



## Diversity of the Vocal Signals of Concave-Eared Torrent Frogs (*Odorrana tormota*): Evidence for Individual Signatures

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### Abstract

Male concave-eared torrent frogs (*Odorrana tormota*) have an unusually large call repertoire and have been shown to communicate ultrasonically. We investigated the individual specificity of male advertisement calls in order to explore the acoustic bases of individual recognition, which was demonstrated in an accompanying study. Vocalizations of 15 marked males were recorded in the field. A quantitative analysis of the signals revealed eight basic call-types. Two of them (the single- and multi-note long-calls) were investigated in more detail. Long-calls were characterized by pronounced and varying frequency modulation patterns, and abundant occurrence of nonlinear phenomena (NLP), i.e., frequency jumps, subharmonics, biphonations and deterministic chaos. The occurrence of NLP was predictable from the contour of the fundamental frequency in the harmonic segment preceding the onset of the NLP, and this prediction showed individual-specific patterns. Fifteen acoustic variables of the long calls were measured, all of which were significantly different among individuals, except biphonic segment duration. Discriminant function analysis (DFA) showed that 54.6% of the calls could be correctly assigned to individual frogs. The correct classification was above chance level, suggesting that individual specificity of calls underlie the ability of males to behaviorally discriminate the vocal signals of their neighbors from those of strangers, a remarkable feat for a frog species with a diverse vocal repertoire. The DFA classification results were lower than those for other anurans, however. We hypothesize that there is a tradeoff between an increase in the fundamental frequency of vocalizations to avoid masking by low-frequency ambient background noise, and a decrease in individual-specific vocal tract information extractable from the signal.

### Introduction

Sound communication plays an important role in the reproduction of anuran amphibians. The advertisement calls of anurans are species-specific and, in most species, are produced by males only.

Advertisement calls confer information about the signaler's species, sexual receptivity and location, and serve to attract gravid females while keeping rivals at bay (Ryan 2001; Gerhardt & Huber 2002; Kelley 2004; Gerhardt & Bee 2007; Wells & Schwartz 2007).

The vocal repertoire size of anurans varies widely; most species have relatively limited repertoires, but a few can produce dozens of complex calls (Narins et al. 2000; Christensen-Dalsgaard et al. 2002). The concave-eared torrent frog, *Odorrana tormota* (previously *Amolops tormotus*) has an extraordinarily diverse vocal repertoire (Feng et al. 2002; Narins et al. 2004). Males of *O. tormota* produce calls with an unusually high fundamental frequency (F0) and numerous harmonics that extend into the ultrasonic range (i.e., >20 kHz). Calls also exhibit strong fundamental frequency modulation (FM) and various nonlinear phenomena (NLP, i.e., frequency jumps, subharmonics, biphonations and deterministic chaos). The occurrence of NLP appears to contribute to signal variability in calls of *O. tormota*, but this tenet has not been directly evaluated, and the abundance of NLP has not been quantitatively determined.

Vocal variability can be beneficial for territorial males if calls are individual-specific. If males can recognize individuals due to their distinct call characteristics, they can focus their energetically expensive aggressive effort on intruding, unfamiliar 'strangers', rather than on familiar neighbors inhabiting adjacent territories with established borders. For example, the American bullfrogs (*Lithobates catesbeiana*) can use acoustic cues to discriminate between neighbors (i.e., 'dear enemies') that are tolerated, and strangers that are attacked (Davis 1987; Bee & Gerhardt 2001a–c). Interestingly, males of *O. tormota* can also discriminate strangers from neighbors acoustically, despite having a very large vocal repertoire (Feng et al. 2009). However, the acoustic basis for neighbor-stranger discrimination by *O. tormota* remains to be determined. The call fundamental frequency and duration are likely parameters coding for individual specificity, but it is unclear whether NLP in the calls of *O. tormota* play a role in distinguishing the calls of individual males as has been shown for vocalizations in mammals (Volodina et al. 2006).

The goals of the current study were to: (1) categorize and analyze the variability of the vocalizations of individual males by defining stereotypically produced call types, (2) quantify acoustic complexity, including the occurrence of NLP, fundamental frequency and temporal characteristics exemplified in one call type, and (3) determine whether the acoustic parameters of this call type are individually distinct, despite their variability. To investigate these goals, we recorded the vocalizations of individually marked male concave-eared torrent frogs over a period of 15 days, and quantitatively

analyzed the spectro-temporal characteristics of their calls.

## Methods

### Marking

Field study was carried out from 12 to 28 May 2007, in Huangshan Hot Springs, China. The ambient temperature and humidity at the frogs' calling sites during this period ranged from 15 to 22.5°C and from 38% to 96%, respectively. The calling activity of males of *O. tormota* was dependent on the ambient temperature and humidity; the calling rate was low when the ambient temperature was  $\geq 22^\circ\text{C}$ , or when the humidity was below 60%. Observations made in other years from Huangshan Hot Springs showed that males' calling activity was very low when the ambient temperature was below 14°C. A transition from a warm and dry evening to a cooler and more humid evening typically produced a robust chorus.

Fifteen actively calling males were captured during the first few nights of the study and individually tagged using two different colors of implantable fluorescent elastomer (Northwest Marine Technologies; Michael Benard, personal communication; Woods & Martin-Smith 2004). The marker was placed on various locations of the frogs' dorsal body surface. Afterward, the frogs were returned to their original calling sites. Each night thereafter we looked for marked males in the vicinity of the original capture sites. Frogs were positively identified using a UV light (Streamlight TwinTask 3C, Streamlight, Inc., Eagleville, PA, USA) before their vocal signals were recorded; the frogs' behavior was visually monitored under the UV light during the recording sessions. The experimental protocol adhered to the ABS Guidelines for the use of animals in research and was approved by the Institutional Animal Care and Use Committees in the Chinese Academy of Science and the University of Illinois at Urbana-Champaign.

Males have a snout-vent length of  $\sim 3.25$  cm and are found in two small geographical regions in the eastern seaboard of China. They breed from mid-April to early June. They inhabit and vocalize from low-lying vegetation alongside streams in mountains and hills at elevations of 150–700 m (Feng et al. 2002); a chorus typically comprises 10–20 males calling interactively. The calling site of a male was generally confined to an area of 4 m<sup>2</sup>. Individual males typically vocalized from the same calling sites from night to night, and as a result we were able to recover many of the tagged frogs (12

of 15) in the vicinity of their original calling/capture sites.

