

AUDITORY TUNING OF THE IBERIAN MIDWIFE TOAD, *ALYTES CISTERNASII*

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The study of auditory tuning in anuran amphibians is useful for understanding their reproductive behaviour. Auditory tuning is known for a relatively large number of anuran species but most of those studied are recently-derived species, rather than ancient. For one of the ancient species analysed, the common midwife toad (*Alytes obstetricans*), an unusual mismatch was found between the dominant frequency in the advertisement call and the tuning of the inner-ear organ that responds to the high frequencies characterizing the call (the basilar papilla). In this paper, the auditory tuning of a closely related species, the Iberian midwife toad (*Alytes cisternasii*), is analysed and the results are discussed in relation to behavioural experiments performed on this species. The results indicate that in *Alytes cisternasii* basilar papilla tuning closely matches the peak frequency in the call, as is common for recently-derived anurans. Furthermore, the tuning is consistent with behavioural measures of call preferences in this species.

Key words: anuran communication, mating behaviour, neurophysiology, vocalization

INTRODUCTION

Calls play a fundamental role in anuran communication in both male-male acoustic competition and female choice. This dependence has provided two effective means by which anuran reproductive behaviour can be studied: playback tests and neurophysiological experiments. The former reveal the behaviours of males or females when exposed to advertisement calls, whereas the latter generally reveal the response of the auditory system when exposed to sounds of different frequencies. In many cases these approaches have provided results that are consistent with each other. Neurophysiology studies have shown the anuran auditory system to be sensitive to frequencies emphasized in conspecific calls (Capranica, 1965; Zakon & Wilczynski, 1988; Gerhardt & Schwartz, 2001), and to indicate frequencies to which both males and females do in fact respond preferentially in playback studies (Capranica, 1965; Ryan *et al.*, 1990; Ryan *et al.*, 1992).

In anurans, the sensitivity to relatively low frequencies is mediated by the amphibian papilla (AP), one of the two inner-ear organs that amphibians possess, whereas sensitivity to relatively high frequencies is mediated by the basilar papilla (BP), the other inner ear organ (see reviews in Zakon & Wilczynski, 1988; Lewis & Narins, 1999). Different anuran species may have calls that stimulate both papillae, or may stimulate either one. It is generally accepted that there is a reasonably good match between the auditory tuning of the inner-ear organ stimulated by the conspecific advertisement call and the band or bands of frequency emphasized in the call (see review in Gerhardt & Schwartz, 2001). However, there may be small, but significant, mismatches

between average tuning and the average advertisement call dominant frequency. Females of several anuran species have shown preferences for calls of lower than average frequency, which are usually related to larger male sizes (Ryan, 1988). For example, male midwife toads show non-random mating success (Márquez, 1993; Raxworthy, 1990), with larger males usually fertilizing more clutches. In addition, for at least three of the four species of *Alytes*, larger males emit advertisement calls with lower frequencies (Márquez & Bosch, 1995; Bush, 1997), and females are preferentially attracted to those lower frequency calls (Márquez, 1995a,b). Neurophysiology studies in the cricket frog (*Acris crepitans*) and the tungara frog (*Physalaemus pustulosus*) have shown that small mismatches between frequency tuning of the females' BP and male call frequency can potentially mediate mate choice for lower frequency calls, and thus directional sexual selection (Ryan *et al.*, 1990, 1992). Therefore, the study of auditory tuning can provide excellent clues to explain the non-random mating success of males frequently observed in nature and the resultant patterns of sexual selection.

Although the auditory tuning of a moderately large number of anuran species has been analysed to date, most of the approximately 25 species studied in any detail are recently-derived species rather than ancient (for reviews see Gerhardt, 1988; Walkowiak, 1988; Feng *et al.*, 1990; Fox, 1995; Gerhardt & Schwartz, 2001). Therefore, our understanding of how calls and tuning relate to each other is based mainly on recently-derived anurans. Wever (1985) used inner ear microphonic potentials to characterize tuning in several ancient amphibians (*Ascaphus truei*, *Leiopelma hochstetteri*, *Rhinophrynus dorsalis*, three *Bombina* species, and three pipids: *Xenopus laevis*, *X. borealis* and *Pipa pipa*), but did not discuss the relationship of tuning and calls in these species. In only two genera of anurans at the basal

