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## Heterospecific call recognition and phonotaxis in the orientation behavior of the marbled newt, *Triturus marmoratus*

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**Abstract** The role of acoustic cues as reference cues for orientation by amphibians has been demonstrated in anurans, the only amphibian group that engages in acoustic communication, but not in urodeles. Orientation responses of marbled newts, *Triturus marmoratus*, were studied to determine whether heterospecific calls elicited positive phonotaxis. The orientation tests consisted in presenting either a familiar acoustic stimulus, the advertisement calls of natterjack toads (*Bufo calamita*), or a control stimulus, the advertisement calls of European green toads (*Bufo viridis*) that the newts would not be expected to recognize. Marbled newts and natterjack toads occur in sympatry, but *T. marmoratus* and *B. viridis* are allopatric species. Thus, *T. marmoratus* is distributed over the northern half of the Iberian Peninsula, whereas *B. viridis* occurs in the Balearic Islands, but not over the Iberian Peninsula. Newts were released in a circular arena while a recorded chorus of natterjack toads or European green toads played outside the arena to determine whether they displayed positive phonotactic orientation. Our results show that marbled newts performed positive phonotaxis when exposed to the breeding calls of natterjack toads, but not to those of European green toads. Newts chose a compass course in the direction of the advertisement calls of *B. calamita*. Acoustic information might improve orientation accuracy. This study is the first to provide evidence of heterospecific call recognition and positive phonotactic response in urodeles.

**Keywords** Acoustic orientation · Homing · Migration · Phonotaxis · *Triturus*

### Introduction

Many species of amphibians engage in remarkable migrations to and from breeding ponds, and several have been found to be adept at homing (Able 1980; Sinsch 1992a). Amphibians may be capable of homing using celestial cues (Landreth and Ferguson 1967; Diego-Rasilla and Luengo 2002), local landmarks (Dole 1965; Adler 1980), odours from ponds (Grubb 1973; Joly and Miaud 1993), acoustic cues (Ferguson and Landreth 1966; Sinsch 1992b) and magnetic cues (Phillips 1986; Fischer et al. 2001).

Available information suggests that demonstrating a particular capability in an animal is only the first step in understanding its orientation behaviour. The ability under study may be only one of many different cues which the animal may rely on, depending on environmental conditions or other factors (Able 1980). Nearly all species rely on multiple environmental cues in orientation and these mechanisms often seem to be related hierarchically (Able 1991). The possible role of acoustic cues in orientation of amphibians has been studied very little (Able 1980). Their use as reference cues for orientation by amphibians has been demonstrated in toads and frogs (Ferguson and Landreth 1966; Sinsch 1992b), but not in newts or salamanders. Although sounds do not provide animals with compass information per se, acoustic cues might provide at least useful back-up input to migrating animals (Able 1980). Acoustic signals might function as beacons for female anurans to locate distant aggregates of calling males (Sun et al. 2000). Thus, female anurans respond phonotactically to the vocalizations of males (Gerhardt 1988, 1994), and laboratory phonotaxis experiments have revealed that male toads preferentially associated with conspecific male calls (Pfenning et al. 2000). On the contrary, urodeles have auditory systems uninfluenced by

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the sexual selective pressures associated with using audition for communication (Wilczynski and Ryan 1988).

Orientation responses of marbled newts, *Triturus marmoratus*, to the advertisement calls of natterjack toads (*Bufo calamita*), and of European green toads (*Bufo viridis*) were studied to determine whether heterospecific calls elicited positive phonotaxis. The hypothesis under investigation is that these particular newts, which normally use celestial cues for locating breeding ponds (Diego-Rasilla and Luengo 2002), may rely on the advertisement calls of anurans that the newts would be expected to recognize, to locate breeding ponds. Namely, urodeles may have evolved some capability to use loud anuran choruses for orientation.