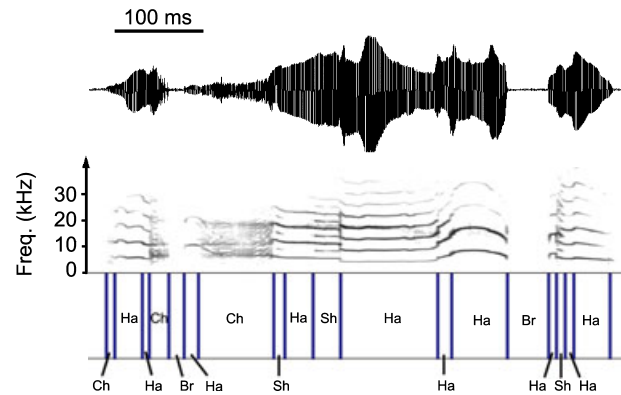
### Sound Recordings

Recordings were made between 18:30 and 24:00 h using a 1/4" wideband omni-directional condenser microphone (G.R.A.S. 40BE, G.R.A.S. Sound & Vibration A/S, Holte, Denmark) and a digital audio recorder (Sound Devices, model 702, Sound Devices, WI, USA; frequency range: 10 Hz–96 kHz) with a sampling rate of 96 or 192 kHz and 16-bit accuracy. The microphone, which had a flat frequency response, was fitted with a 3" conical reflector to provide a directional sensitivity of ~15–20 dB at 5–10 kHz. Oral comments during the recording sessions were spoken to the same recording track.

### Call Analysis

Initial analysis of vocal signals was based on narrow-band spectrogram of the signal using SELENA, a custom-designed program (Feng et al. 2006; Shen et al. 2008). Criteria for categorizing a call into a unique entity (i.e., call-type) were the call duration, F0 range and the presence of small interruptions (breaks). Breaks are periods of silence of 10–200 ms durations—breaks longer than 200 ms were taken as gaps between separate calls. If one or more breaks were present the call was categorized as a multi-note call type. Calls were additionally sorted by intensity which showed a bimodal distribution (low: <75 dB SPL; high: 75–106 dB SPL at 0.5 m). Two of the call-types were categorized as 'long calls', comprising single- and multi-note calls that are characterized by high sound levels and abundant presence of NLP. Further acoustic analysis was carried out on 324 long calls from eight males, each with a sample size of >10 calls.

We first identified the different temporal segments of a call (Fig. 1) on the basis of their acoustic regimes with PRAAT (Boersma & Weenick 2008). Segmentation was based on visual inspection of narrowband spectrograms (Herzel 1993) and associated Fourier frequency spectra, following earlier studies (Riede et al. 2000, 2004; Titze et al. 2008; Zollinger et al. 2008). Segment borders were positioned at *bifurcations*, which refer to the boundaries between different dynamic regimes. Regimes were categorized as no phonation, harmonic phonation, subharmonics, biphonation, apparent deterministic chaos and breaks (Riede et al. 1997; Wilden et al. 1998; Fitch et al. 2002). Subharmonics can be found at 0.5, 0.33, 0.25,



**Fig. 1:** Waveform and spectrogram of a long-call. Segmentation is indicated in the lowest panel. The segmented call is shown to possess several harmonic segments [Ha] having multiple harmonics with energy extending into the ultrasonic range, a signal break [Br], as well as two of the nonlinear characteristics (i.e., chaos [Ch] and subharmonics [Sh]).

0.2 or 0.167 F0. In some cases, bifurcations also occurred between identical regimes when the segments were qualitatively different enough and the change between them occurred suddenly; for example, a 'frequency jump' marked the bifurcation between two harmonic segments with different F0s.

After segmentation was completed, the time and frequency of occurrence of each segment were noted. In addition, total call duration, duration of the breaks, and durations of the harmonic, subharmonic, biphonic and chaotic segments were measured (all in seconds). Segment and break durations were also calculated as percentage of the total call duration.

The F0 was tracked for each harmonic segment using the 'pitch tracking' mode in PRAAT with 1-ms intervals. F0 analysis was not carried out for nonlinear segments, thus F0 values in a call containing NLP refer to the F0 in the harmonic segments only. Resulting F0 tracks were inspected after being overlaid on corresponding narrowband spectrograms. Where the F0 track deviated from the first harmonic, tracks were recomputed using different thresholds and octave cost values in PRAAT until the deviation was eliminated. If tracking problems persisted, which most often occurred at the beginning or the end of a harmonic segment when the signal-to-noise ratio was low, the F0 extraction was restricted to portions of segments in which tracking was successful. The mean, maximum and minimum F0, and the difference between the maximum and minimum values, were measured. Thus, a total of 15 temporal and spectral parameters were calculated for each analyzed call.

To quantify call variability, we calculated the within- and between-individual coefficient of variance for each acoustic parameter, i.e.,  $CV_w$  and  $CV_b$ , respectively. Part of the within-individual variation can likely be explained by the change in ambient temperature, as vocal production in anurans is temperature-dependent (Gerhardt 1978). We recorded many calls over wide ranges of temperature (between 15 and 22.5°C) for two frogs. The data from these frogs (not presented here) show significant negative correlations between temperature and total call duration, but inconsistent correlations between temperature and fundamental frequency. To exclude the potential effect of ambient temperature we focused our analysis on 252 calls from six frogs recorded from a narrow temperature range of 19–21°C.

### Statistical Analysis

The main goal of this analysis was to identify the acoustic basis of vocally mediated neighbor-stranger discrimination by *O. tormota* males (Feng et al. 2009). To determine whether vocal signals of individual males are distinct and contain individual signatures, we performed: (1) multivariate ANOVA to determine whether calls from different individuals were significantly different, (2) univariate ANOVA to determine which of the 15 call variables were significantly different between individuals, (3) a stepwise discriminant function analysis (DFA) to predict group membership for each call—the result was a percentage documenting the average correct assignment to individuals. To make the sample size of the DFA more uniform, we took the first 20 calls from frogs that had a larger sample size, resulting in a sample of 111 calls from six frogs.

The *a priori* probability of correct assignment by the DFA was based on the total number of groups, and thus the probability that a call belonged to 1 of 6 individuals was 100%/7, or 16.6%. However, this procedure did not take the characteristics of the specific data into account (Titus et al. 1984). We calculated an alternative chance level based on a randomized version of the actual data set (Solow 1990). A 'sequence generator' function (provided at <http://www.random.org>) produced a random sequence of the numbers from 1 to 111 which was linked to the original data set, and the data were sorted accordingly. The number of calls per individual remained the same. The randomized set was then subjected to DFA resulting in an average correct assignment. We tested the hypothesis that the correct assignment of

the randomized data set was smaller than that of the original data set. We repeated the randomization procedure four times in order to estimate a range in which the average assignment varied if group membership was randomized.

We next addressed whether the occurrence of NLP could be predicted from the F0 contour by comparing the last F0 measurement from a harmonic segment before a bifurcation with the mean value for the entire harmonic segment. In addition, the rate of F0 change before a bifurcation was estimated by regressing F0 before a bifurcation against distance (in this case time) from the bifurcation. A 10-ms period before the bifurcation was analyzed (time resolution of the signal was 1 ms). We tested the hypothesis that the slope of the regression line was different from 0 with a paired *t*-test.

