The phonotactic reaction by males of *Oophaga histrionica* **(Anura: Dendrobatidae) is adjusted to stimulus sound pressure level and fine temporal traits.**

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Resumen

Una pregunta central en comunicación animal es qué tanta información proveen las señales. Las señales auditivas deben permitir una eficiente estimación de la distancia de las fuentes de sonido, así como la decodificación de información sobre el emisor, el tamaño o su estado motivacional. En los contextos de defensa territorial las reacciones diferenciales hacia diferentes parámetros de la señal pueden ser importantes al permitir ajustes comportamentales hacia los intrusos de acuerdo a la información disponible en sus cantos. Los machos de la rana venenosa *Oophaga histrionica* son territoriales y responden agresivamente a sus conspecíficos. En este estudio, investigamos las relaciones entre las características de la llamada y la longitud corporal (LRC) de los machos para saber si las señales pueden proporcionar información sobre el tamaño. Además, por medio de experimentos de playback intentamos entender la codificación auditiva de esta especie estudiando las reacciones fonotácticas de los machos. Al manipular la presión de sonido (SPL), y las características temporales gruesas (intervalo silencioso entre cantos) y finas (número de pulsos por canto) de las señales sintéticas, simulamos intrusos lejanos y cercanos con tamaños o estados motivacionales variables. Nuestros resultados sugieren que las características de la llamada no proveen información sobre LRC. Ni la presión de sonido ni las características temporales indican si un macho residente ataca o no a un oponente. Sin embargo, los machos ajustan su reacción comportamental al reconocer las variaciones en la intensidad del sonido (SPL) y en el número de pulsos por llamada. Logramos distinguir las características temporales finas como pistas funcionalmente importantes para la defensa territorial. Además, la magnitud de la respuesta no dependió del tamaño corporal del macho residente. Nuestros resultados sugieren que en condiciones de campo, los machos usan la intensidad de sonido y las características temporales finas de las señales auditivas y como una fuente de información potencialmente útil para ajustar la respuesta agresiva durante los encuentros territoriales. Discutimos el posible papel de las características finas de la llamada en la transmisión de información sobre el estado motivacional durante encuentros agonistas. El reconocimiento simultáneo e independiente tanto de la distancia, como del número de pulsos del canto del intruso puede incrementar la eficiencia de los machos durante la defensa territorial.

Abstract

A central question in communication is how much information animal signals convey. Auditory signals contain information that might allow receivers to estimate the distance to the sound source, as well as the size or the aggressive motivational state of the sender. Proper differential reactions by receivers in aggressive contexts might be important for territorial species, because it allows behavioral adjustments towards intruders according to the perceived risk of losing a territory. Males of the dart-poison frog *Oophaga histrionica* are territorial and respond aggressively towards conespecific male intruders. To know whether signals convey information on male body size, first we investigated the relationships between advertisement-call traits and the snout vent length (SVL) of males. Then, we used playback experiments to understand the auditory coding scheme of this species. By manipulating sound pressure level (SPL), gross (intercall intervals) and fine temporal (number of pulses) structure of the synthetic signals, we simulated near and far intruders with potentially variable sizes or motivational states. Our results suggest that call traits do not communicate information about SVL. Moreover, sound intensity and temporal traits do not indicate whether or not a resident attack an opponent. However, males of *O. histrionica* recognize and use the variations of the SPL and in the number of pulses per call, to adjust its behavioral reaction accordingly. We distinguish the fine temporal trait as a functionally important cue for territorial defense. The response of males to stimuli was not affected by resident body length (SVL). Our results suggest that, under field conditions, males use SPL and temporal traits of auditory signals as a source of information potentially useful to adjust an aggressive reaction. We discuss the possible role of the fine temporal traits of calls on the transmission of information about the motivation to fight. Simultaneous and independent recognition of both distance and fine temporal traits might improve the efficiency of males during territorial defense.

Keywords: territoriality, vocalization, information transfer, phonotactic response, number of pulses, motivational state.

Introduction

In communication systems of amphibian anurans and orthopterans insects, males emit a single signal with the double function of attracting females and repelling rival males (reviewed in Gerhardt & Huber 2002). The physical properties of the advertisement calls are thus used for species recognition in both mating and agonistic contexts (Gerhardt 1988, reviewed in Gerhardt & Bee 2007). How much information is encoded in animal signals is therefore, a central question in studies of communication. Accordingly, some sender traits correlate with signal features that are thought to contain information related to sender's spatial location, identity, size (fight ability) and motivational state. This information can determine the establishment, defense and spacing of territories.

As the signals degrade (lose energy) during propagation from sender to receiver, some features of the remaining signal (e.g. reverberations, overall loss of amplitude, loss of intensity of relative higher frequencies and temporal degradation) might allow receivers to estimate sender location. (Wiley & Richards 1978, 1982; Kim et al. 2000; Naguib & Willey 2001; Castellano et al. 2004). Furthermore in male – male interactions, signals might communicate aggression in two ways. First, signals might inform reliably and honestly the resource holding potential (RHP, i.e. the competitive abilities of contestants) or the fighting ability of senders. Spectral or temporal features of calls are expected to correlate with some physical attributes of males, ensuring honesty through the costs or physical constraints for signal production (Enquist & Leimar 1990; Petersen & Hardy 1996). Accordingly for some frog species, the negative correlations between the dominant frequency of calls and body size might allow receivers accurate estimations of the size of rivals (e.g. Ryan 1985; Wagner 1992); such estimations might have important consequences in territorial encounters since larger males tend to win more fights (Wagner 1989; Burmeister et al. 2002; Bee 2002;

