosing such sites, the tree frogs avoided the high temperatures of the main water mass exposed to strong insulation; during the daytime the water temperature in the channels rose to just above 25°C, and in the evening it was still between 23.9 and 25.1°C. Not uncommonly two males would call in alternation (Fig. 2). The two males were usually neighbours, but could be as much as 5 m apart. Each positioned its pulse groups precisely in the intervals between those of the other male. During this antiphony the intervals inserted by the two partners were very regular but somewhat longer than those of males calling singly. When the calling activity declined in late evening, even males considerably further (up to 20 m) apart called in alternation.

Fig. 2. Antiphonal calling of two tree frogs. The pulse groups with lower amplitude belong to the call of the partner further away from the microphone.

Towards midnight the number of calling frogs decreased as they began wandering away, until finally calling ended.

3.2. The Advertisement call

The advertisement calls consist of pulse groups (Figs. 1, 2), of which the number per call varied widely. In the recordings examined here a call comprises on average 28.79 pulse groups, with a range from 12 to 49 (Table

1). Depending on the number of pulse groups, the duration of the calls also varies considerably. The pulse groups are composed of an average of 8.55 pulses. Each group begins with a very quiet pulse (Figs. 1, 2), after which the pulse amplitude rises continually, reaching a maximum with the penultimate or final pulse.

Table 1: Results of the statistical calculations. Significance level: *, P < 0.1, **, P < 0.01, ***, P < 0.001. n.s. not significant.

Variable	*N*	r^2	Regression equation or $\bar{x} \pm$ Std. Deviation
H. intermedia:			
Duration of pulse groups	28	0.75***	y = 132.40 – 3.83x
Interval between pulse groups	28	0.60***	y = 182.30 – 5.87x
Pulses / pulse group	28	0.06 n.s.	$\bar{x} = 8.55 \pm 0.97$
Pulse groups / call	28	0.01 n.s.	$\bar{x} = 28.79 \pm 11.72$
Call duration	28	0.09 n.s.	$\bar{x} = 4707.00 \pm 2157.00$
H. a. arborea :			
Duration of pulse groups	91	0.75***	y = 125.10 – 3.02x
Interval between pulse groups	92	0.57***	y = 237.20 – 7.90x
Pulses / pulse group	82	0.02 n.s.	$\bar{x} = 9.10 \pm 0.41$

The three parameters call duration, number of pulse groups per call and number of pulses per group were all unaffected by air temperature (Table 1). However, two others – pulse-group duration and inter-group interval – were negatively correlated with air temperature (Figs. 4, 5). These changes conform to linear regressions. The shortening of the pulse groups is brought about by an increase in the pulse repetition rate, associated with a truncation of the damped phase of the preceding pulse. When the air temperature rises from 9°C to 20°C, the pulse groups and intervals are shortened by 43% and 50%, respectively.

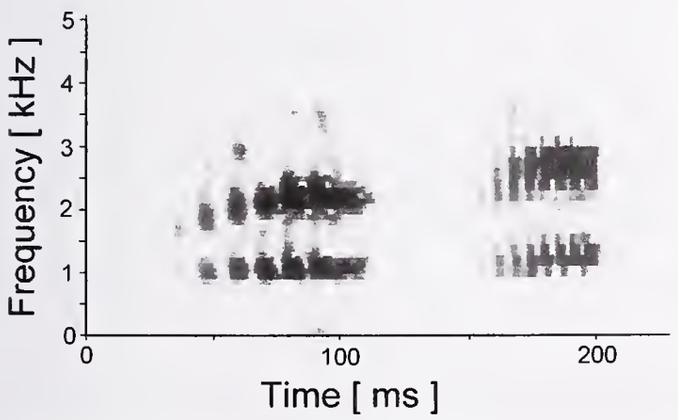
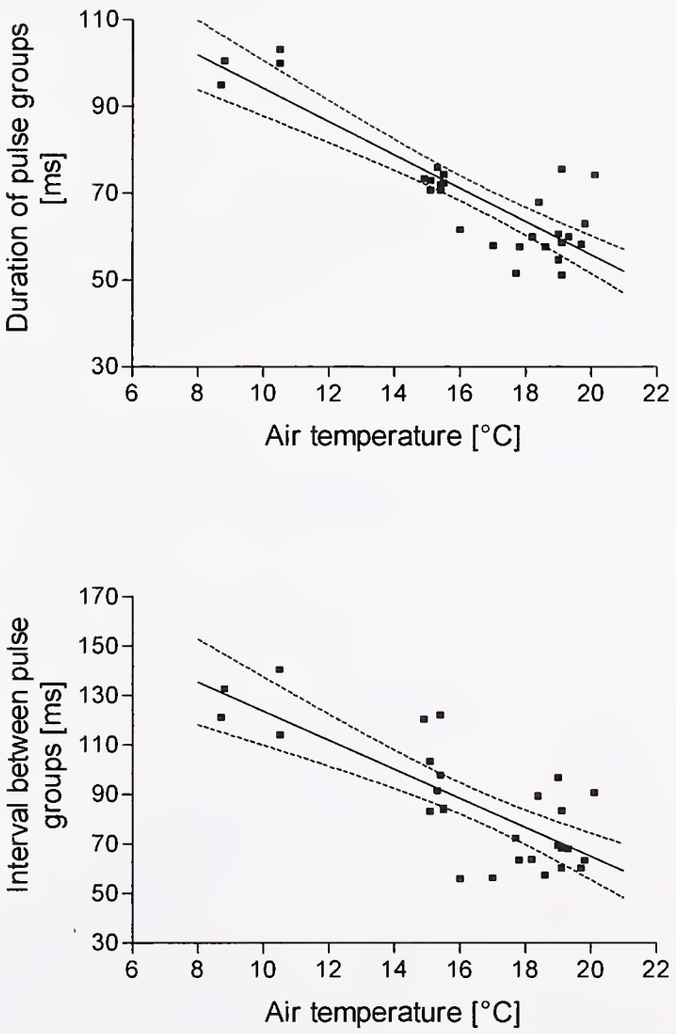