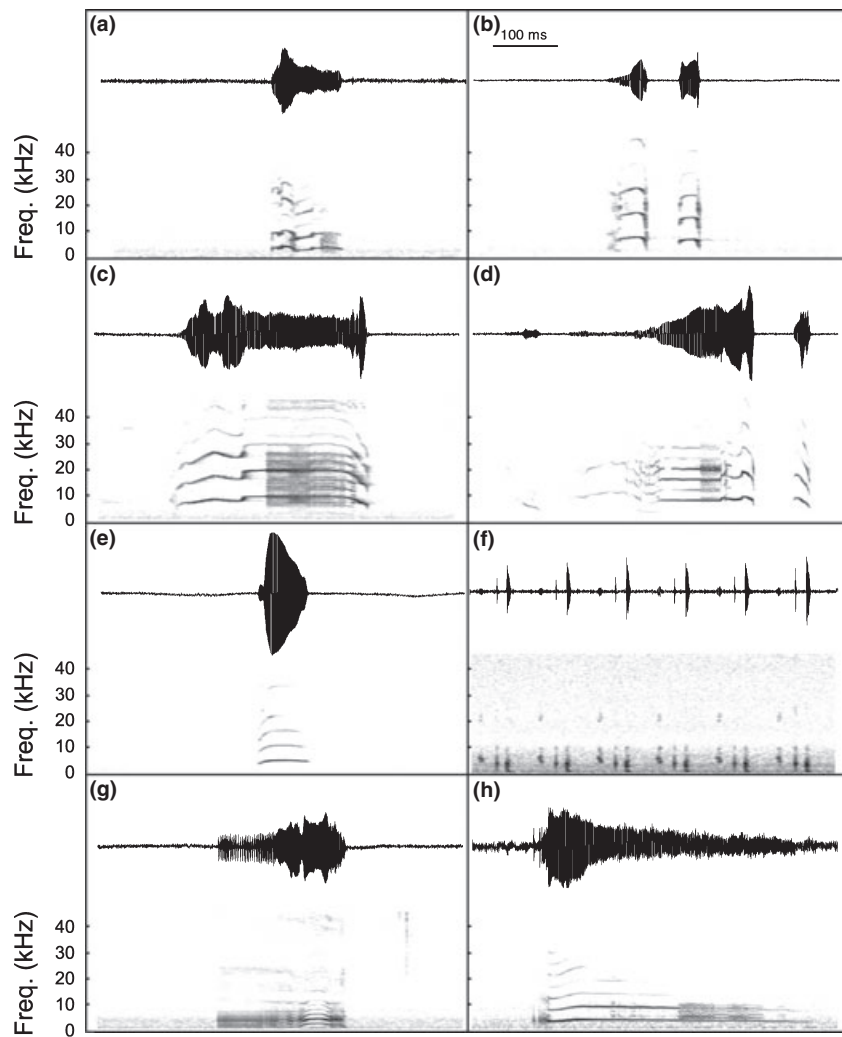
## Results

### Call Repertoire

Vocal signals of male *O. tormota* were highly variable and diverse. We identified eight distinct call-types (Table 1): single- and multi-note calls having short (<200 ms; Fig. 2a,b) or long (>200 ms; Fig. 2c,d) duration, short tone-pips (Fig. 2e), low-level staccato calls (Fig. 2f), low-level 'meow' calls (Fig. 2g), and low-level shallow-frequency modulated (FM) calls (Fig. 2h). Single- and multi-note calls (long and short) and short tone-pips were tonal or whistle-like, with a distinct F0 in the range of 5–9 kHz and multiple harmonics that extended into the ultrasonic range; these calls showed pronounced and diverse FM patterns. Staccato calls comprised a train of clustered sound pulses (double or triple pulses each with a duration of ~3 ms) repeated at a rate of ~14 Hz over an average period of  $3.9 \pm 1.2$  s. Their bandwidth was narrow, limited to 2–6 kHz. Low-level 'meow' calls were characterized by a low F0 (<2 kHz), containing subharmonics over much of

**Table 1:** Call-types of males' vocalizations

Call-type	F0 range (kHz)	Call duration
One-note short call	5–9	<200 ms
One-note long call	5–9	>200 ms
Multi-note short call	5–9	Each note <150 ms
Multi-note long call	5–9	Each note >200 ms
Short tone-pip	5–9	100–200 ms
Low-level staccato call	≈2	Each pulse ≈3 ms
Low-level 'meow' call	<2	50–220 ms
Low-level shallow-FM call	4–5	180–400 ms



**Fig. 2:** The eight basic call-types of males' vocalizations showing their spectrograms and waveforms: single-note short call (a), multi-note short call (b), single-note long call (c), multi-note long call (d), short tone-pip (e), low-level staccato call (f), low-level 'meow' call (g), and low-level shallow-FM call (h).

the duration of the call. These calls had a duration of 44–212 ms (average:  $149.5 \pm 49.5$  ms) and a limited spectral energy (<4 kHz); they sometimes showed a brief upward FM followed by a downward FM over a short segment of the call. Low-level shallow-FM calls were characterized by: (1) a gradual upward FM followed by a gradual downward FM over the entire duration of the call, (2) a higher F0 (~4–5 kHz), and (3) a longer duration (range: 183–402 ms; average:  $286.0 \pm 47.3$  ms). Unlike the 'meow' calls, they sometimes contained a subharmonic segment but over a small portion of the call only and there was no evidence of other NLP.

Tone-pips, short calls and low-level shallow-FM calls were the most common call types; these were heard in the study site whether the chorus was robust or not. Whereas short calls were almost always emitted in isolation or in response to acoustic playback of conspecific calls, tone-pips were gener-

ally emitted in a series of 3–7 calls over 4–5 s, often after broadcasting a long call. Following the emission of a series of tone-pips, some frogs produced a low-level shallow-FM call. High-level long calls (single- or multi-note) and low-level staccato calls were broadcast only on nights when the chorus was robust. The wide propagation of the long call (>30 m away) makes it an effective signal for sexual advertisement and territorial defense. The remainder of our study was focused on long calls (both single- and multi-note) from eight males.

#### Nonlinear Call Characteristics

The long calls (single- or multi-note;  $n = 324$ ) of male *O. tormota* contained many NLP, i.e., frequency jumps, deterministic chaos, subharmonics, and biphonation (Figs 1 and 2c,d). The number of calls comprising purely harmonic segments without any NLP

**Table 2:** Frequency of occurrence of the various NLP in the frogs' long calls

Frog ID	N	Pure Ha	Pure Ha (%)	FJ	FJ (%)	Sh	Sh (%)	BP	BP (%)	Ch	Ch (%)
2	20	0	0	17	85.0	18	90.0	1	5.0	19	95.0
8	18	0	0	12	66.6	13	72.2	4	22.2	7	38.9
9	91	2	2.0	52	59.1	73	82.9	12	13.6	61	69.3
13	17	0	0	8	47.0	16	94.1	4	23.5	14	82.3
16	36	0	0	14	38.9	29	80.6	3	8.3	33	91.7
18	74	12	16.2	43	69.3	38	61.3	8	12.9	23	37.1
25	16	0	0	16	100.0	9	56.3	4	25.0	2	12.5
28	53	9	17.0	29	65.9	39	88.6	2	4.5	14	31.8

N, number of calls; Ha, harmonic component; FJ, frequency jump; Sh, subharmonic; BP, biphonation; Ch, chaos.

was very small (Table 2). A great majority ( $n = 301$ ; 92.9%) of male long calls contained at least one type of NLP. For five frogs, *all* calls contained one, or more commonly multiple, NLP. It is notable that the number of calls containing chaotic segments ranged widely from one frog to another (from 12.5% to 95%) and appeared to be individual specific (Table 2).