reviewed in Gerhardt & Huber 2002). Second, signals might inform motivation to fight, through call traits that are not related to RHP (i. e. do not inform about the size of opponent) (Enquist & Leimar 1990; Brown et al. 2006). Althoug h controversial, honest information about the motivational state might also be ensured by a higher cost of signal production (Enquist 1985, 1990; Johnstone & Norris 1993). In s ome frog species, the temporal traits (which tend to be independent of size) might reflec t changes in the aggressive intent (Wager 1989; Burmeister et al 2002). However, some anuran species have also shown temporal patterns correlated with body size (McClelland et a l. 1996). Although morphologically and physiologically less constrained than the spectral domain, the production of temporal traits may be energetically expensive in species having high c alling rates (Wells and Taigen 1989; Bevier 1997). In this way, changes in temporal trai ts may reflect the motivational state of the senders (Burmeister et al. 2002; Owen & Gordon 2005).

However, receivers may use only a fraction of the a vailable information in the emitted signal (reviewed in Bradbury & Vehrencamp 1998; Dall et al . 2005). Therefore, a distinction is needed between the available information in signals and that one actually used by receivers (e.g. signal design and decision mechanisms) (Endle r & Basolo 1998; Cate & Rowe 2007). For that reason, the differential reaction of recei vers towards call features may be as important as the signal features in the evolution o f communication systems (Amézquita et al. 2005). Most studies have been conducted in frog spe cies in which males form leks or choruses to breed, investigating either f emale's preferences (Schwartz 1987; Ryan & Rand 2003), male's call timing patterns (reviewed in Wells & Schwartz 2007) , discrimination of call types (Brenowitz et al. 2001), and in informat ion transfer during agonistic interactions (Burmeister et al. 2002 ; reviewed in Gerhard & Bee 2007). However, far few er studies have investigated differential reactions in territorial breeding anurans, a fundamentally di fferent

mating system (Thery 1992; Pröhl 2005). In lekking species, females that fail to recognize conespecific males might severely affect their reproductive investment, while errors in species recognition by males would have less serious consequences, possibly just time and effort; therefore, males should tend to be less demanding in their responses to signal variation than females (Bernal et al. 2007). On the contrary, the territoriality of dendrobatid males might imply higher selective pressures for discrimination of signals, because the defense of critical resources is needed for individual survival or reproduction (Roithmair 1992, 1994; Pröhl 2005). Therefore, males that do not detect and react against calling intruders might lose mating opportunities or even the whole territory; moreover, unnecessary aggressive reactions towards males that do not represent a serious threat might increase predation risks as well as a waste of time and energy (Amézquita et al. 2005). Recent studies of dendrobatid calls have been used to test male calling variation (Pröhl 2003), individual recognition (Bee 2003), sensorial modalities for territorial defense (Narins et al 2003), auditory matching and masking interference (Amézquita et al 2005; 2006). Despite this, the current knowledge about the information content of calls during agonistic encounters in dendrobatid frogs is limited.

To evaluate the actual use of information by receivers during agonistic contexts, a playback test under natural conditions is easier to interpret by far (Amézquita et al. 2005). As is usual for playback experiments, signal perception and recognition is presumed when males perform unambiguous behavioral responses towards the played-back sounds (Narins & Zelick 1988). Moreover, the playback experiment should be conducted on a species in which abilities to recognize and react to conespecific calling intruders has strong fitness consequences (Amézquita et al. 2005). Territorial dendrobatid frogs fulfill these criteria. Males exhibit site tenacity, advertisement calling, and aggressive announcement of long-term multi-purpose territories against competitors, which usually involve physical combat (Wells 1980; Pröhl

2005). The defended places include sites used for calling, courtship, oviposition and tadpole rearing (Roithmair 1992; 1994; Pröhl & Hödl 1999; Pröhl 2005). The defense of places for reproduction without interference from other males is important for dendrobatids, because it enhances their access to receptive females (Roithmair 1992, 1994; Pröhl 2005). Males of the poison frog *Oophaga histrionica* (= *Dendrobates histrionicus*) are territorial and respond to conespecific intruders (Silverstone 1973). Males hold the same territory for long periods, often more than a year (Gómez & Amézquita, unpublished data). Moreover, as in other dendrobatid species (Roithmair 1992, 1994), the possession and defense of a territory and the uttering of advertisement calls are necessary to attract females.

Here we test the information content of calls and the actual capacity of receivers to use information about distance (sound intensity) and whether variation in the gross and fine temporal traits affects the responsiveness of males during aggressive contests. To know whether or not calls might convey information on male body length (RHP), we investigated the relationships among temporal and spectral call traits and body length of males (snout–vent length = SVL). Our second goal was to understand the auditory coding scheme of this species. We used the differential reactions of males to playback experiments under natural conditions to (1) describe the behavioral responses of males to an intruder caller, and (2) test experimentally the effect of variations of sound intensity (dB SPL), silent intervals between calls (intercall interval) and number of pulses per call in males' reaction in the context of aggression. We predicted that (i) a stronger reaction of receivers towards high intensity calls would provide evidence that males estimate sound source distance and adjust their behavioral reaction according to it. As changes in call temporal structure (higher pulse rates, or number of pulses), may allow the recognition of call variation within a species (Brenowitz & Rose 1994) or may inform aggressive messages to males or represent escalated competition

(Wagner 1989, 1992; Burmeister et al. 2002; Owen 2005), we predicted that (ii) males should adjust their reaction, being progressively more res ponsive to calls with higher number of pulses. On the other hand, as the ability of males to signal for longer periods o f time (endurance rivalry; increase of number of call grou ps) might explain variation in male mating success and may influence interactions between male s, in the close related *Oophaga pumilio* (Pröhl 2003; Bee 2003), is reasonable that males ca lling more vigorously might defend territories more efficiently. Therefore we also pre dicted (iii) that males should be more responsive to calls with shorter intercall interval s, simulating a vigorous caller.