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level of amphibians, *Bombina* (*B. variegata*, *B. orientalis*, and *B. bombina*) and *Alytes* (*A. obstetricans*), has the relationship of calls and tuning been addressed (Mohneke & Schneider, 1979; Walkowiak, 1980, 1988; see also Wever, 1985, for tuning data in *Bombina*). Findings in *Bombina* and *A. obstetricans* are inconsistent. Mohneke & Schneider (1979) found a BP best excitatory frequency (BEF) of 1800 Hz. This value is very far above the mean call frequency known for this species (around 1280 Hz in Spanish populations; Márquez & Bosch, 1995) and, more importantly, this mismatch is in the opposite direction to the observed female preference for calls of lower than average frequency (Márquez, 1995a,b). It is thus opposite to the expected deviation that could explain directional sexual selection to low frequency calls. In contrast, in *B. variegata* there is a good match between frequency tuning of the BP and the animal's call frequency (Mohneke & Schneider, 1979).

Since the mismatch found in *A. obstetricans* by Mohneke & Schneider (1979) between frequency tuning of the BP and call frequency is unusual, in this paper we looked at the closely related species *A. cisternasii* to see if tuning far above the dominant call is characteristic of this genus. The Iberian midwife toad, *A. cisternasii*, is one of the three species of midwife toad that occur in the Iberian Peninsula, where it is confined to the southwestern quarter. In this species, males emit very simple tonal advertisement calls, which overlap substantially with *A. obstetricans* calls in frequency and duration (Márquez & Bosch, 1995; Fig. 1). In some areas, the two species are sympatric (see Márquez & Bosch, 1997, for a distribution map within the Iberian Peninsula). In addition to reporting neurophysiological tuning of the auditory system in *A. cisternasii*, we discuss the results in the context of the existing data on sexual selection for this species.

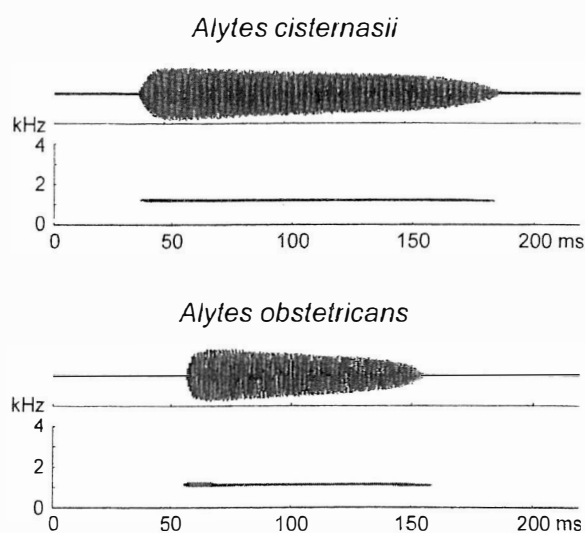


FIG. 1. Oscillograms (above) and audiospectrograms (below) of characteristic male advertisement calls of *A. cisternasii* and *A. obstetricans* from the studied sympatric population (see Márquez & Bosch, 1997 for details).

METHODS

Ten adults of *A. cisternasii* were collected in January 1998 in Aliste (Zamora, West Northern Spain), from a population sympatric with *A. obstetricans*. The capture took place outside the breeding season, and the animals were transported alive to the University of Texas at Austin, USA, and maintained in captivity. Both the animal room and the room in which the neurophysiology experiments were done were kept at 21°C.

Neurophysiological experiments were performed between May 1998 and August 1999 using methods previously used successfully with other small anurans (Ryan *et al.* 1990; Wilczynski *et al.*, 1993; Wilczynski *et al.*, 2001). All procedures were approved by The University of Texas Institutional Animal Care and Use Committee.

Animals were anaesthetized by immersion in a 2.5% aqueous solution of urethane. The midbrain was exposed by opening a skin flap on the top of the head and drilling a hole in the skull. The underlying meninges were then carefully removed. The surgical opening was then covered with a small piece of tissue paper, the skin flap was repositioned, and the animal was allowed to recover from the surgery for 1–2 days.