## Methods

### Subjects and study site

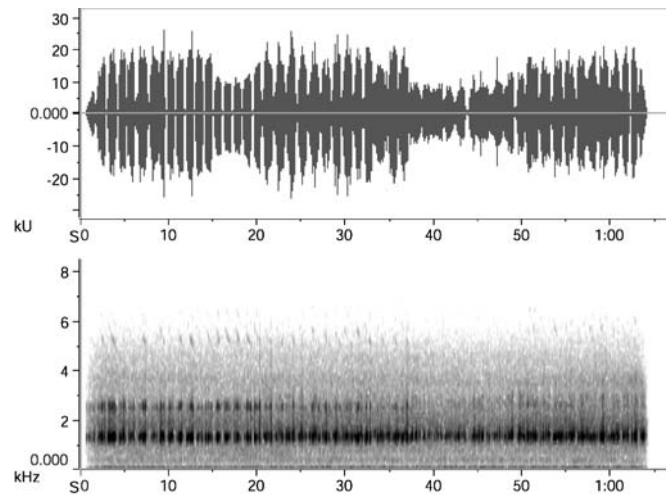
We collected 38 marbled newts, 13 adult females and 25 adult males, from a temporary pond of 270 m<sup>2</sup>, situated in Valdeajos (Burgos Province, northern Spain; 42°44'32"N, 3°54'41"W), at an elevation of 1,040 m. Newts shared the pond with a population of *B. calamita*, and no other species of amphibians were found in the pond. In this area, marbled newts usually begin their migration to their breeding pond in mid-March (Diego-Rasilla and Luengo 2002). Test specimens were collected during spring 2003 (11 April) between 1930 and 2200 hours (GMT), placed in opaque plastic containers (54×35×21 cm) in which the water depth was 1 cm, and taken to the indoor testing arena by car. Animals were tested between 1 and 8 h after being captured and they were returned to their pond just after testing.

### Procedure

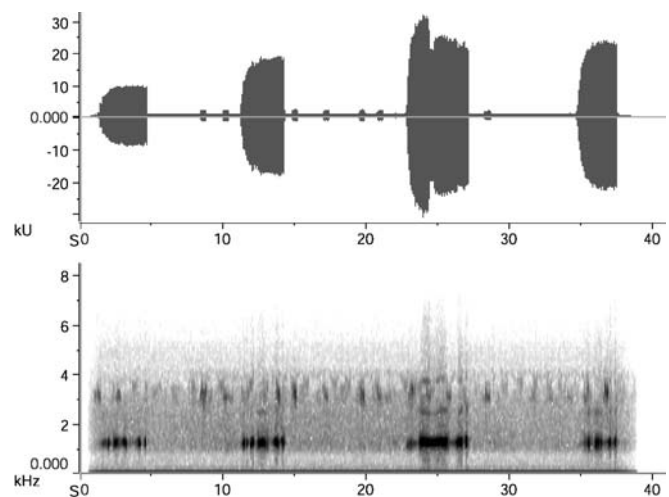
Newts were tested indoors. The room temperature during the tests was 10.71±0.27°C, and relative humidity was 80.96±0.34%. The test arena consisted of a circular plastic container (57 cm diameter, 28 cm high). The arena floor and walls were thoroughly wiped with a damp sponge after each trial to eliminate directional olfactory cues (Fischer et al. 2001), and then they were wiped dry using paper towels.

Just prior to their individual testing session, newts were kept individually for 5 min in opaque plastic containers (34×24×16 cm) in which the water depth was 1 cm. For testing, a newt was removed individually from its plastic container, and put in the arena centre in darkness beneath an opaque, cylindrical plastic container (9 cm diameter, 14.5 cm high). Then we presented the acoustic stimulus, i.e. the advertisement calls of toads (see details below). Newts could not be seen through the container wall and they were kept in the container for 1 min to overcome effects of handling before the cover was lifted. Once the cylindrical plastic container was lifted, each newt was permitted to move about the arena, but animals remaining motionless in the arena centre for 5 min were excluded from the analyses. To further minimize disturbance during the experiments, we left the testing site, leaving each animal alone for 5 min. The newt's directional response was later recorded by the moist trails it left on the floor of the arena. In all cases, the animals moved directly from their initial position to the arena walls, tapped against the wall and then, clinging to the wall, proceeded to circle around the arena. Directional responses were recorded to 5° accuracy at the first point where an animal tapped against the wall.