Methods

Study system

This study was conducted on El Amargal private rese rve (77°30'W; 5°34'N), about 5 Km southwest of the village of Arusí (Chocó, Colombia). Males of *O. histrionica* are diurnal and use trunks, fallen trees and slightly elevated perc hes (±50 cm.) as calling sites. Calling activity peaks in the morning (6:00–11:00) and in t he afternoon (13:00–15:00) (Silverstone 1973; pers. obs).

The simple advertisement calls of *O. histrionica* consist of short pulsed calls of one note (Myers & Daly 1976; A. Vélez, unpublished data; this study). The advertisement calls are repeated in discrete call groups or "bouts" that co nsist of a few calls to several hundred of consecutive calls (Fig. 1). Each call lasts 164.51 ± 25.3 ms (mean±SD), and consists of 48 ± 13 pulses, uttered with a dominant frequency of $2.8 88 \pm 0.145$ kHz. Calls are emitted at a rate of 3.026 ± 0.324 calls/sec and used in mating and a ggressive contexts. In contrast to other frog species (e.g. Marshall et al 2003), *O. histrionica* males lacks distinctive aggressive

signals (Myers & Daly 1976; A. Vélez, unpublished data; this study). We used previously recorded males to analyze call traits. Then, we obtained the mean and standard deviations for the spectral and temporal characters of calls. These latter measurements served to calculate the coefficient of variation (CV=100% X SD/mean) from the variability observed between males (Gerhardt 1991; Bee and Gerhardt 2001). In this way, we were able to classify the static (peak frequency, CV=5.58%; call rate, CV=8.28%) and dynamic (call duration, CV=13.98%; number of pulses, CV=19.70%) properties of calls. This information allowed us to confirm the dynamic nature of temporal traits as in other dendrobatids (Pröhl 2003) and we took advantage of this, to test the effect of variations of temporal traits on male's reaction.

Synthesis of experimental stimuli

To obtain call parameters necessary to synthesize the stimuli, we analyzed call of 13 males previously recorded in the field (A. Vélez, unpublished data) with a Sony WM-D6C tape recorder loaded with Sony superchrome UX-S tapes; the microphone was positioned at 0.5–1 m in front of the focal male (A. Vélez, unpublished data). Ten call groups of each of these 13 individuals were digitized (16 bit; 22.05 kHz) and then analyzed using Raven Pro 1.3 software (Charif et al. 2008). We measured call duration, number of pulses and call rate (calls/s) as well as peak (dominant) frequency following definitions and procedures defined by Crocroft & Ryan (1995). Three to five calls were averaged to describe the call traits of each frog, which represents the statistical and biological unit of analysis. To evaluate the actual use of information on agonistic encounters, we assembled synthetic calls varying simultaneously in their sound pressure level (SPL), their intercall interval as well as the number of pulses per call. Consequently, we unavoidably modified call rate because it is a parameter covarying with both the intercall interval, and the number of pulses per call.

All stimulus calls were synthesized using the software SoundEdit 2.0.3 (Weary & Weisman 1993) on a Mac computer. We varied the number of pulses per call (call duration) and intercall interval independently of each other in our test stimuli. We assembled synthetic stimuli that varied simultaneously on SPL, intercall interval, and number of pulses per call (and unavoidably, call rate) as follows. The average values of temporal (intercall intervals and number of pulses) and spectral traits were used to construct the first stimulus call, designated as the "average call". Then, another group of synthetic calls with modified intercall intervals and number of pulses were prepared by varying 1.5 standard deviations (positive and negative) around the average temporal parameters (Amézquita et al. 2005). Therefore, stimulus temporal traits vary both in magnitude and in direction (above or below the average values). We devised nine experimental treatments in a fully factorial design: three levels of intercall intervals (low=0.114s, average=0.163s and high=0.210s) and three levels of number of pulses per call (low= 28 pulses, call duration= 0.103 s; average= 48 pulses, 0.171s; high=68 pulses, 0.241s). As the call rate is a composite parameter determined by the sum of the durations of both the calls and the silent intervals between the calls (1/call duration+Intercall interval), we automatically obtained call rates ranging between 2.22 and 4.67 calls/s (Fig. 2). We further constructed three replicates of each stimulus treatment by randomly modifying their dominant frequency within 0.5 standard deviations around the mean. To mimic the envelope shape of the natural calls, the option fade-in effect and then the option fade-out effect were applied in SoundEdit 16 to each stimulus.

We repeated the latter procedure to obtain a new combination of nine treatments that differ in their lower amplitude. We used the option amplitude tool in SoundEdit to reduce in 36% the original amplitude, which corresponds to a 9 dB reduction in SPL or a 1.5 times increase in the simulated distance between the receiver and the simulated sender. These latter stimuli

constituted the low intensity treatments. The factorial combination of call parameters resulted in a total of 18 treatments (Fig. 2).