Following recovery, the animal was immobilized with an intramuscular injection of d-turbo-cuararine chloride dissolved in a 20 mg/ml aqueous solution. The drug dose was approximately 10 µg/g body mass. The surgical opening was swabbed with lidocaine and the skin flap retracted to re-expose the dorsal surface of the midbrain. A drawn-glass micropipette filled with 3M KCl was placed into the midbrain and moved down into the torus semicircularis (inferior colliculus) using a Kopf hydraulic microdrive. Multi-unit auditory activity was isolated using a multi-tone search stimulus delivered to the ear contralateral to the recording site via a custom-made earphone assembly. Evoked auditory activity was monitored visually on a storage oscilloscope and acoustically using stereo earphones. With Wavetek stimulus generators and resistive attenuators, stimulus frequency and amplitude were controlled manually. Custom-made stimulus-control hardware was used to shape stimulus duration, rise/fall time, and repetition rate. Sound amplitude delivered through the earphone was calibrated with a B&K 2300 precision digital sound pressure meter at the end of a recording session by sealing the end of the earphone assembly over the meter microphone.

Once the electrode reached a location where robust evoked auditory activity was isolated, midbrain auditory thresholds were determined to frequencies from 100 to 4000 Hz in 100 Hz steps using 150 ms tone bursts repeated every 1.5 s. Threshold was defined as the minimum sound amplitude which could evoke discernible multi-unit neural activity. Once these thresholds were obtained, the process was repeated in smaller frequency and amplitude steps to obtain a more precise estimate of the best excitatory frequencies (BEF; fre-

quency with lowest threshold) of the two sensitivity peaks apparent in the recordings: the lower one we presume represents amphibian papilla tuning and the higher one we presume represents basilar papilla tuning (Wilczynski & Capranica, 1984). The procedure was repeated at three to six different electrode positions within the midbrain. Means were calculated for each animal based on dB values without converting to absolute pressure. Recordings at different positions sometimes resulted in different thresholds, but gave no differences in the shape of the audiogram or the positions of the threshold minima. This is probably due to the fact that the low impedance electrodes used for obtaining the multi-unit recordings summed activity over an area that was large relative to the small size of the toad midbrain, and is also consistent with previous reports indicating little or no tonotopic organization apparent in neurophysiological investigations of the anuran midbrain (Wilczynski & Capranica, 1984).

Neurophysiological experiments were completed successfully only on five animals. All the experimental animals were immediately euthanized by an overdose of the anesthetic MS222 (tricaine methyl sulfonate), either at the end of the neurophysiological experiment or when we could not obtain consistent, clearly recognizable, auditory-evoked activity above the background activity during the recording session. Snout-vent length was measured and sex was determined by dissecting the animal and looking at its gonads.

RESULTS

The five animals analysed were found to be males. Their snout-vent length (SVL) ranged from 31 to 37.9

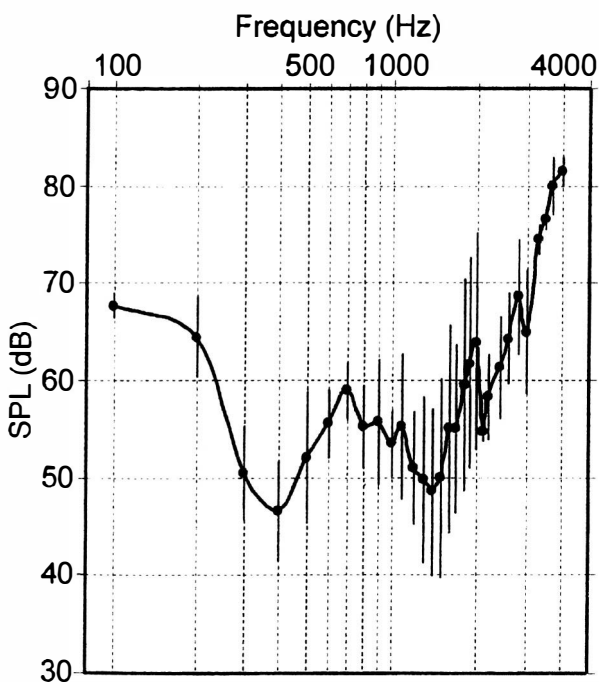


FIG. 2. Average audiogram (mean \pm SE) for five *A. cisternasii* males. The average AP BEF is 417 Hz, while the average BP BEF is 1365 Hz.

mm (average 34.7 mm), slightly below the population average size (38.8 ± 3.1 mm; Márquez & Bosch, 1997).

