Sound radiation pattern of the advertisement call of the highly territorial poison frog *Allobates femoralis*

Camilo Rodríguez⁎, Walter Hödl²

1. Introduction

In acoustically communicating species sound production and signal detection is critical for species-specific recognition, where selective forces (e.g. predators, sexual selection) and constraints (e.g. physiological, morphological, environmental constraints) may affect the evolution of the signal (Amézquita et al., 2006; Andersson & McGregor, 1999; Forrest, 1994; Narins & Zelick, 1988). Acoustic communication in frogs is an important factor in reproductive biology, where males produce calls to attract females. In territorial frogs (e.g. Dendrobatidae) the calling behavior also allows the males to advertise a territory (Pröhl, 2005).

The effectiveness of frog communication depends on sound-propagation patterns, the sound-pressure level at the caller position, and the sensory system of the receiver (Bernal et al., 2009). The sound-propagation pattern of the acoustic signals has ecological implications for both, the sender and the receiver in a heterogeneous environment (Hunter et al., 1986). On the one hand, calling with spatially uniform radiation allows information to be transmitted equally into all directions (Gerhardt, 1975; Narins & Hurley, 1982). On the other hand, directionally emitted calls could optimize energy expenditure, reduce predation or unwanted intraspecific aggression by addressing only receivers in specific directions, and improve the orientation of the receiver to the sender (Narins & Hurley, 1982; Hunter et al., 1986). Sound-radiation patterns have been studied in frog species with a lek mating system (Bernal et al., 2009; Brenowitz et al., 1984; Gerhardt, 1975; Greer & Wells, 1980; Narins & Hurley, 1982; Parris, 2002; Table 1), but have been largely ignored in territorial species where sound propagation is critical for territory advertisement.

Most of the neotropical poison frogs are well known to be highly territorial (Lotters and Mutschmann, 2007; Wells, 2007). For instance, males of the brilliant-thighed poison frog, *Allobates femoralis* Boulenger, 1883 (Dendrobatidae: Aromobatinae; AmphibiaWeb, 2018; Pyron & Wiens, 2011; Grant et al., 2017) announce territory ownership against intruders with advertisement calls and defend it with physical attacks (Narins et al., 2003; Ringler et al., 2011; Roithmair, 1992). Usually, males rotate phonotactically on the same spot towards calling neighbours and produce antiphonal calls when the amplitude of the received signal is between 56 and 68 dB. Signals above ~68 dB evoke an aggressive response (Hödl, 1982, 1983, 1987). The behavioural significance of the head-body turning behaviour might be to direct the call towards specific targets, as has been shown in several species of songbird (Breitwisch & Whitesides 1987; Glutz von Blotzheim & Bauer 1997; Brumm, 2002). However, when calling alone, males usually call without rotating, suggesting that the signal spreads uniformly in all directions.

If the main functions of the advertisement call of *A. femoralis* are to announce the ownership of a territory to neighbouring males and to attract females, then we expect that an omnidirectional sound radiation pattern would be advantageous to attract females from and repel intruders in surrounding territories in all directions, within highly structured environments. We thus aimed to test whether territorial
frogs are more likely to have an omnidirectional sound propagation pattern. Since frogs could also address the spectral parameters of the call as a function of sound direction and position of the caller (Reviewed in Bee & Christensen-Dalsgaard, 2016), we additionally tested how spectral features of the advertisement call could change as a function of the direction of the sound source.

2. Methods

2.1. Study species and study area

We conducted our study with the *A. femoralis* population in the field camp ‘Pararé’, located in the Natural Reserve ‘Les Nouragues’ in French Guiana (4°02’N - 52°41’W; Bongers et al., 2013; see Ringler et al., 2015 for a description of the population characteristics), during the early reproductive season (February to April) in 2018 and 2019. The measurements were taken between 1500–1800 h, which corresponds to the peak calling activity of this species (Kafer et al., 2012). The advertisement call of *A. femoralis* usually consists of four notes with a small ascending frequency sweep and a mean-call peak frequency of 3582 ± 213.5 Hz (mean ± SD, n = 12; Fig. 1).