Of the 301 calls showing NLP, 191 calls (63.4%) displayed one or more frequency jumps, 235 (78.1%) contained one or more subharmonic segments, 173 (57.5%) had one or more chaotic segments, and 38 (12.6%) exhibited biphonation. Table 3 shows the relative durations of these NLP, along with those of the harmonic and signal break segments, revealing the variability in calls between males. We found that 236 calls (78.4%) showed at least two types of NLP, with various different combinations. The average number of bifurcations in a call was significantly correlated with the average call duration (Pearson correlation,  $r = 0.86$ ,  $p = 0.005$ ,  $n = 8$  frogs).

### Relationship Between FM and NLP

We investigated whether there is a causal relationship between FM pattern and the occurrence of NLP

by determining the behavior of the F0 in the harmonic segment before a bifurcation (i.e., prior to the occurrence of NLP). We found that the average F0 in the segment before a bifurcation was significantly lower than the last F0 measurement preceding a bifurcation (one-tailed paired t-test,  $n = 667$ ;  $t = 2.88$ ,  $p < 0.01$ ). We carried out separate t-test comparisons for each type of NLP, which included 273 cases of subharmonics (one-tailed paired t-test,  $t = -0.092$ ,  $p = 0.536$ ), 123 cases of deterministic chaos (one-tailed paired t-test,  $t = 1.85$ ,  $p = 0.033$ ), and 266 cases of frequency jumps (one-tailed paired t-test,  $t = 3.15$ ,  $p < 0.001$ ). These separate tests suggest that in the cases of deterministic chaos and frequency jumps, the F0 increased above the segment's mean F0 before the bifurcation.

To investigate this finding further, the slope of the F0 contour before a bifurcation was evaluated. The mean slope of the F0 contours in harmonic segments before a bifurcation into deterministic chaos was  $-7.4$  kHz/s (SD = 71.4), which was not significantly different from 0 (one-sided paired t-test,  $n = 140$ ,  $t = -1.2$ ,  $p = 0.22$ ). Frequency jumps were differentiated according to the direction of the jump. The mean slope of F0 contours in harmonic segments prior to a downward frequency jump was  $12.4$  kHz/s (SD = 47.0), which was significantly greater than 0

**Table 3:** The call duration and the relative durations ( $\bar{x} \pm$  SD) of the harmonic segment, various nonlinear segments and signal break in the calls of male *O. tormota*

Frog ID	n	Duration (s)	Ha duration (%)	Sh duration (%)	BP duration (%)	Ch duration (%)	Br duration (%)
2	20	0.49 ± 0.12	50.0 ± 12.6	9.4 ± 5.4	0.09 ± 0.4	20.7 ± 13.4	20.1 ± 12.1
8	18	0.28 ± 0.09	80.6 ± 13.9	7.4 ± 6.7	1.7 ± 3.4	4.5 ± 7.4	5.6 ± 10.7
9	90	0.35 ± 0.09	68.5 ± 19.2	13.7 ± 14.7	1.1 ± 3.6	10.2 ± 11.8	6.2 ± 8.7
13	17	0.46 ± 0.11	49.2 ± 17.2	13.9 ± 8.3	0.9 ± 1.8	21.0 ± 13.2	14.9 ± 7.9
16	36	0.33 ± 0.1	50.5 ± 17.7	10.1 ± 9.1	0.5 ± 1.6	19.5 ± 12.6	18.5 ± 11.2
18	74	0.26 ± 0.06	73.3 ± 15.7	4.9 ± 7.5	0.8 ± 3.8	3.3 ± 7.4	17.4 ± 15.0
25	16	0.31 ± 0.1	85.0 ± 11.2	4.1 ± 5.6	1.8 ± 3.6	1.3 ± 4.9	7.8 ± 11.9
28	53	0.38 ± 0.09	70.6 ± 19.0	15.1 ± 16.7	0.2 ± 0.8	2.3 ± 4.9	11.4 ± 12.4

Ha, harmonic segment; Sh, subharmonic segment; BP, biphonic segment; Ch, chaotic segment; Br, signal break segment.

(one-sided paired t-test,  $n = 114$ ,  $t = 2.9$ ,  $p < 0.01$ ). The mean slope of F0 contours in harmonic segments prior to an upward frequency jump was  $-27.8$  kHz/s ( $SD = 37.2$ ), which was significantly smaller than 0 (one-sided paired t-test,  $n = 115$ ,  $t = -8.04$ ,  $p < 0.001$ ).

Given that F0 did not behave randomly before a bifurcation, we tested whether this non-random behavior prior to a bifurcation is individual specific. We found that, in some individuals, a bifurcation occurred only in certain F0 ranges. For example, for frogs #2, 13 and 16, we observed three types of NLP (frequency jumps, deterministic chaos, subharmonics) in mutually exclusive F0 ranges (Fig. 3). However, the F0 ranges for the three types of NLP overlapped in long calls from frogs #9, 18, and 25 (Fig. 3). For frogs #8 and 25, deterministic chaos and subharmonic regimes occurred at different F0s than the other two NLP (Fig. 3). Results of a two-way ANOVA indicated that the last F0 before a bifurcation was specific to individual and the NLP type (variable 'frog ID':  $df = 7.14$ ,  $F = 31.4$ ,  $p < 0.001$ ; variable 'NLP type':  $df = 2.14$ ,  $F = 3.0$ ,  $p < 0.05$ ).

**Call Variability**

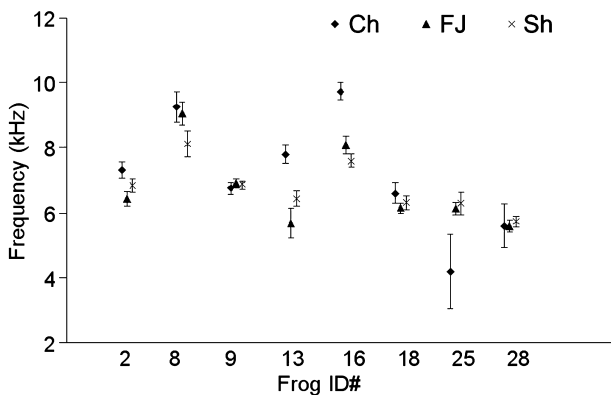
In addition to the frequency of occurrence of NLP, the time of occurrence and the duration of each of the NLP varied from one call to the next, and the amplitude- and frequency-modulation patterns were also variable. Figure 4 shows four representative calls from four males recorded in one evening to illustrate the intra- and inter-individual variability. No two long calls were identical, even in frogs from which we had >50 calls (Fig. 4). For example, calls

of frog #9 (Fig. 4b) while displaying a similar overall FM pattern showed subtle differences in the FM pattern as well as in the timing and frequency of occurrence of subharmonic segments.