The average audiogram for the five analysed animals appears in Fig. 2. As expected, the tuning curve shows a bimodal pattern. We presume that the area of tuning from 100 to 1000 Hz represents the sensitivity profile of the amphibian papilla (AP). The second band of increased sensitivity, 1000–2000 Hz, represents the sensitivity profile of the basilar papilla (BP). In the five animals tested, the average AP BEF was 417 Hz, while the average BP BEF was 1365 Hz.

DISCUSSION

The bimodal appearance of the audiogram obtained for *A. cisternasii* is typical for neurophysiological recordings of auditory sensitivity in the anuran midbrain (Wilczynski *et al.*, 1984, 1993, 2001; Gerhardt & Schwartz, 2001). The two areas of frequency sensitivity have been shown to represent the contributions from the two inner-ear auditory organs, the lower frequency amphibian papilla (AP) and the higher frequency basilar papilla (BP). The area of greatest sensitivity, or best excitatory frequency (BEF), obtained for the BP matched the average male call dominant frequency for the population (1328 ± 64 Hz; Márquez & Bosch, 1997). The BEF obtained for the AP confirms that this inner-ear organ in *A. cisternasii* is not likely to be involved in the recognition of acoustic species-specific signals, being related to detecting low frequencies not present in the conspecific call. Several small, recently-derived anurans (Neobatrachia) show the same pattern of specialization of the inner-ear organs seen in the present study for this more ancient species (Narins & Capranica, 1976; Wilczynski *et al.*, 1984, 1993; Ryan *et al.*, 1992).

Although the sample size for these experiments is not very high, the consistent and expected bimodal tuning curve obtained indicates reasonable accuracy for the purpose of this study. Furthermore, the studied species does not present the problem of having a call with a broad-band spectrum with substantial acoustic energy spurs which characterizes many anuran advertisement calls. Instead, the total power in the *A. cisternasii* call is concentrated in a narrow frequency band with no harmonics and no frequency modulation.

The BEF obtained for the BP indicates that males in the studied population are matched or tuned slightly higher in frequency than the call. This is consistent with the majority of studies in recently-derived anurans (Neobatrachia) showing that BP tuning roughly matches call frequency. The small difference between BP BEF and call frequency found here is within the methodological sample error both in the acoustical analysis of male call frequency and the neurophysiological experiments. In fact, the standard deviation for the dominant frequency of the population is 64 Hz (over a sample size of 16 males recorded; Márquez & Bosch, 1997), double the difference found here. Therefore we are confident that there is no major mismatch between auditory tuning and call frequency in males of this species.

Although the results obtained here are only for males, in this species we can tentatively extend the conclusions to females. Despite the existence of good evidence for sexual differences in tuning, mediated in large part by size differences in other species (see Narins & Capranica, 1976; Wilczynski, 1986; Keddy-Hector, Wilczynski & Ryan, 1992; Wilczynski *et al.*, 1992), the sexes in *A. cisternasii* are similar in size, contrary to most anuran species. In typical habitat (i.e. Mérida, Extremadura, West Central Spain), the average SVL of males is 37.8 mm while that of females is 39.8 mm (Bosch & Márquez, 1996). Additionally, Mohnke & Schneider (1979) did not find significant differences between sexes in auditory tuning in *A. obstetricans*, although size ranges of males and females did not overlap. It will be necessary to obtain neurophysiology data from females before definitive conclusions can be reached, but given the data we obtained and extrapolations from the literature on other anuran species, it is very unlikely that females would be tuned greatly above males and therefore unlikely that they are tuned far above the conspecific call.