2.2. Sound radiation pattern

To determine the sound-radiation pattern of the advertisement call of *A. femoralis*, we measured the Sound-Pressure Levels (SPL) of the advertisement call of 10 males from the four cardinal directions (0/360º = front, 90º = right side, 180º = back and 270º = left side) with a SPL-meter (Voltcraft 329, Conrad Electronic SE, Wenberg Köblitz, Germany). Since the sound can be attenuated due to scattering by vegetation (Forrest, 1994), we carefully cleaned the path from the groundcover vegetation with garden clippers, between the frog and the SPL-meter to avoid variation in the SPL measurements due to differential vegetation effects. We used low-intensity playbacks from the four cardinal directions to orientate the focal individual phonotactically into the direction of the speaker at its calling perch. Upon playback cessation the individual started to call without changing its calling site and position (Hödl, 1982). The SPL-meter was mounted on a full-size tripod, aligned parallel to the ground, and pointing towards the calling frog. We adjusted the height of the SPL-meter according to the calling-perch height of the focal frog (average ± SD of the calling-perch height of the 10 measured frogs = 9.7 ± 3 cm). All SPL measurements were taken at a fixed distance of 1 m from the frog using the peak detector of the SPL-meter in the C-weighting curve and on its fast setting. The frogs generally start calling with “warm-up calls”, defined as suboptimal advertisement calls of less than steady-state SPL (Jameson, 1954; Toledo et al., 2014); therefore, we measured the SPL from the 5th four-note call in a call series consisting of at least 15 consecutive four-note calls and we took the average of three SPL measurements after a linear conversion to Pascals (Pa = 20μPa10ΔB/20). SPL values were standardized within the z-distribution (with mean = 0 and SD = 1) and the z-scores were used in subsequent analyses.

2.3. Call recordings and analysis

Acoustic recordings of the advertisement call of 12 males of *A. femoralis* were taken with directional microphones (Sennheiser ME64 or ME66, Sennheiser, Wedemark, Germany) on digital recorders (H4n, Zoom, Tokyo, Japan) in three non-consecutive days. We recorded between 10 and 29 consecutive four-note calls with the microphone positioned at a distance of 0.5 m to 1.0 m from each male, parallel to the ground, and pointing towards the calling frog. We calculated the signal’s peak frequency using Praat (Boersma and Weenink, 2019) by averaging the measurements from three calls on every recording. We also registered the cardinal position of each frog in reference to the microphone, being 0/360º the position of the microphone in the cardinal plane. To allow individual body-size measurements (snout-urostyle length mean = 2.94 ± 0.05, n = 12) and identification, we captured and photographed males after each call recording. Additionally, we measured the air temperature at the exact spot where the males were calling to control the effect of temperature-dependent variation in the call frequency.

2.4. Statistical analysis

To establish the sound radiation pattern, we first transformed the

---

### Table 1

| Family       | Species                          | Sound radiation pattern | Δ 0º-180º | Reference       |
|--------------|----------------------------------|-------------------------|-----------|-----------------|
| Hylidae      | *Pseudacris* (Hyla) crucifer     | Omnidirectional         | −0.5 dB   | (Gerhardt, 1975) |
| Hyla chrysocelis |                              | Directional              | −6 dB     |                 |
| Bufonidae    | *Anaxyrus* (Bufo) americanus    | Directional              | −3 dB     |                 |
| Leptodactylidae | *Engystomops* (Physalaemus) pustulosus | Directional              | −6 dB*    | (Bernal et al., 2009) |
| Dendrobatidae| *Allobates* femoralis           | Nearly omnidirectional   | −2 dB     | This study       |

* Highest radiated SPL above the frog at ~90º from the horizontal direction.

---

**Fig. 1.** Temporal and spectral properties of the advertisement call of *A. femoralis*. Numbers in brackets are time in milliseconds (mean ± SD).
four directions into a circular variable by using the “circular” function within the circular package in R (R Core Team, 2017). Then, we predicted the SPL from a simulated vector of 300 angle values from 0° to 360° by splitting the circular predictor in the sine and cosine components of the circular variable and running a simple linear model (Pewsey et al., 2013).

To determine differences in the SPL between the four directions, we performed a general linear mixed model (LMM) using the function “lmer” within the lme4 package (Bates et al., 2015) using SPL as the response variable, the angles as the fixed factor, and the individual as the random factor. In the same way, to evaluate the relationship between the calling direction and the peak frequency, we performed a LMM using the peak frequency as the response variable, the sine and cosine components of the calling angle as fixed factors and the individual as the random effect.

3. Results and discussion

At a distance of 1 m the advertisement call of A. femoralis showed a nearly omnidirectional radiation pattern, decreasing by ~1.5 dB towards the sides (LMM: $t_{0.05} = 2.04, P = 0.05$; $t_{0.01} = 2.84, P = 0.008$; $N = 10$; Fig. 2A & B) and ~2 dB towards the back (LMM: $t_{0.05} = 8.44, P < 0.001$; $N = 10$; Fig. 2A & B) of the calling frog, compared to the front (0°). Linear regression analyses showed that neither the temperature ($R^2 = 0, F = 0.016, P = 0.89$), nor the body size ($R^2 = 0, F = 0.75, P = 0.39$) had an effect on the peak frequency of the advertisement call in A. femoralis. The peak frequency of the signal did not change in function to the directionality of the call (LMM: $cos(\theta) = -98.49, P = 0.06$; $sin(\theta) = -21.71, P = 0.67$; $N = 12$; Fig. 2C).