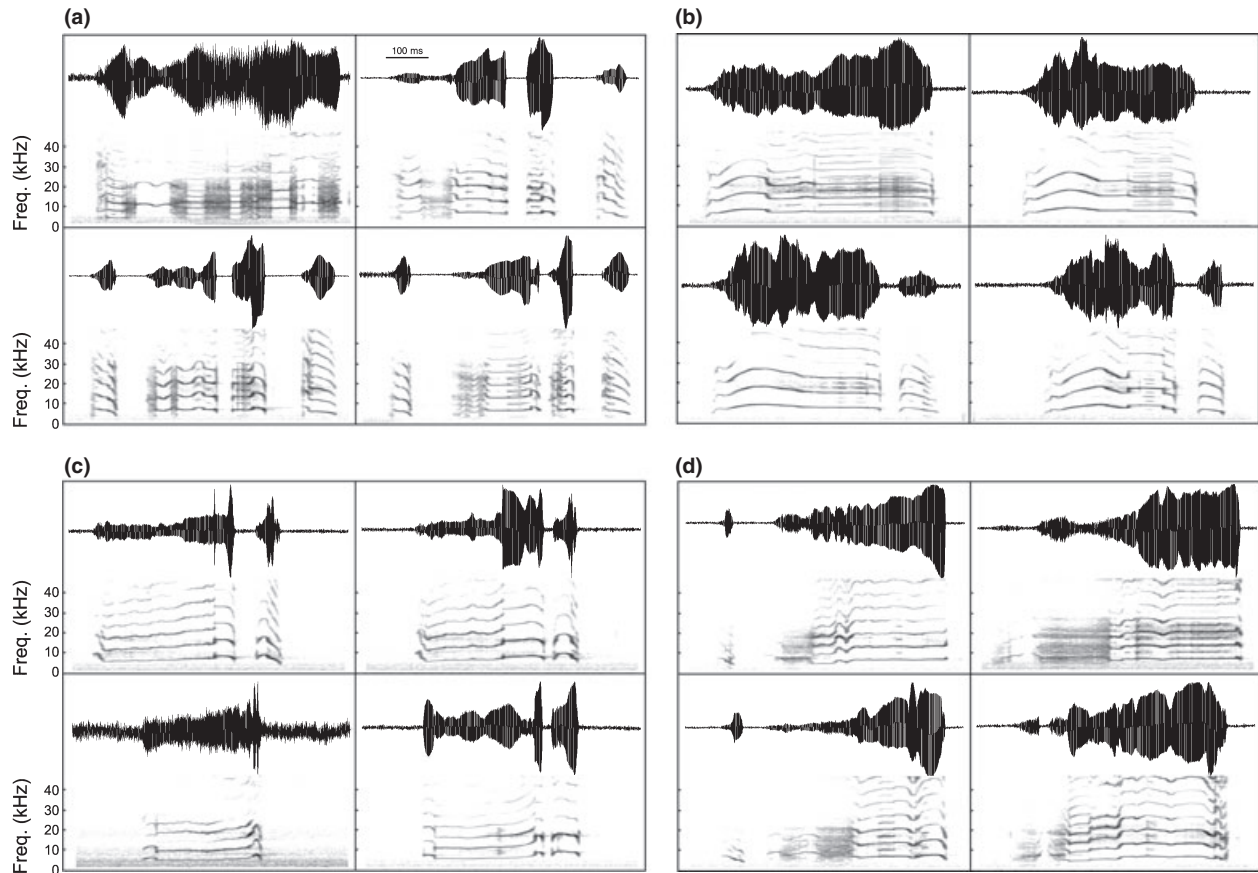
A qualitative inspection of long calls of individual frogs indicated that whereas the call spectrograms for each male showed large intra-individual differences, the inter-individual differences appeared to be greater (Fig. 4). For example, calls of frog #2 featured multiple short chaotic segments and multiple short notes, with 1–2 subharmonic segments (Fig. 4a). Calls of frog #9 consisted of a single long note with upward and downward frequency excursions. The calls contain multiple subharmonic segments, but few if any chaotic regimes (Fig. 4b). Calls of frog #25 showed no subharmonic segments, few or no chaotic segments, and exhibited one or more prominent frequency jumps of 1–5 kHz (Fig. 4c). Calls of frog #13 comprised a long note led by a long chaotic segment (Fig. 4d) that was often preceded by a brief FM note and a signal break. Importantly, calls of individual frogs appeared to be individual-specific in terms of the F0 (for a quantitative description see Table 4), and the timing and frequency of occurrences of the various NLP and signal breaks.

Table 3 gives quantifications of the call durations and the relative durations of various NLP for eight males, showing the intra- and inter-individual variability. Table 4 shows a quantitative description of the spectrum of individual frogs that includes the average, minimum, maximum and max–min F0.

To determine whether the qualitative assessment of greater inter-individual than intra-individual variability is valid, we measured the between- ( $CV_b$ ) and within-individual coefficients of variations ( $CV_w$ ) of all acoustic parameters for the complete data set from eight males (525 calls). These data are shown in Table 5, along with the coefficients of variation for the smaller sample that controlled for temperature-effects (111 calls from six males). There was a direct relationship ( $r = 0.97$ ,  $p < 0.0001$ ) between  $CV_b$  and  $CV_w$ . For example, for the average F0, which had the lowest  $CV_w$  ( $\bar{x} = 13.1\%$ ), the  $CV_b$  were also small, compared to those of other parameters (Table 5). Call parameters that were more variable intra-individually also tended to vary more between males (e.g., durations of biphonic, subharmonic and chaotic segments; Table 5). In most cases, the intra-individual variability approximated the inter-individual variability; the ratios of  $CV_b/CV_w$  approached 1.0, except for that of the duration of the chaotic regimes (Table 5). The same applied to the smaller sample with control for temperature



**Fig. 3:** The last F0 ( $\bar{x}$  and standard deviation) before a bifurcation into three types of NLP (deterministic chaos, Ch; frequency jump, FJ; subharmonics, Sh) for eight individual frogs.



**Fig. 4:** Four representative vocal signals from four males *O. tormota* (a–d) showing qualitative differences in the amplitude and frequency modulation patterns, absence/presence as well as timing and frequency of NLP. Sound spectrograms were derived using Praat. (a) Calls of frog #2. (b) Calls of frog #9. (c) Calls of frog #25. (d) Calls of frog #13.

effects (comparing the  $CV_b/CV_w$  ratios for the large and the small sample; paired t-test,  $t = 0.12$ ,  $p = 0.9$ ).