The orientation tests consisted of presenting either a familiar acoustic stimulus, the calls of *B. calamita*, or a control stimulus, the calls of *B. viridis*, that *T. marmoratus* would not be expected to recognize. *T. marmoratus* and *B. calamita* occur in sympatry, but *T.*



**Fig. 1** Waveform and spectrogram views of *Bufo calamita* calls. Waveform view (*top*): the *x*-axis indicates time in seconds, while the *y*-axis denotes amplitude in kilounits (kU). The “units” displayed on the vertical axis of the waveform are the actual sample values in the signal, which are proportional to the sound pressure at the microphone when the sound was recorded. The Spectrogram view (*bottom*) represents time on the horizontal axis, frequency on the vertical axis, and relative intensity at each time and frequency as a greyscale value



**Fig. 2** Waveform and spectrogram views of *B. viridis* calls. Waveform view (*top*): the *x*-axis indicates time in seconds, while the *y*-axis denotes amplitude in kilounits (kU). The “units” displayed on the vertical axis of the waveform are the actual sample values in the signal, which are proportional to the sound pressure at the microphone when the sound was recorded. The Spectrogram view (*bottom*) represents time on the horizontal axis, frequency on the vertical axis, and relative intensity at each time and frequency as a greyscale value

*marmoratus* and *B. viridis* are allopatric species, since they occupy exclusive geographical areas. *T. marmoratus* is distributed over the northern half of the Iberian Peninsula, whereas *B. viridis* occurs in the Balearic Islands, but not over the Iberian Peninsula.

The calls of *B. calamita* (Fig. 1) and *B. viridis* (Fig. 2) were obtained from Márquez and Mateu (1995) and were stored on a notebook computer (MITAC 5033). The calls were broadcast to subjects using Tsunami EA-968 speakers (Tsunami, Guangdong,

**Table 1** Directional responses of the marbled newts

Orientation directions of the acoustic stimuli	Absolute bearing	Acoustic bearing
Control acoustic stimulus ( <i>Bufo viridis</i> )		
180°	330°	150°
180°	140°	320°
180°	15°	195°
180°	10°	190°
180°	170°	350°
180°	120°	300°
270°	335°	65°
270°	260°	350°
270°	220°	310°
270°	200°	290°
270°	295°	25°
270°	135°	225°
270°	350°	80°
270°	180°	270°
90°	25°	295°
90°	100°	10°
90°	225°	135°
90°	90°	0°
90°	140°	50°
90°	360°	270°
90°	10°	280°
360°	200°	200°
360°	225°	225°
360°	210°	210°
360°	350°	350°
360°	75°	75°
360°	90°	90°
360°	145°	145°
360°	105°	105°
Familiar acoustic stimulus ( <i>B. calamita</i> )		
180°	320°	140°
180°	310°	130°
180°	165°	345°
180°	150°	330°
180°	315°	135°
180°	175°	355°
180°	135°	315°
270°	30°	120°
270°	110°	200°
270°	280°	10°
270°	270°	0°
270°	235°	325°
270°	270°	0°
270°	215°	305°
90°	95°	5°
90°	40°	310°
90°	100°	10°
90°	125°	35°
90°	300°	210°
90°	360°	270°
90°	240°	150°
360°	10°	10°
360°	15°	15°
360°	350°	350°
360°	310°	310°
360°	90°	90°
360°	25°	25°
360°	55°	55°

China) and Cool Edit 96 acoustics software (Syntrillium Software Corporation, Phoenix, USA). The overall absolute sound pressure level (dB SPL) of the calls, measured 20 cm from the speakers (i.e. the distance of the newt to the speakers) with a Digital Sound Level Meter (Electro Tools ET 9901, Guijarro Hermanos, Madrid, Spain),

was 57 dB. As newts were situated 20 cm from the sound sources, amplitude attenuation was minimal.

The acoustic stimuli were presented outside of the circular arena from four different compass orientation directions (0°, 90°, 180° and 270°). We randomised the order of the stimulus presentation for each individual in each experimental condition. The first four newts were tested with the control acoustic stimulus (i.e. the calls of *B. viridis*), followed by four fresh newts (i.e. previously untested) tested with the familiar acoustic stimulus (i.e. the calls of *B. calamita*). This sequence was repeated until all the newts had been exposed once to the familiar acoustic stimulus and once to the control stimulus. Also, the first individual was tested with the acoustic stimulus from 180°, the next individual was tested with the acoustic stimulus from 270°, followed by one tested with the stimulus from 90°, and one tested with the stimulus from 0°. The order of the four acoustic stimuli directions (180°, 270°, 90°, 0°) was determined using a random number sequence. If a newt did not reach the orientation criterion within the appropriate time interval, the next individual was tested following the established sequence. This sequence was repeated until the test had been completed. Accordingly, data pooled from an entire test series included roughly equal numbers of bearings from newts tested in each of the four symmetrical orientation directions of the acoustic stimuli.