Fig. 3. Frequency spectrogram of two pulse groups, at the air temperatures 8.7°C (left) and 19.8°C (right).

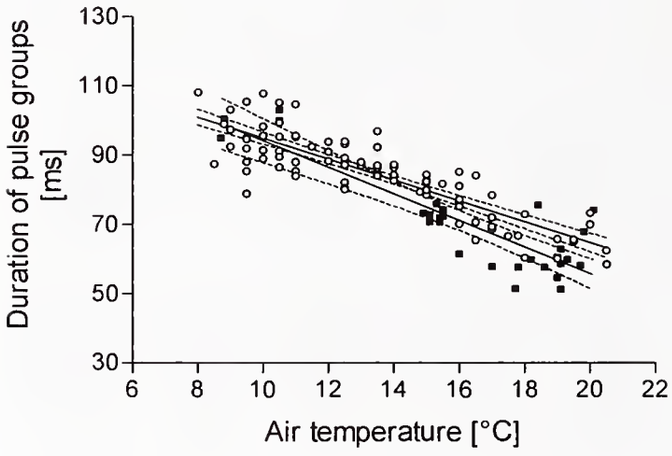
The frequency spectrum exhibits two temperature-dependent dominant frequency bands (Fig. 3). At 9°C air temperature the first lies between 900 and 1100 Hz, while the second is between 1600 and 2500 Hz. When the air temperature rises to 19.8°C, both bands shift upward by 50-100 Hz.

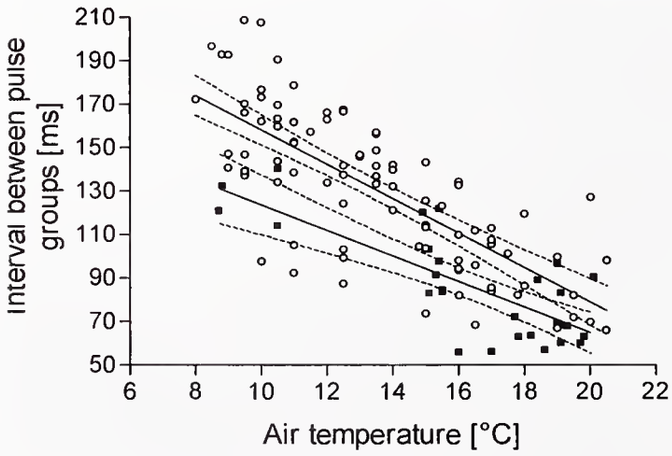


Figs. 4-5. Duration of the pulse groups (Fig. 4) and the intervals between the pulse groups (Fig. 5) as a function of air temperature. Regression line with 95% confidence interval.

3.3. Comparison between *H. intermedia* and *H. arborea*

Until the species *H. intermedia* was established, Italy was counted as part of the range of *H. a. arborea*. Hence a comparison of the advertisement calls of the two taxa seemed to be indicated. For comparison the data from tree frogs in southwestern Germany were used (SCHNEIDER 1967). Regarding the duration of the pulse groups, there are only minor differences between these species (Fig. 6). At 9°C air temperature the pulse-group durations calculated from the regression equations are 97.93 ms for *H. intermedia*, and 97.92 ms for *H. a. arborea* – the two values agree. At 20.0°C air temperature the corresponding values are 55.80 ms for *H. intermedia* and 64.70 ms for *H. a. arborea*. The difference is 8.90 ms. The statistical comparison of the linear regression models revealed that the slopes did not differ significantly (*H. intermdia* -3.80 ± 0.40 versus *H. a. arborea* -3.00 ± 0.20; ANOVA, P>0.05), whereas the differences among the intercepts were sigificant (*H. intermedia* 132.40 ± 7.20 versus *H. a. arborea* 125.10 ± 2.60; ANOVA P=0.0002).