Playback experiments

Playback experiments were conducted between December 2007 and January 2008 (6:00 - 17:00 hours). Territorial males of *O. histrionica* were found by their calling activity and individually identified through their easily recognizable color patterns. To avoid pseudoreplication, we conducted 55 playback experiments with 55 different stimuli on 55 males, each focal male being tested only once with a unique signal (Kroodsma 1990, 2001; Amézquita et al. 2005). Males were never manipulated before or during the experiment. A speaker-amplifier (Sony SRS-M30) connected to a music player (Markvision, wav format; 16 bit; 22.05 kHz) was placed on the forest floor at about 2 m from the focal male. During the whole experiment, a microphone (Shure KSM9) connected to a digital recorder (Marantz PMD660) was placed at the same distance, forming an approximately equilateral triangle between the focal male, the loudspeaker and the recorder. Preliminary trials were used to adjust the group of *high-intensity* stimuli to 62 dB (re 20 μPa) measured with a sound level meter (RO-1350) at 2 m from the loudspeaker in a flat zone without obstacles. Therefore, the group of *low intensity* stimuli corresponded to 53 dB approximately.

The experiments consisted basically of two periods of 300 seconds each: before and during the stimulus playback. In those cases in which males did not approach within 30 cm of the loudspeaker during the experimental time, we added a third 300s period to conduct *a posteriori* controls of the male's motivational state by broadcasting an average call. Only those males that reached the loudspeaker during either the stimulus or the *a posteriori* control periods (positive phonotactic response) were included in the analyses. For each trial and period, a single observer measured the latency of first movements and calls, number of

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orientations, jumps, and walks. Simultaneously, the calling behavior of males was recorded during the whole experiment. At the end of each experiment we measured the distance (cm) between the initial male's position and the loudspeaker, the sound pressure level (dB) of the stimulus as perceived from the male's initial position and the body length of each male (snout–vent lengths: SVL). To characterize male calling behavior during each experimental period, we analyzed the temporal and spectral characteristics of ten calls for each individual, using Raven Pro 1.3 software (Charif et al. 2008). We measured latency time to first call, number of calls, bout duration, call rate, intercall interval, number of pulses per call, call duration and peak (dominant) call frequency.

Statistical analysis

We ran univariate Pearson correlation analysis in order to test for relationships between call traits (number of pulses, call duration, intercall interval, call rate and peak frequency) and the snout–vent lengths (SVL) of males.

To assess the behavioral reactions of males in response to intruders, we calculated a response scores as the difference in all measured variables between two experimental periods: during stimulus minus before stimulus. Response scores thus represent our estimate of the behavioral changes attributable to the effect of synthetic calls, taking into account the initial or basal behavioral state. Comparing male's behavior before and during the playback of average calls (i.e. control) allowed us to characterize males' reaction to an average intruder. Only those variables that significantly differed in the corresponding Paired–T test were used as dependent variables in subsequent analyses. The significance level was adjusted using the Bonferroni correction for the number of tests.

To test for the effect the call rate (obtained as a consequence of varying independently both the intercall interval and the number of pulses per call) on the behavioral scores of males, we ran a univariate Pearson correlation analysis. Next, we examined the effect of SPL, intercall interval and number of pulses on the proportion of positive and negative phonotactic responses of the receivers. We used chi-square to test whether the proportion of phonotactic responses varied with the levels of SPL and temporal parameters of the stimuli. At a finer level of analysis we tested the effect of SPL and temporal parameters on the response scores of males. We ran a Multivariate Analysis of Variance (MANOVA) introducing SPL, intercall interval and number of pulses of stimulus as independent factors; we used receiver body length (SVL) as covariate. As both, sound pressure level attenuation and temporal degradation could serve as cues for distance perception or for estimating threat level (Naguib & Willey 2001; Owen & Gordon 2005) and this traits might interact with each other (Alder & Rose 1998), we examined simultaneously the effect of sound pressure level and number of pulses, introducing the interaction term (SPL x number of pulses). We used the previously chosen response scores as dependent variables. All statistical tests were conducted using the software SPSS 15.0 for Windows.

Results

Relationships between male body length and call traits

Male body length (SVL) of the subjects ranged from 31.5 to 35.5 mm (mean: 33.145, $\pm$SD=0.841), encompassing the range of sizes in previous studies (Velez, unpublished data). Although larger males tended to produce shorter calls consisting of fewer pulses per call (Table 1), only a small amount of information (13% of the population variation) about body length might be available in the number of pulses per call emitted by males (Pearson correlation: r_{55}= -0.358, *P*< 0.01). However, neither intercall interval nor call rate was

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correlated with SVL. Moreover, SVL was not correlated with dominant frequency, as reported in previous surveys on this species (Vélez 2002 unpublished data). Temporal traits were correlated with each other in an intuitive way: longer calls contained more pulses, and call rate (controlling for bout duration) was significantly lower in calls with longer intercall intervals. In addition, peak frequency was positively correlated with call rate (Table 1).

Behavioral reactions in response to intruders

Experiments were carried out on 55 different *Oophaga histrionica* males, 39 of which reached the loudspeaker during the stimulus period (i.e. they exhibited unambiguous positive phonotactic response) and 16 during the a *posteriori* controls. All males called within the period before stimulation. Males' reaction to average (i.e. control) calls consisted typically of changes in the temporal but not in the spectral traits of their calls as well as re-orienting, walking and jumping in a zigzag way toward the loudspeaker (Table 2). The effects of control stimuli on males' reaction were detected as changes in the behavioral scores (the difference between periods before and during stimulus playback). Males increased their movements (number of jumps, latency time to first orientation, number of orientations) and the number of pulses per call (Paired–Ttest; Table 2).