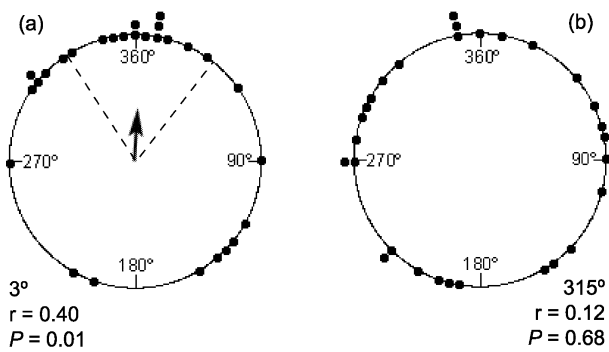
The advantage of this type of design is that the data can be pooled by rotating the four distributions so that the directions of the acoustic stimuli coincide (see Phillips 1986). Thus, in subsequent analysis, data from the four conditions are combined by rotating the bearings so that the acoustic stimuli compass directions coincide at 0° (i.e. 90° is subtracted from the actual headings of newts tested with the acoustic stimuli from 90°, 180° from the headings of newts tested with acoustic stimuli from 180°, and 270° from the headings of newts tested with acoustic stimuli from 270°) (Table 1). Therefore, if the newts are exhibiting a consistent directional response relative to the acoustic stimuli, the pooled data will be non-randomly distributed, and the 95% confidence interval for the mean vector bearing will include 0° (Batschelet 1981).

#### Statistical analyses

Data were analysed using standard circular statistics (Batschelet 1981; Fisher 1995). Mean vectors were calculated by vector addition and tested for significance using the Rayleigh test for a non-random distribution. Ninety-five percent confidence intervals were used to determine whether the mean for the distribution included the acoustic stimulus direction (see Batschelet 1981; Fisher 1995). Also, a modified Rayleigh test, the *V* test, was used to test closeness to expected orientation (i.e. the direction of the acoustic stimuli, 0°), and the Watson *U*<sup>2</sup>-test was used to test for differences between distributions (Batschelet 1981).

## Results

The Rayleigh test of uniformity of a circular distribution showed a significant preferred orientation of newts exposed to the familiar calls of *B. calamita* ( $\mu=3^\circ$ ,  $r=0.40$ ,  $n=28$ ,  $P=0.01$ ). The 95% confidence interval (327°, 40°) for the mean vector bearing ( $\mu$ ) includes the direction of the acoustic stimuli ( $\mu_0=0^\circ$ ), and the *V* test also shows that the newts clearly headed in the direction of the acoustic stimuli ( $P=0.001$ , *V* test with expected direction = 0°), indicating that the newts performed positive phonotaxis when exposed to the advertisement calls of natterjack toads (Fig. 3a). However, they oriented randomly when exposed to the control acoustic stimulus (i.e. the calls of *B. viridis*), and we could not reject the null hypothesis of uniformity ( $\mu=315^\circ$ ,  $r=0.12$ ,  $n=29$ ,



**Fig. 3a, b** Orientation responses of the marbled newts, *Triturus marmoratus*, during the experiments. **a** Orientation responses of marbled newts to the advertisement calls of natterjack toads (*B. calamita*). **b** Orientation responses of marbled newts to the advertisement calls of European green toads (*B. viridis*). Symbols indicate the direction of movement of each individual newt tested only once in one of four symmetrical acoustic stimuli orientation directions (i.e. 0°, 90°, 180°, 270°). The arrow at the centre of the diagram indicates the mean direction of orientation; the length of the arrow is proportional to the mean vector length ( $r$ ). Dashed lines indicate the 95% confidence intervals for the mean vector length; the radius of each diagram corresponds to  $r=1$

$P=0.68$ , Rayleigh test;  $P=0.27$ ,  $V$  test with expected direction = 0° (Fig. 3b). The distributions of the orientation data under the two treatments did not differ ( $U^2=0.141$ ,  $P>0.10$ , Watson  $U^2$ ).