Many animal communication systems present directionality in the radiation of the acoustic signals, allowing them to optimize energy expenditure by focusing the signal to a specific target (e.g. cicadas (Michelsen & Fonseca, 2000), frogs (Bernal et al., 2009), birds (Hunter et al., 1986) and dogs (Frommolt & Gebler, 2004)). However, omnidirectional emitted sounds could improve the effective communication in complex frog assemblages, attracting females from and advertising to neighbouring males in all directions (Gerhardt, 1975; Narins & Hurley, 1982). Previous studies in other anuran species showed that frogs are able to discriminate sound-pressure level differences of 2 to 6 dB (Fay & Simmons, 1999). Our study found that the differences in SPL for A. femoralis in the four cardinal directions are equal to or below 2 dB (see Fig. 2A & B). Frogs might be using the small differences in SPL to accurately orientate phonotactically towards a conspecific caller (Gerhardt and Rheinlaender, 1980). In fact, A. femoralis often turn on the same axis when responding towards an acoustically active neighbour (Hödl, 1982, 1983, 1987). The same rotating behaviour has been found in Dendrobates granuliferus, another neotropical poison frog, suggesting the same effective vocal transmission in all directions (Goodman, 1971). Furthermore, directing the advertisement call to specific targets (i.e. conspecific neighbouring males or females) from elevated perches also increase the visibility of the calling male using the vocal sac as a multimodal signalling tool (Narins et al., 2003). Thus, we suggest that the rotating behavior in A. femoralis toward the calling rival could be driven by both, the perception of small differences in the SPL of neighbouring males and the display of the vocal sac as a visual signal.

Although the peak frequency of the advertisement call of A. femoralis did not change in function of the direction of the call, a slight difference of about 0.1 kHz can be noticed in azimuth to the microphone within 110°-130° (Fig. 2C). Previous studies in the green tree frog (Hyla chrysoscelis) showed that females can localize with better accuracy sound sources consisting of call components of only 0.9 kHz (Rheinlaender et al., 1979). At present, we can only speculate that the small differences in peak frequency related to the direction of the call in A. femoralis are meaningless for the accuracy of localization by females or neighbouring males. Further research on the tympanum sensitivity in A. femoralis is needed to give insight on how call frequencies could provide information about acoustic localization of neighbouring frogs.

Overall, our results suggest that A. femoralis males present a nearly omnidirectional radiation pattern, which grants the males to advertise the territory and attract females from all directions. However, antiphonal calling neighbours elicit body rotation towards each other, suggesting directivity of the call and the visual field to specific targets.

Acknowledgements

We thank the Nouragues research field station (managed by CNRS) which benefits from “Investissement d’Avenir” grants managed by Agence Nationale de la Recherche (AnaEE FranceANR-11-INBS-0001; Labex CEBA ANR-10-LABX-25-01). This work was funded by the Austrian Science Fund (FWF): W1262-B29. For providing field assistance we are grateful to A. Lembens and V. Canoine. For providing help in call recordings and posterior analysis we especially thank G. Raboisseion.

References

Amézquita, A., Hödl, W., Lima, A.P., Castellanos, L., Erdtmann, L., de Araújo, M.C., 2006. Masking interference and the evolution of the acoustic communication system in the Amazonian dendrobatid frog Aplodontia rana. Evolution 60 (9), 1874–1887.
AmphibiaWeb, 2018. AmphibiaWeb: Information on amphibian biology and conservation. University of California, Berkeley, CA, USA last checked 07.11.2018. www.amphibiaweb.org.
Andersson, S., McGregor, P.K., 1999. Animal communication: what is the signal to noise ratio? Trends in Ecology & Evolution 14 (5), 174–175.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67 (1), 1–48.
Bernal, X.E., Page, R.A., Ryan, M.J., Argo, T.F., Wilson, P.S., 2009. Acoustic radiation
patterns of mating calls of the túngara frog (Physalaemus pustulosus): Implications for multiple receivers. The Journal of the Acoustical Society of America 126 (5), 2757–2767.

Boersma, P., Weenink, D., 2019. Praat: doing phonetics by computer [Computer program]. Version 6.1.03. retrieved 1 September 2019 from. http://www.praat.org/.

Bongers, F., Charles-Dominique, P., Forget, P.-M., Théry, M., 2013. Nouragues: dynamics and plant-animal interactions in a neotropical rainforest Vol. 80 Springer Science & Business Media.

Boonlenger, G.A., 1983. On a collection of frogs from Yurimaguas, Huallaga River, northern Peru. Proceedings of the Zoological Society of London 635–638 Vol. 51. Bretwisch, R., Whiteside, G.H., 1987. Directionality of singing and non-singing behavior of mated and unmated northern mockingbirds, Minus polyglottos. Animal Behaviour 35 (2), 311–339.