Our results contrast with those obtained by Mohnke & Schneider (1979), who found a considerable mismatch between the BEF of the BP and the dominant frequency for *A. obstetricans* advertisement calls. Since the animals they tested ranged from 36.5 mm to 45.5 mm, it is difficult to assume that the difference found occurred because the studied animals were significantly smaller than the average in their population. It is not clear from either the behaviour or the environmental conditions of *A. obstetricans* why that species should have such a great mismatch.

The closeness of BP tuning to the call dominant found here in *A. cisternasii* suggests that males from the studied population would be most sensitive, and presumably respond preferentially, to average calls from their own population. If females from this population were tuned about the same as males, they too would prefer average calls. This hypothesis is consistent with the behavioural experiments performed with females from this population by Márquez & Bosch (1997). Female preferences for calls with lower than average frequency have been found in several other populations of midwife toads (Márquez, 1995a,b), but not in females from the sympatric population whose animals we used for the present study. This could be because in the sympatric populations choosing a low frequency, the call may result in a heterospecific male being chosen (i.e. *A. obstetricans*), whose males produce a call with a slightly lower dominant frequency than that of the *A. cisternasii* call. This possibility should exert selection pressure against preferring lower frequency calls (Márquez & Bosch, 1997). Therefore, under this consideration, we would not expect to find a BEF lower than the average of male call frequency in the studied population. The behavioural results showing that preferences for low frequency calls occur in other, allopatric populations of

A. cisternasii suggest that there may be interesting population-level differences in the tuning of the auditory system in this species.

Further neurophysiological studies to determine whether *A. cisternasii* frequency-tuning is lower in allopatric populations than in sympatric populations could elucidate whether auditory tuning perfectly explains female *Alytes* preferences. Furthermore, additional studies of the auditory systems of other ancient anurans would be helpful in understanding whether the close relationship between calls and auditory tuning is as much a characteristic of basal anurans as it is of recently-derived taxa.

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REFERENCES

- Bosch, J. & Márquez, R. (1996). Discriminant functions for sex identification in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Herpetological Journal* **6**, 105-109.
- Bush, S. L. (1997). Vocal behavior of males and females in the Majorcan midwife toad. *Journal of Herpetology* **31**, 251-257.
- Capranica, R. R. (1965) *The Evoked Vocal Response of the Bullfrog: A Study of Communication by Sound*. Cambridge, MA: MIT Press.
- Feng, A. S., Hall, J. C. & Gooler, D. M. (1990). Neural basis of sound pattern recognition in anurans. *Progress in Neurobiology* **34**, 313-329.
- Fox, J. H. (1995). Morphological correlates of auditory sensitivity in anuran amphibians. *Brain, Behavior and Evolution* **45**, 327-338.
- Gerhardt, H. C. (1988). Acoustic properties used in call recognition by frogs and toads. In *The Evolution of the Amphibian Auditory System*, 455-484. Fritzsche, B., Ryan, M. J., Wilczynski, W., Hetherington, T. E. and Walkowiak, W. (Eds.). New York: John Wiley & Sons.
- Gerhardt, H. C. & Schwartz, J. J. (2001). Auditory tuning and frequency preferences in anurans. In *Anuran Communication*, 73-85. Ryan, M. J. (Ed). Washington: Smithsonian Institution Press.
- Keddy-Hector, A. C., Wilczynski, W. & Ryan, M. J. (1992). Call patterns and basilar papilla tuning in cricket frogs. II. Intrapopulation variation and allometry. *Brain, Behaviour and Evolution* **39**, 238-246.
- Lewis, E. R. & Narins, P. M. (1999) The acoustic periphery of amphibians: Anatomy and physiology. In *Comparative Hearing: Fish and Amphibians*, 101-