We next tested the hypothesis that a change in total call duration is reflected in absolute and relative segment duration. In all eight subjects the correlation between absolute and relative segment duration was significant (Pearson correlations,

$p < 0.001$ ,  $r \geq 0.81$ ) for subharmonic, biphonic, chaotic and break segments. However, the correlation for harmonic segment duration was not significant ( $p > 0.05$ ) in three frogs and was significant ( $p < 0.01$ ) in the others (correlation coefficient between 0.59 and 0.71). This suggests that individuals exploit different mechanisms to alter total call duration. While the duration of the harmonic

**Table 4:** Quantitative description of the spectral characteristics ( $\bar{x} \pm SD$ ) of the calls of male *O. tormota*

Frog ID	n	Average F0 (Hz)	F0 <sub>max</sub> (Hz)	F0 <sub>min</sub> (Hz)	F0 <sub>max</sub> – F0 <sub>min</sub> (Hz)
2	20	6604 ± 1062	8940 ± 1469	4205 ± 1418	4734 ± 1727
8	18	8327 ± 1166	10307 ± 1253	6041 ± 1175	4266 ± 1270
9	90	6632 ± 918	8039 ± 1201	4702 ± 1118	3336 ± 1248
13	17	6709 ± 706	8793 ± 1107	4229 ± 1022	4563 ± 1109
16	36	7844 ± 887	10425 ± 1474	4839 ± 796	5585 ± 1749
18	74	6026 ± 495	7689 ± 1012	4160 ± 714	3528 ± 1127
25	16	6462 ± 1020	8450 ± 1612	4632 ± 722	3817 ± 1207
28	53	5493 ± 806	6916 ± 1175	4438 ± 710	2478 ± 941



**Table 5:** Coefficients of variations of call duration and durations of various harmonic and NLP segments (absolute measure only) of long calls (525 calls from eight males)

Parameter	Mean CV <sub>w</sub>	Mean CV <sub>b</sub>	CV <sub>b</sub> /CV <sub>w</sub>	CV <sub>b</sub> /CV <sub>w</sub>
Total duration	26.9 ± 3.9 (23.1–32.3)	26.6	0.99	0.98
Duration of Ha	33.8 ± 4.5 (26.1–40.9)	33.7	0.99	0.95
Duration of Sh	108.1 ± 4.8 (50–200)	103	0.95	0.77
Duration of BP	331.3 ± 148.1 (166–500)	241	0.73	0.84
Duration of Ch	156 ± 85.9 (70–300)	90.3	0.58	0.59
Duration of Br	107.4 ± 51.6 (42.8–200)	83.3	0.77	0.85
Average F0	13.1 ± 2.8 (8.2–16.1)	13.1	1.0	0.99
F0 <sub>max</sub>	18.5 ± 3.2 (15.0–24.9)	18.3	0.99	1.0
F0 <sub>min</sub>	20.8 ± 6.2 (15.6–33.7)	20.6	0.99	0.99
F0 <sub>max</sub> – F0 <sub>min</sub>	32.6 ± 4.6 (24.3–37.9)	32.1	0.98	0.98

Shown are the within-male (CV<sub>w</sub>) and between-male (CV<sub>b</sub>) coefficients of variation; the range of CV<sub>w</sub> is given in the bracket. The CV<sub>b</sub>/CV<sub>w</sub> ratios for the smaller temperature-effect controlled sample (111 calls from six males) are shown in the last column. See Table 3 for abbreviations.

segment was generally (i.e., in seven of the eight frogs studied) correlated with the total call duration, the durations of subharmonic, biphonic, chaotic and break segments were not consistently correlated with the total call duration (Table 6).

### Individual Signatures

Multivariate ANOVA of 111 calls from six frogs revealed significant differences between calls from different individuals (Wilks'  $\lambda = 0.060$ ,  $F = 4.579$ ,  $p < 0.001$ ). Subsequent univariate tests showed that all variables except 'biphonic segment duration' were significantly different (Table 7). Importantly, the absolute and relative durations of subharmonic and chaotic segments differed between individuals,

**Table 6:** Correlations between total call duration and absolute harmonic (Ha), subharmonic (Sh), biphonic (Bp), chaotic (Ch) and break (Break) segment duration

Frog ID & n	Ha	Sh	Bp	Ch	Break
#2, n = 20	$r = 0.58$ $p = 0.007$	$r = 0.60$ $p = 0.005$	$r = 0.06$ $p = 0.79$	$r = 0.54$ $p = 0.01$	$r = 0.43$ $p = 0.055$
#8, n = 18	$r = 0.89$ $p < 0.001$	$r = 0.06$ $p = 0.79$	$r = 0.16$ $p = 0.51$	$r = 0.39$ $p = 0.11$	$r = 0.13$ $p = 0.60$
#9, n = 89	$r = 0.48$ $p < 0.001$	$r = 0.51$ $p < 0.001$	$r = 0.30$ $p = 0.003$	$r = 0.22$ $p = 0.04$	$r = 0.11$ $p = 0.31$
#13, n = 17	$r = 0.45$ $p = 0.06$	$r = 0.56$ $p = 0.02$	$r = 0.10$ $p = 0.69$	$r = 0.37$ $p = 0.015$	$r = 0.22$ $p = 0.38$
#16, n = 36	$r = 0.65$ $p < 0.001$	$r = 0.53$ $p < 0.001$	$r = -0.04$ $p = 0.81$	$r = 0.34$ $p = 0.04$	$r = 0.52$ $p = 0.001$
#18, n = 74	$r = 0.74$ $p < 0.001$	$r = 0.10$ $p = 0.37$	$r = 0.15$ $p = 0.19$	$r = 0.25$ $p = 0.03$	$r = 0.18$ $p = 0.11$
#25, n = 16	$r = 0.91$ $p < 0.001$	$r = 0.47$ $p = 0.06$	$r = 0.39$ $p = 0.12$	$r = -0.25$ $p = 0.34$	$r = 0.22$ $p = 0.42$
#28, n = 53	$r = 0.49$ $p < 0.001$	$r = 0.50$ $p < 0.001$	$r = 0.03$ $p = 0.82$	$r = 0.52$ $p < 0.001$	$r = -0.12$ $p = 0.37$

Significance level  $p < 0.05$ .

as did the total call duration, absolute and relative durations of the harmonic segments and signal breaks, and the average, minimum, maximum, and max–min F0.

We used a stepwise forward DFA to identify the call variables that contributed most to discrimination of individuals. For this, we removed 9 of the original 15 variables from the data set, leaving a subset of 6 uncorrelated variables (total call duration, duration of the subharmonic segments, duration of the chaotic regimes, duration of signal breaks, average F0, and minimum F0). Stepwise DFA revealed that the average correct assignment of the original data set was 54.6%, i.e., on average 54.6% of the calls were correctly classified to six individuals. The average correct assignment of the five randomized samples used to determine the chance level of correct assignment ranged between

**Table 7:** Results of model II ANOVAs examining between-male variability (n = 111, df = 5)

Acoustic variable	F	p
*Total duration (s)	14.5	<0.001
Harmonic segment duration (s)	13.3	<0.001
Harmonic segment duration (%)	21.1	<0.001
Subharmonic segment duration (s)	6.2	<0.001
*Subharmonic segment duration (%)	6.2	<0.001
Biphonic segments duration (s)	1.2	0.307
Biphonic segments duration (%)	1.0	0.408
*Chaotic segments duration (s)	22.3	<0.001
Chaotic segments duration (%)	22.9	<0.001
Break duration (s)	5.6	<0.001
Break duration (%)	3.73	<0.001
*Average F0	12.6	<0.001
F0 <sub>max</sub>	11.6	<0.001
*F0 <sub>min</sub>	3.29	<0.001
F0 <sub>max</sub> – F0 <sub>min</sub>	10.0	<0.001

Asterisk (\*) indicates the parameters used for the DFA.