## Discussion

Since newts performed positive phonotaxis when exposed to the advertisement calls of natterjack toads, our results indicate that marbled newts might rely on acoustic cues from *B. calamita* to locate breeding ponds. Also, present findings support the hypothesis that the newts are capable of recognizing the advertisement calls of anurans with which they share a breeding pond. In fact, the calls of *B. viridis* did not elicit any orientation response. This may not be surprising, since in Spain this toad only occurs on the Balearic Islands, and a newt recognizing a familiar breeding call would not be expected to recognize the call of other allopatric anuran species. This study is the first to provide evidence of heterospecific call recognition and positive phonotactic response in urodeles.

Although newts lack middle and external ears, they have inner ears that can process sound (Hetherington 2001). As previously demonstrated in earless frogs and toads (Lindquist et al. 1998; Hetherington and Lindquist 1999), the lateral body wall and lungs of newts may function in sound reception, especially at relatively low frequencies (i.e. peak motion ranging from 1,600 to 2,500 Hz in small newts, and from 1,250 to 1,600 Hz in larger salamanders) (Hetherington 2001). Sound causes the newt's chest to vibrate, and the vibrations are carried by air from the lungs to the newt's inner ear for processing. Since thinner body walls respond more readily to sound, it may be that the lungs can capture a

wide range of frequencies only in small animals (Hetherington 2001). These findings suggest that the lung-based hearing system of earless amphibians may represent the retention of the first auditory mechanism used by early tetrapod vertebrates for detection of airborne sound (Hetherington and Lindquist 1999; Hetherington 2001).

The use of acoustic cues in marbled newts' orientation may be limited, if compared with celestial orientation (Diego-Rasilla and Luengo 2002). It is advantageous for a newt to return to a site where that species has bred successfully (Joly and Miaud 1989), and celestial orientation best suits this purpose, whereas movement to new sites, presumably in response to calls, may accomplish dispersal (Ferguson and Landreth 1966).

Evidence from many species suggests that a hierarchy of cues may be used for orientation (Able 1991). Toads of the genus *Bufo* employ a variety of orientation cues to relocate the breeding site following experimental displacement (Sinsch 1990). Initial orientation is based on a hierarchical order of the different sensory cues according to their relative availability within the habitat, and to the distance of pondward migration, as suggested by Sinsch's (1990) findings from three toad species. Indeed, the newts often migrate on rainy nights, when cloud cover would preclude the use of celestial cues (Joly and Miaud 1993). Moreover, marbled newts seem to be unable to orient themselves in the absence of celestial cues (Diego-Rasilla and Luengo 2002). Hence, *T. marmoratus* might use breeding calls of toads as an alternative guidance mechanism under an overcast sky. The use of conspecific calls for orientation on rainy or overcast nights has been suggested in anurans. Ferguson and Landreth (1966) demonstrated the role of conspecific calls as a guidance mechanism for migrating individuals of *B. fowleri* and they suggested that calls would provide a useable mechanism when it is cloudy and an alternative mechanism on clear nights. In addition, female toads and frogs use phonotaxis to determine the directionality of male calls in order to locate mates (Castellano and Giacoma 1998; Bosch et al. 2000, 2003; Schwartz et al. 2001).

However, the perception of acoustic cues a long way from the breeding site is improbable because acoustic signals change over distance due to loss of amplitude and change in the frequency spectrum. Changes in both signal amplitude and frequency spectrum over distance can degrade signal efficacy (Kime et al. 2000). Thus, sufficient loss of amplitude can decrease signal-to-noise ratio to a level at which the receiver cannot detect the signal. Moreover, selective attenuation of certain frequencies can make the signal unrecognisable to the newts (Kime et al. 2000). Therefore, the use of the toad's calls as orientation cues may only be useful from relatively close to the pond. Even with clear conditions, auditory cues may increase the accuracy of orientation during the final approach to the breeding pond. Hence, celestial orientation would be enhanced with an orientation toward the advertisement calls of the natterjack toads when entering a zone around the pond. Acoustic information

might improve accuracy, mainly by pinpointing the precise direction of the pond.

Hence, our results show that migrating newts can use the calls of toads as an alternative guidance mechanism, particularly under an overcast sky. Thus, meteorological conditions might govern the relative importance of each type of cue in providing useful orientation information (Able 1980; Ferguson and Landreth 1966).

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