Effect of SPL, intercall interval and number of pulses on male's reaction

In our study, call rate was a composite parameter that unavoidably covaried with our main target call features (intercall interval and number of pulses). Therefore, we tested the relationships between the resulting call rate variation and the response scores of receiver males. We found no relationships between the stimulus call rate and the change in the number of pulses per call emitted by males (Pearson's r_{55}: -0.270, ns) neither in the change in

movements of receivers (number of jumps: Pearson's r_{55}: -0.190, ns; number of walks, r_{55}: -0.049, ns; latency to first orientation, r_{55}: -0.059, ns; number of orientations, r_{55}: -0.136, ns). The variation of SPL, intercall interval and number of pulses did not affected the proportion of phonotactic responses (a gross measure) of receivers (chi-square test: $\chi^2 = 2.875$, df=1, $P=0.080$; $\chi^2=0.909$, df=2, $P=0.635$; $\chi^2=4.836$, df=2, $P=0.089$, respectively, Fig 3). However, the variations of the stimulus SPL affected the response scores of males (a finer measure) (MANOVA: $F_{4.600}$, $P=0.0020$; Table 3). Louder calls increased more the number of body orientations with respect to the period before stimulation than softer calls (ANOVA: $F_{20.632}$, $P<0.001$; Table 3; Fig 4a). Moreover, males produced longer calls containing more pulses in response to louder stimuli (ANOVA: $F_{4.578}$, $P=0.0378$; Table 3; Fig. 4b). Variations on stimulus intercall interval did not affect the response scores of males (MANOVA: $F_{1.250}$, $P=0.2724$; Table 3). Otherwise, the response scores were also affected by the stimulus number of pulses (MANOVA: $F_{2.332}$, $P=0.0177$; Table 3). Stimulus number of pulses, increased significantly the number of orientations performed by males (ANOVA: $F_{3.797}$, $P=0.0299$; Table 3; Fig. 5a). Although average number of pulses values produced the maximal change in the number of orientations, the *post-hoc* test revealed that average and high number of pulses treatments formed an indiscernible group, while average differed from low number of pulses (Post-hoc test: $P=0.132$, $P=0.037$, respectively). Stimulus number of pulses also affected the number of walks (ANOVA: $F_{3.413}$, $P=0.0417$; Table 3; Fig. 5b). The change in the number of walks differ between average and high number of pulses, but not between low and average levels (Post-hoc test: $P=0.045$; $P=0.232$, respectively). The interaction term SPL X number of pulses did not affected the response scores of males (MANOVA: $F_{0.585}$, $P=0.8216$; Table 3). Finally, males' response did not depended on receiver body length (SVL) (MANOVA: $F_{0.700}$, $P=0.6264$; Table 3).

Discussion

Our study reveals that only a small amount of information (13%) about body length might be available on the number of pulses per call. Although larger males tend to produce fewer pulses per call, males might not use call traits (temporal or spectral) to assess opponent body length. Males exposed to simulated intruders reacts by changing the temporal but not the spectral traits of their calls, additionally they increased the movement rate. Although neither sound intensity (dB SPL) nor intercall interval or number of pulses had any detectable influence on the proportions of phonotactic response (positive or negative) of males, the stimulus sound intensity and the number of pulses, explain the changes in the movements and in the number of pulses per call evoked by receiver males. Altogether, the results suggest that males adjust its behavioral reactions accordingly to intruder's distance and to the variations in the number of pulses of calls. The territorial defense in *O. histrionica* males might be ruled by relatively simple mechanisms such as sound intensity (distance) estimation. The differential reactions to variations in the number of pulses per call may only reflect the effectiveness of each type of stimulus in the auditory system, within the recognition space. However, we discuss the possible role of fine temporal traits of calls on the information transmission about the motivation to fight.

We only found a small amount of information about the SVL that could be encoded in the number of pulses per call (Table 1). This suggests that it is unlikely that males can achieve an accurate estimation of the body lengths of the opponents. The slight but significant negative relationship between SVL and the number of pulses per call may result from allometric effects of the structures involved in the biomechanics of call generation, similar to those found in other frog species (e.g. *Acris crepitans*, McClelland et al 1996). However, the general lack of information on the dendrobatid sound production mechanisms deserves

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further study. The low predictability for the *size – number of pulses* correlation suggest that call traits are arbitrary with respect to the resou rce holding potential (RHP). The ecological significance is that males should use simpler mecha nisms to achieve the resolution of territorial encounters.

The behavioral reaction of male *O. histrionica* towards simulated intruders consist of an increase in the number of movements (orienting, walking more) and in the number of pulses per call, but without changing the peak frequency o f calls (Table 2). O ther dendrobatid frogs such as *Rheobates palmatus* (Lüddecke 1999), *Silverstoneia nubicola* (Summers 2000), *Allobates femoralis* and *Ameerega trivittata* , (Narins et al. 2003; Amézquita et al. 2005, 2006) present a rapid and highly stereotypic "all or none " response, with a sudden stop of calling. On the contrary, males *O. Histrionica* lacks of such fast response. Instead, males interspersed their approaches with calling until th ey reach the loudspeaker or simply stopped the approach and continued calling at higher rates midway to the broadcasting speaker. Additionally, we do not detect any influence of the experimental variations of sound intensity, silent intervals between calls (intercal l interval) or number of pulses in the proportions of phonotactic reactions (positive or n egative) of males (Fig 3). As result, the absence of an all or none response of *O. histrionica* males becomes evident. These call traits might provide incomplete information about whether an intruder male will attack or not. As in other dendrobatid frogs (Narins et al. 2003), ma les of *O. histrionica* may require additional visual cues to start physical attacks. The rapid al l or none responses of other dendrobatid frogs may allow an efficient territorial defense th at should compensate its energetic and ecological implications (Amézquita 2005). Thus, und er what conditions behavioral reactions such as those of *O. histrionica* may be favored? A recent study of the sister taxon *Oophaga lehmanni* with captive individuals (Rojas, unpublished data) suggest that conflict resolution in this species is well explained through the confl ict resolution model of sequential