Brenowitz, E.A., Wilczynski, W., Zakon, H.H., 1984. Acoustic communication in spring peepers. Environmental and behavioral aspects. Journal of Comparative Physiology A 155 (5), 585–592.

Brumm, H., 2002. Sound radiation patterns in nightingale (Luscinia megarhynchos) songs. Journal of Ornithology. 143 (4), 468–471.

Bee, M.A., Christensen-Dalsgaard, J., 2016. Source sound localization and segregation with internally coupled ears: the treefrog model. Biological Cybernetics. 110 (4–5), 271–290.

Fay, R.R., Simmons, A.M., 1999. The sense of hearing in some North American frogs and toads. Journal of Comparative Physiology 102 (1), 354–365.

Forrest, T.G., 1994. From sender to receiver: propagation and environmental effects on acoustic signals. American Zoologist 34 (6), 644–654.

Frommolt, K.-H., Gebler, A., 2004. Directionality of dog vocalizations. The Journal of the Acoustical Society of America 116 (1), 561–569.

Gerhardt, H.C., Rheinlaender, J., 1980. Territorial and reproductive behavior of the tropical Grant, T., Rada, M., Anganoy-Criollo, M., Batista, A., Dias, P.H., Jeckel, A.M., et al., 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatidae). Proceedings of the 4th Ordinary General Meeting of the Societas Europaea Herpetologica 201–204.

Hunt, T., Roberts, J., Vuillermoz, M., 1986. Directionality of avian vocalizations: a laboratory study. Condor 371–375.

Jameson, D.L., 1954. Social Patterns in the Leptodactylid Frogs Syrrhopus and Eleutherodactylus. Copeia 1954 (1), 36–38.

Kaefer, I.L., Montanarin, A., Da Costa, R.S., Lima, A.P., 2012. Temporal patterns of reproductivity activity and site attachment of the brilliant-thighed frog Allobates femoralis from central Amazonia. Journal of Herpetology 46 (4), 549–554.

Lotters, S., Münchmann, F., 2007. Poison Frogs: Biology, Species and Captive Care. Edition Chimaira.

Michelsen, A., Fonseca, P., 2000. Spherical sound radiation patterns of singing grass cricetines. Typosialis gaulis. Journal of Comparative Physiology A 186 (2), 163–168.

Narins, P.M., Hurley, D.D., 1982. The Relationship between Call Intensity and Function in the Puerto Rican Coqui (Anura: Leptodactylidae). Herpetochelys 38 (2), 287–295.

Narins, P.M., Zelick, R., 1988. The effects of noise on auditory processing and behavior in amphibians. The Evolution of the Amphibian Auditory System. Wiley, New York, pp. 511–536.

Narins, P.M., Hödl, W., Grabul, D.S., 2003. Bimodal signal requisite for agonistic behavior in a dart-poison frog, Epipedobates femoralis. Proceedings of the National Academy of Sciences 100 (2), 577–580.

Parrish, K.M., 2002. More bang for your buck: The effect of caller position, habitat and chorus noise on the efficiency of calling in the spring peeper. Ecological Modelling 156 (2–3), 213–224.

Pewsey, A., Neuhäuser, M., Ruxton, G.D., 2013. Circular statistics in R. Oxford University Press.

Pröhl, H., 2005. Territorial Behavior in Dendrobatid Frogs. Journal of Herpetology 39 (3), 354–365.

Pyron, R.A., Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61 (2), 543–583.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. Vienna, Austria. Retrieved from. https://www.r-project.org/.

Rheinlaender, J., Gerhardt, H.C., Yager, D.D., Capranica, R.R., 1979. Accuracy of phototaxis by the green treefrog (Hyla cinerea). Journal of Comparative Physiology 133 (4), 247–255.

Ringler, M., Ringler, E., Mendoza, D.M., Hödl, W., 2011. Intrusion experiments to measure territory size: development of the method, tests through simulations, and application in the frog Allobates femoralis. Pisces One 6 (10), e25844.

Ringler, M., Hödl, W., Ringler, E., 2015. Populations, pools, and pecarías: simulating the impact of ecosystem engineers on rainforest frogs. Behavioral Ecology 26 (2), 340–349.

Rothhammer, M.E., 1992. Territoriality and Male Mating Success in the Dart-poison Frog, Epipedobates femoralis (Dendrobatidae, Anura). Ethology 92 (4), 318–326.

Toledo, L.F., Martins, I.A., Bruschi, D.P., Passos, M.A., Alexandre, C., Haddad, C.F.B., 2014. The anuran calling repertoire in the light of social context. Acta Ethologica 18 (2), 87–99.

Wells, K.D., 2007. The Ecology and Behavior of Amphibians. University of Chicago Press.