Figs. 6-7. Duration of the pulse groups (Fig. 6) and intervals (Fig. 7) in *H. intermedia* (■) and *H. a. arborea* (○) as a function of air temperature. Regression lines with 95% confidence intervals.

In the case of the intervals between pulse groups the differences are somewhat greater. The results are as follows: for *H. intermedia*, 129.47 ms at 9°C air temperature and 64.90 ms at 20.0°C air temperature; for *H. a. arborea*, 166.10 ms and 79.20 ms at the same temperatures. The differences are thus 36.63 ms at 9°C and 14.30 ms at 20.0°C air temperature (Fig. 7; Table 1). Again the slopes did not differ between the two taxa (*H. intermedia* -5.70 ± 0.90 versus *H. a. arborea* -7.70 ± 0.70; ANOVA, P>0.05), but the intercepts did (*H. intermedia* 182.30 ± 15.70 versus *H. a. arborea* 235.20 ± 10.00; ANOVA, P<<0.0001).

The comparison of means for pulses per pulse group revealed a statistically significant difference between the means of the two samples (*H. intermedia* 8.50 ± 0.18 versus *H. a. arborea* 9.10 ± 0.05; (mean ± std. error) (ANOVA, P=0.0001).

In the previous study on the calls of *H. a. arborea* (Schneider 1967) the statistics for the number of pulse groups per call and the call duration were not calculated, because these were not correlated with air temperature. Most of the calls were composed of 15-30 pulse groups. During the main spawning season the calls became much longer, comprising 100-180 pulse groups or occasionally as many as 244-362.

4. DISCUSSION

The advertisement call of *H. intermedia* has a structure typical of the *H. arborea* group. The duration of the pulse groups and the intervals between pulse groups are short, and both parameters are negatively correlated with air temperature. There are few pulses per group, and their number is uninfluenced by temperature (Table 2). In *H. intermedia* calls were recorded over a temperature span of 11.1°C. This broad range allows inferences

Table 2: Parameters of the advertisement calls of various tree frogs at 15°C air temperature. The values for *H. savignyi* and *H. meridionalis* (a Tenerife, b Camargue) are based on equations published by Schneider (1977).

Taxon	Duration of pulse groups [ms]	Interval [ms]	Pulses/pulse group [N]
H. intermedia	74.95	94.25	8.55
H. a. arborea	79.80	118.70	9.10
H. savignyi	147.95	194.19	19.92
H. meridionalis	431.82[a]	2062.60[a]	39.04[b]

to be drawn about the changes in call features that are correlated with air temperature. There is some scatter in the values recorded between 16 und 20.1°C, which is probably ascribable to the temperature conditions. On the days when the calls were recorded, the air tempera-

ture was above the water temperature during the daytime, but became lower than that of the water at night. The decisive influence on amphibians is exerted by the temperature of the air, because it is the temperature on the head that determines calling behaviour, as found in *Bombina bombina* (SCHNEIDER 1976). However, when individual tree frogs were in close contact with the water while calling, the higher water temperature may have affected the calls to some extent.

Although some differences between the advertisement calls of *H. intermedia* and *H. a. arborea* are significant they are slight. The duration of the pulse groups is almost the same in both species, with no difference in the lower part of the temperature range and only a small one in the higher part. The confidence intervals overlap over a large proportion of the measurement region (Fig. 6). *H. intermedia* has 8.55 pulses per pulse group; although this is lower than the 9.10 pulses/group found for *H. a. arborea*, this difference does not noticeably affect the duration of the pulse groups. The main detectable difference is that of the intervals separating the pulse groups (Fig. 7). The intervals are somewhat shorter in *H. intermedia* than in *H. a. arborea.* The bioacoustic studies of the advertisment calls appear not to support convincingly the new species, *H. intermedia*.

That the differences between the advertisement calls of *H. intermedia* and *H. a. arborea* are quite small is best shown by comparison with the tree frogs *H. savignyi* and *H. meridionalis* (Table. 2). These two species have very specific advertisment calls, which distinguish them unequivocally from *H. a. arborea*. The original assignment of species status to these two tree frogs was based on the striking differences in their mating calls (SCHNEIDER 1967, 1968, SCHNEIDER & NEVO 1972). The specificity of the advertisement calls enables the females of *H. meridionalis* and *H. savigny* to distinguish a conspecific male reliably from a male *H. a. arborea* (SCHNEIDER, 1982, SCHNEIDER et al. 1984). Conversely, the fact that the differences between the advertisment calls of *H. intermedia* and *H. a. arborea* are so slight raises the crucial question whether the females of the two species are able to distinguish between the advertisement calls of their own species and that of the other one.

According to NASCETTI et al. (1995), *H. intermedia* and *H. a. arborea* are parapatric in the east – the boundaries of their ranges coincide quite closely with the borders between Italy and Slovenia. Hence it is unlikely that the advertisement calls of the two species there would be more different from one another than they are in the populations studied here.

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