**Table 8:** Correct classification of 111 calls from six frogs based on six parameters

Frog ID	N	Original data set	Random data set #1	Random data set #2	Random data set #3	Random data set #4	Random data set #5
9	20	65	5	10	35	15	5
13	16	62.5	37.5	40	37.5	12.5	6.25
16	20	65	40	0	5	0	5
18	20	65	45	37.5	35	10	60
25	16	18.7	43.7	0	31.3	31.3	18.7
28	20	70	25	35	25	70	50
Mean correct assignment (%)		51.4	18.9	4.9	13.5	8.1	10.3
One-sided paired t-test			t = 2.12 p = 0.04	t = 4.88 p = 0.002	t = 2.97 p = 0.01	t = 2.61 p = 0.02	t = 2.89 p = 0.01

Shown is the correct classification for the original data set as well as of randomized data sets (#1–#5). The one-sided paired t-test compares the correct classifications of the original data set with the respective random data sets. Significance level  $p < 0.05$ .

4.9% and 18.9% (Table 8). For all five cases, this was significantly smaller than the correct assignment of the original sample (Table 8), showing that the correct assignment of 54.6% was significantly above chance level.

Figure 5 is a plot of long calls of individual males in a two-dimensional signal space defined by the first two discriminant functions (or canonical scores). We found that there was overlap among individual males, which explains the low  $CV_w/CV_b$  ratio. However, the overlap was minimal enough to allow the above-chance assignment of the DFA.

## Discussion

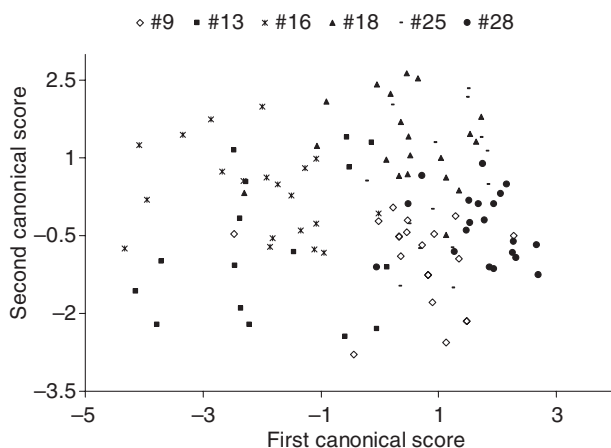
Results of the present study provide four new contributions to the understanding of the acoustic communication system of *O. tormota*. First, these frogs possess a repertoire of at least eight distinct call-

types, a subset of which shows highly diverse call duration and F0, and abundant presence and various combinations of NLP. This is in contrast to the vocal signals of the great majority of anurans, which are generally stereotyped and restricted to a limited spectral range (Gerhardt & Bee 2007; Wells & Schwartz 2007). Second, a large number of acoustic parameters can be used to discriminate calls of individual males reliably above the chance level. Third, individual call differences can be described at two levels: spectral and temporal characteristics, and the occurrence of NLP. Fourth, the occurrence of NLP is predictable from the contour of the F0 in the harmonic segment, and this prediction shows individual-specific patterns. Together with results from a playback study (Feng et al. 2009), our data suggest that the neighbor-stranger discrimination by *O. tormota* males is vocally mediated.

## Call Variability and Abundance of NLP

Vocal signals of male *O. tormota* are highly diverse. The calls have variable durations and they can be emitted in isolation, or combined to form multi-note calls. In addition, the frogs' calls show varying FM patterns and NLP combinations. Results of the present study of individually marked frogs support the findings in earlier field studies involving unmarked frogs (Feng et al. 2002; Narins et al. 2004). In prior studies, however, the staccato, 'meow' and shallow-FM calls were excluded from the samples due to their low signal levels and uncertainty about the emitters. The low-level staccato calls and shallow-FM calls were only positively identified recently in phonotactic experiments carried out in a laboratory (Shen et al. 2008).

The present study provides the first quantitative description of NLP in a frog species. Almost all long



**Fig. 5:** Plot of the long-call of individual male frogs in a two-dimensional signal space defined by the first two canonical scores.

calls (92.9%) of *O. tormota* contain at least one NLP, and the majority (78.4%) of these calls has multiple types of NLP combined in various ways. NLP have been observed in the vocal signals of birds (Fee et al. 1998; Beckers & ten Cate 2006; Zollinger et al. 2008) and mammals (Riede et al. 1997, 2000, 2004; Wilden et al. 1998; Tokuda et al. 2002; Peters et al. 2004; O'Shea & Poche 2006; Volodina et al. 2006), including humans (Mende et al. 1990; Herzel 1993; Herzel et al. 1994; Titze et al. 2008). In these vertebrate groups, each vocal signal usually contains just one type of NLP. Calls containing multiple NLP are not common, for example comprising only 8% of chimpanzee pant hoot calls (Riede et al. 2004) and 12% of chimpanzee scream calls (Riede et al. 2007). The abundance and diversity of NLP combinations in the vocal signals of *O. tormota* appear to be rare and representative traits of the species' communication system. Future playback experiments are required to address specifically the behavioral significance of individually distinct acoustic features, for example by digitally manipulating the playback signal to see which features are used by the receiver to discriminate individuals.

#### Individual Specificity in the Vocal Signals of Male *O. tormota*

Discriminant function analysis of long calls of *O. tormota* revealed that such calls can be discriminated with a probability above the chance level. On average, stepwise DFA classifies 54.6% of the calls correctly to six individuals. Classification levels of stereotyped male advertisement calls in the American bullfrog (72% for 27 males and up to 92% for a smaller sample with only five males; Bee & Gerhardt 2001a) and green frog (52–100%; Bee et al. 2001) are comparatively higher, but those in the Aromobatid frog (Gasser et al. 2009) are comparable to those in *O. tormota*. This variability of classification results within a taxonomic group is comparable to others, for example in mammals, which range between 56% and 94% (Zimmermann & Lerch 1993; Riede 1997; Fischer et al. 2002; Blumstein & Muñoz 2005; Grilliam et al. 2008).

The low ratio of  $CV_b/CV_w$  for all measured parameters of *O. tormota* long calls indicates that there is substantial intra-individual variability in the frogs' vocalizations. This is in contrast to the American bullfrog which shows comparatively little within-individual variability, and reaches a  $CV_b/CV_w$  value of 4.7 for F0 and dominant frequency (Bee & Gerhardt 2001a). These data suggest that individual-

specific classification of calls in *O. tormota* is more challenging than in the American bullfrog. Interestingly, despite the large intra-individual variability of their calls, *O. tormota* males are capable of discriminating between neighbors and strangers based on acoustic cues (Feng et al. 2009), as shown also for male bullfrogs (Bee & Gerhardt 2002).