assessment (in which the fighting ability is initia lly uncertain and estimated during the contest)(Enquist & Leimar 1990; reviewed in Bradbu ry & Vehrencamp 1998). The persistent response of males of *O. histrionica* might be consistent with that interpretation. More over in a previous study, Zimmermann (1990) reported (though no quantitatively) that the calls of *O. histrionica* might grade into aggressive calls. If so, males a ssessing rivals through such persistent display may reduce the chance of escalat e encounters into fights as hypothesized for graded communication systems (Laurilia et al. 2 004; Owen & Gordon 2005). Simultaneously, the aposematic coloration may balan ce the cost of such persistent displays by males, lessening of the likelihood of attraction of predators, an emerging constraint for graded responses (Laurilia et al. 2004; Owen & Gord on 2005).

As expected, the variations in the perceived distan ce of the simulated intruder (stimulus intensity) modified the response of resident males. It is possible that males have perceived low and high SPL stimuli as equally threatening, bu t were unable to find the sound source when the low intensity stimulus were broadcasted. A lternatively, males might interpret more distant intruders as less threatening. We believe t his latter to be more likely: males adjusted its response to louder stimuli producing longer cal ls (containing more pulses) and increasing the number of body orientations (Table 3; Fig 4) th us, supporting our first prediction. In the close related *O. pumilio,* the magnitude of the response of resident males wa s much lower when the stimulus was delivered from inside the nei ghbouring territory than when it was broadcasted midway between territories (Bee 2003). These results also agree those of previous research on the effect of perceived proxim ity on male behavior (Robertson 1984, 1986; Wagner 1989; Morton 1998; Burmeister et al. 2 002; Owen & Gordon 2005). The ability to estimate the distance to a sound source is particularly important in the dendrobatid mating system. Female distribution is related to th e location of adequate tadpole-rearing sites

and thus, males seems to compete for the establishment of territories in areas where female density is high (Pröhl 2003, 2005). In consequence, distance perception might enhance territorial defense, avoiding an excessive boundary overlap, and therefore avoiding chances of a physical contest.

The increase in the number of pulses per call in response to nearer intruders (high sound intensities), might not allow the assessment of body length by receivers. However, in order to achieve such response males might invest a considerable effort. In other species, energetic expenditure is correlated with calling (Wells & Taigen 1986; Grafe et al. 1992) and aggressive calling may impose significant additional demands on signaling males (Marshall et al. 2003). Although the assumption that increase the number of pulses is energetically expensive in *O. histrionica* requires testing, the fact is that males recognize variations in the stimulus number of pulses per call (Table 3, Fig 5). In our experiment, average and higher than average number of pulses per call, captures the attention (orientations) of the resident male significantly more than the lowers values; additionally, males walked in a greater extent towards the loudspeaker in response to calls containing lower and average pulses than in response to higher values of pulses. These differential reactions to variations in the number of pulses per call might only reflect the effectiveness of stimulus variation in the auditory system, within the recognition space. In female choice contexts, species-specific temporal information in the call is necessary to attract females (Schwartz 1987; reviewed in Gerhardt & Huber 2002). Sibling cohabitating species (e.g. *Hyla chrysoscelis* and *H. versicolor*) often differ in call features such as repetition rate of signal units allowing the use of different call recognition mechanisms to avoid mating errors (Shull & Bush 2002). Heterospecific signals may represent a source of masking noise therefore, recognition of temporal (and spectral) traits is necessary for increasing conespecific detection and reducing the likelihood of

heterospecific interference (Amézquita 2005, 2006). However at our study site, the acoustic environment was fairly poor; we did not identify any co-occurring species signalling within the temporal (or spectral) range of *O. histrionica* calls. Therefore, is unlikely that the temporal recognition might function as a mechanism to avoid heterospecific interference. Moreover, aggressive calls do not function in species recognition (Littlejohn 2001). Although *O. histrionica* lacks the discrete call types (advertisement call structurally different from aggressive signals) characteristics of some graded communication systems (Schwartz 1989; Marshall et al 2003), the significant increase in the number of pulses per call and its recognition by males during simulated territorial encounters may be functionally analogous, and may confirm the previous qualitative reports of the use of aggressive calls by male *O. histrionica* during territorial defense.

No effects of variation of the silent intervals between calls (intercall interval) or in the call rate of stimuli were found in the reaction of males (Table 3; see results). These gross temporal traits probably might not be useful to resolve short range agonistic encounters. However, previous studies (Pröhl 2003; Frosman & Hagman 2006) suggest that gross temporal traits are probably important for females in order to find mates or during courtship to assess prospective mates and as consequence, male mating success is correlated with overall calling activity. Our results allow the distinction of the number of pulses per call as a functionally important cue, independent from the intercall interval during short-range encounters between males of *O. histrionica*.