154. Fay, R. R. and Popper, A. N. (Eds.). New York: Springer-Verlag.
- Márquez, R. (1993). Male reproductive success in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Behavioral Ecology and Sociobiology* **32**, 283-291.
- Márquez, R. (1995a). Female choice in the midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Behaviour* **132**, 151-161.
- Márquez, R. (1995b). Preferencia de las hembras por cantos de frecuencia dominante baja en el sapo partero común *Alytes obstetricans* (Anura, Discoglossidae). Experimentos *in situ*. *Revista Española de Herpetología* **9**, 77-83.
- Márquez, R. & Bosch, J. (1995). Advertisement calls of the midwife toads *Alytes* (Amphibia, Anura, Discoglossidae) in continental Spain. *Journal of Zoological Systematics and Evolutionary Research* **33**, 185-192.
- Márquez, R. & Bosch, J. (1997). Male advertisement call and female preference in sympatric and allopatric midwife toads (*Alytes obstetricans* and *Alytes cisternasii*). *Animal Behaviour* **54**, 1333-1345.
- Mohneke, R. & Schneider, H. (1979). Effect of temperature upon auditory thresholds in two anuran species, *Bombina v. variegata* and *Alytes o. obstetricans* (Amphibia, Discoglossidae). *Journal of Comparative Physiology A* **130**, 9-16.
- Narins, P. M. & Capranica, R. R. (1976). Sexual differences in the auditory system of the treefrog, *Elutherodactylus coqui*. *Science* **192**, 378-380.
- Raxworthy, C. J. (1990). Non-random mating by size in the midwife toad *Alytes obstetricans*: bigger males carry more eggs. *Amphibia-Reptilia* **11**, 247-252.
- Ryan, M. J. (1988). Constraints and patterns in the evolution of anuran communication. In *The Evolution of the Amphibian Auditory System*, 637-677. Frittsch, B., Ryan, M. J., Wilczynski, W., Hetherington, T. E. and Walkowiak, W. (Eds.). New York: John Wiley & Sons.
- Ryan, M. J., Fox, H. H., Wilczynski, W. & Rand, A. S. (1990). Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* **343**, 66-67.
- Ryan, M. J., Perrill, S. A. & Wilczynski, W. (1992). Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *American Naturalist* **139**, 1370-1383.
- Walkowiak, W. (1980). The coding of auditory signals in the torus semicircularis of the fire-bellied toad and the grassfrog: Responses to simple stimuli and to conspecific calls. *Journal of Comparative Physiology A* **138**, 131-148.
- Walkowiak, W. (1988). Neuroethology of call recognition. In *The Evolution of the Amphibian Auditory System*, 485-510. Frittsch, B., Ryan, M. J., Wilczynski, W., Hetherington, T. E. and Walkowiak, W. (Eds.). New York: John Wiley & Sons.
- Wever, E. G. (1985). *The Amphibian Ear*. Princeton NJ: Princeton University Press.
- Wilczynski, W. (1986). Sexual differences in neural tuning and their effect on active space. *Brain Behavior and Evolution* **28**, 83-94.
- Wilczynski, W. & Capranica, R. R. (1984). The auditory system of anuran amphibians. *Progress in Neurobiology* **22**, 1-38.
- Wilczynski, W., Keddy-Hector, A. C. & Ryan, M. J. (1992). Call patterns and basilar papilla tuning in cricket frogs. I. Differences among populations and between sexes. *Brain Behavior and Evolution* **39**, 229-237.
- Wilczynski, W., McClelland, B. E. & Rand, A. S. (1993). Acoustic, auditory, and morphological divergence in three species of neotropical frog. *Journal of Comparative Physiology A* **172**, 425-438.
- Wilczynski, W., Rand, A. S. & Ryan, M. J. (2001). Evolution of calls and auditory tuning in the *Physalaemus pustulosus* species group. *Brain, Behavior and Evolution* **58**, 137-151.
- Wilczynski, W., Zakon, H. H. & Brenowitz, E. A. (1984). Acoustic communication in spring peepers: call characteristics and neurophysical aspects. *Journal of Comparative Physiology A* **155**, 577-587.
- Zakon, H. H. & Wilczynski, W. (1988). The physiology of the anuran eighth nerve. In *The Evolution of the Amphibian Auditory System*, 125-155. Frittsch, B., Ryan, M. J., Wilczynski, W., Hetherington, T. E. and Walkowiak, W. (Eds.). New York: John Wiley & Sons.

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