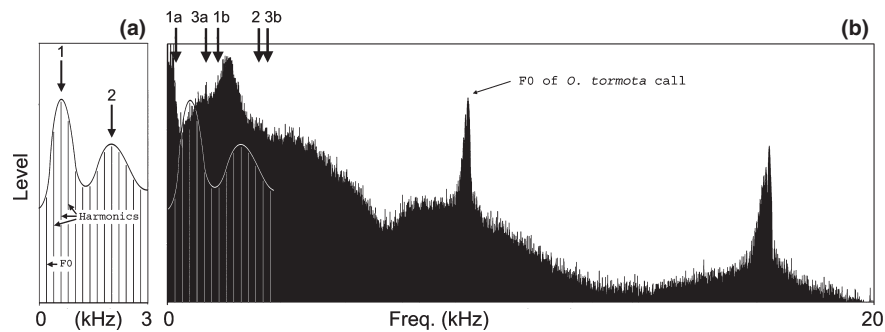
We found that differences in the calls of individual males of *O. tormota* occur at two levels: in the spectral and temporal characteristics of the calls (the average, minimum, maximum and max–min F0, the total call duration) and in the occurrence of NLP. It has been suggested that the occurrence of NLP in vocalizations contributes to individual specificity (Wilden et al. 1998; Fitch et al. 2002; Volodina et al. 2006). Indeed, the durations of subharmonic and chaotic segments are among the parameters discriminating individual males, in spite of the fact that the  $CV_b/CV_w$  ratios for these parameters are not high compared to the ratios for the F0 parameters. In the present study we considered only the temporal components of NLP; future studies need to take into account the spectral characteristics of NLP, a task that is quite challenging (Tokuda et al. 2002).

#### Sources of Individual Specificity

Despite the large variability of the vocal signals of *O. tormota*, we were able to isolate 8 call-types with distinct acoustic features, suggesting that these call-types are under differential neural controls. Individual-specific variation within any of those call types is therefore most likely attributed to morphological features of the sound production system.

According to the source-filter theory of voice production (Fant 1960), the spectral structure of vocalizations results from the combination of producing sound at a sound source and the subsequent filtering of the sound in the vocal tract. The source signal is generated by the vibration of the vocal folds during the passage of air through the glottis. This mechanism determines the F0 (and associated harmonics) of the vocalization (Fig. 6a). The source signal passes through the supra-laryngeal vocal tract that acts as a filter, shaping the sound spectrum and providing optimal transfer for specific resonance frequencies (Fig. 6a).

In the American bullfrog and the green frog, the call's F0 is among important individual-specific acoustic parameters (Bee & Gerhardt 2001a; Bee et al. 2001). Similarly, in *O. tormota*, the average and minimum F0 are relevant acoustic parameters differentiating individuals' vocalizations. In addition,



**Fig. 6:** a: Schematic diagram of a spectrum of a frog call (an arbitrary North American species) showing the relationships between the F0 and its harmonics, and how their amplitudes are shaped by the resonance characteristics of the vocal tract (arrows '1' and '2' indicate the first and second resonance frequencies). b: A spectrum of the background noise (averaged over a 10 s period) in the habitat of *O. tormota* overlaid with the spectrum of an *O. tormota* long-call (F0 = 8.5 kHz). The resonance frequencies for three North American frog species are indicated by arrows: *Rana catesbeiana* (Arrows 1a and 1b indicate the 'dominant frequency' at 219 Hz and the 'secondary frequency' at 1301 Hz from Bee & Gerhardt 2001); *Pseudacris streckeri* (arrow 2 at 2 200 Hz from Gridi-Papp 2008); *Hyla cinerea* (arrows 3a and 3b at 850 Hz and 2 500 Hz from Gridi-Papp 2008). Note, that the sound of frog calls with low F0 (as schematized in a), like for example those of *Lithobates catesbeiana*, *Pseudacris streckeri* or *Hyla cinerea*, are buried in the background noise in the *O. tormota* habitat. The ambient noise in Huangshan Hot Springs (China) is frequency dependent and the peak amplitude occurs at frequencies below 3 kHz and ranges from 55 to 90 dB SPL (Feng, unpub data). Call amplitudes for North American frogs (Gerhardt 1975) were approximated relative to the ambient noise. The call amplitudes for *O. tormota* and North American frogs are comparable.

*O. tormota* calls display individually distinct durations of two NLP. The mechanism underlying NLP production in *O. tormota* is not fully understood, but the results of a recent study by Suthers et al. (2006) suggest that the vocal folds are the source of the NLP.

The role played by the vocal tract in differentiating the calls of individual *O. tormota* is not clear. The anuran vocal tract and sound radiator do not seem to be a classical cavity resonator (Rand & Dudley 1993). For many frogs, specific structures of the body (e.g., the vocal sac) help radiate the sound, and the resonances of these structures as well as other parts of the vocal tract shape the signal (Gridi-Papp 2008). In the American bullfrog, the 'dominant frequency' and 'secondary frequency', two variables likely representing resonance frequencies, are among those that are highly individual-specific (Bee & Gerhardt 2001a). The high F0 of the long calls of *O. tormota* made it difficult to identify resonance frequencies; it is possible that these resonance frequencies fell entirely below the spectral composition of the calls.

#### A Tradeoff Between Being Heard in a Noisy Environment and Being Individual-specific

It has been suggested that the high F0 of *O. tormota* calls is an adaptation for acoustic communication in the presence of intense, predominantly low-frequency background noise (Narins et al. 2004; Feng et al. 2006). The frogs' high-frequency calls indeed

confer a favorable signal-to-noise ratio over the ambient noise (Fig. 6b), reducing the potential for acoustic masking. However, this upward shift of the signal's F0 may eliminate one potential source of individual specificity: vocal tract resonances. Vocal tract resonances are an important component of the acoustic basis of individual specificity in frog calls (Bee & Gerhardt 2001a) and in many other taxas vocalizations (Owren & Rendall 2001). For frogs investigated so far, resonances are all below 3 kHz (Gridi-Papp 2008) (Fig. 6b). The F0 of *O. tormota* long calls is well above 3 kHz; in other words, the calls do not contain acoustic energy in the expected resonance range. Thus, we suggest that there may be a tradeoff between escaping environmental noise by producing calls with a high F0, and losing individual-specific call features based on vocal tract resonance. This tradeoff may explain why the DFA classification percentage of *O. tormota* long calls was lower than that of American bullfrogs and green frogs, both of which produce calls with F0s below 200 Hz.

Two questions are not resolved for the trade-off hypothesis: (1) What are the resonant frequencies of the vocal tract of *O. tormota*? If they are found, like in other anurans, below 3 kHz, a source signal with a F0 greater than 3 kHz would indeed not be sufficient to provide energy to make them audible; (2) To which acoustic variables does the receiver attend? In the American bullfrog, resonance frequencies were among the most individual-specific variables

(Bee & Gerhardt 2001a), however it seems they are not among those selected by the receiver (Bee & Gerhardt 2002).

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