In our experiment, body length (SVL) was not a significant predictor of males' reaction having at best only a week influence on receiver's response. Reproductively active males defending territories efficiently, may reach similar sizes. Therefore, the information assessment of motivational states might be critical for resolving agonistic interactions.

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Additionally, call traits may have a fairly low predictability to assess SVL. Therefore, both male's signals and the cues used during territorial defence by receivers appear non informative with respect to RHP. Although body mass estimated thought body condition indexes (Jakob et al 1996) is a good determinant of RHP in many animals (Shackleton et al. 2005; Brown et al 2006), other studies of information transfer in anurans have also used the snout vent-length (SVL) of males (e.g. Burmeister et al 2002; Owen & Gordon 2005) therefore, our data might be comparative. The interaction term (SPL x number of pulses) did not explained the behavioral scores of males (Table 3). However, the differential reactions to SPL and to the number of pulses may be similar to those founded in studies examining graded changes in temporal properties of calls in frogs (Schwartz and Wells 1984; Schwartz 1989; Wagner 1989; Grafe 1995). In natural encounters, males might use both sources of information independently.

Our data might confirm previous descriptions (Zimmermann 1990) in which advertisement calls may grade into aggressive calls in *O. histrionica*. By definition, graded aggressive signals increase in magnitude as the contest progresses and the aggressiveness vary, according to context (Wagner 1989). For some species, long and short-range aggressive calls represent two ends of a continuum (reviewed in Wells & Schwartz 2007; Rose & Brenowitz 1997; Laurilia et al. 2004) in which the conditions (for an appropriate response) are represented along a continuous condition axis (reviewed in Bradbury and Vehrencamp 1998). Our data may agree that definition; however, graded communication systems are usually described as having discrete advertisement and aggressive variants (but see Burmeister et al. 2002) therefore, we are cautious of this appreciation and further studies should be conducted in order to confirm it.

Together, the above factors suggest that if the calls of *O. histrionica* actually grade into aggressive calls (a graded signaling system), males are potentially signaling its level of motivation to fight (Hurd 2006). It is likely that males may transmit such information since calls are not related with physical attributes of males (i.e. they arbitrary with respect to RHP) a condition to indicate that sort of information (Maynard Smith 1976; 1982; Enquist 1985). Additional work will be required to test the hypothesis of motivation on dendrobatid frogs through experiments that take into account the effect of variation in fine temporal traits and the presence of a valuable resource (e.g. females) (Kotiaho et al. 1999; Brown et al. 2006) on the differential reaction of males.

In conclusion, our results suggest that call traits do not communicate information about fighting ability or RHP. Moreover, males of *O. histrionica* recognize and use the variations of the SPL and in the number of pulses per call, to adjust its behavioral reaction accordingly. Although *O. histrionica* lacks a distinctly discrete aggressive call, in response to variations of SPL males increase the number of pulses. Therefore, we distinguish this fine temporal trait as a functionally important cue for territorial defense. As a whole, the behavioral reaction of males might be consistent with the sequential assessment model of conflict resolution. Additionally, receiver body length do not influenced male's reaction. Taken together, our results may be in agreement with previous reports about the use of aggressive calls in this species. In consequence, an untested prediction that follows from our study is that male calls may encode information about motivation to fight that may potentially influence territorial defense.

Acknowledgments

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Table 1. Pearson correlation matrix between call characteristics (temporal: number of pulses, call duration intercall interval; spectral: peak frequency) and the body size of males (snout-vent length –SVL).

	Snout-vent length (SVL)	Number of pulses per call	Call duration (s)	Intercall interval (s)	Call rate (calls/bout duration)	Total Call rate (Calls/300s)
Number of pulses per call	-0.3582 **					
Call duration (s)	-0.3147 *	0.6117 **				
Intercall interval (s)	-0.2614	0.0771	-0.0845			
Call rate (calls/bout duration)	0.2218	-0.0876	-0.1693	-0.5149 **		
Total Call rate (Calls/300s)	-0.0729	0.1219	0.0828	-0.1617	-0.0761	
Peak Frequency (kHz)	-0.0864	0.1813	0.0596	-0.0404	0.3066 *	0.3764 **

N=55; Pearson's correlation r; *P<0.05; **P<0.01.

Table 2. Reaction of males of *O. histrionica* to playback experiments simulating average calling intruders. Eleven behavioural parameters were analysed comparing the behaviour of males before and during playback (Paired–T test). The summary of all tests is presented for each variable. Significance levels are assessed after Bonferroni's correction for the number of test (0.05/ number of tests = 0.0045): ** statistically significant.

Dependent	Control stimuli			
	Difference between experimental periods (Before - during)			
	n	*t*	*p*	
Number of jumps	6	-9.082	0.0003	**
Number of pulses per call	6	-6.968	0.0009	**
Number of orientations	6	-5.818	0.0021	**
Number of steps	4	-6.908	0.0022	**
Latency time to first orientation (s)	4	-8517	0.0034	**
Bout duration (s)	6	-3.275	0.0221	
Call rate (calls/300 s)	6	-2.799	0.0380	
Latency time to first jump (s)	6	-2.720	0.0418	
Call rate (calls/bout duration)	5	2.623	0.0470	
Intercall interval (s)	6	-1.837	0.1256	
Peak frequency (Hz)	6	-0.774	0.4737	

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Table 3. Summary of the MANOVA analysis (top panel) to test the effect of stimulus SPL, intercall interval, number of pulses per call, the interaction of SPL x number of pulses and body size (SVL) in the behavioral scores of males of *O. histrionica*. Bottom panel shows an overview of the complementary ANOVA test of effects of the stimulus variables on each behavioral score. Asterisks highlight statistical significance: *<0.05; **<0.0001.

Independent	Pillai's Trace	F	Hypothesis df	Error df	P
Stimulus sound intensity (dB)	0.359	4.600	5	41	0.0020 **
Stimulus intercall interval (s)	0.259	1.250	10	84	0.2724
Stimulus number of pulses	0.435	2.332	10	84	0.0177 *
Stimulus sound intensity X Stimulus number of pulses	0.130	0.585	10	84	0.8216
Snout–vent length (SVL)	0.079	0.700	5	41	0.6264

							Dependent									
Independent	Change in number of Jumps			Change in number of Walks			Latency time to first Orientation			Change in number of Orientations			Change in number of Pulses per call		
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Stimulus sound intensity (dB)	1	0.0421	0.8383	1	2.785	0.1021	1	1.130	0.2934	1	20.632	<0.0001 **	1	4.578	0.0378 *
Stimulus intercall interval (s)	2	1.636	0.2062	2	2.225	0.1198	2	1.128	0.3326	2	0.218	0.8052	2	0.444	0.6443
Stimulus number of pulses	2	2.777	0.0729	2	3.413	0.0417 *	2	0.748	0.4792	2	3.797	0.0299 *	2	2.175	0.1254
Stimulus sound intensity X number of pulses	2	0.194	0.8240	2	0.999	0.3761	2	0.067	0.9352	2	0.970	0.3869	2	0.207	0.8140
Snout–vent length (SVL)	1	0.119	0.7312	1	0.163	0.6882	1	0.471	0.4959	1	0.269	0.6069	1	2.900	0.0955

(a) Call Bouts

(b) Calls

(c) Call

Pulse

Figure 1.

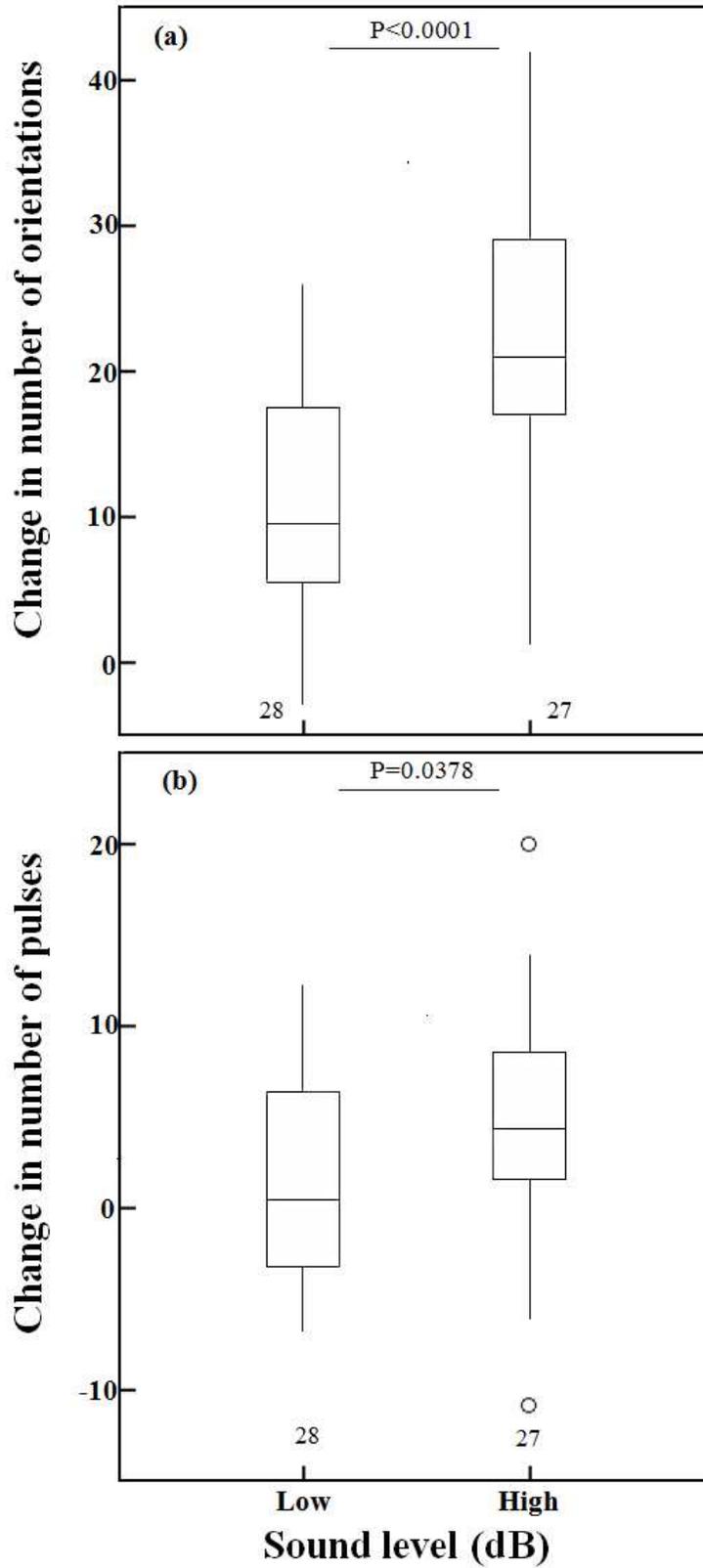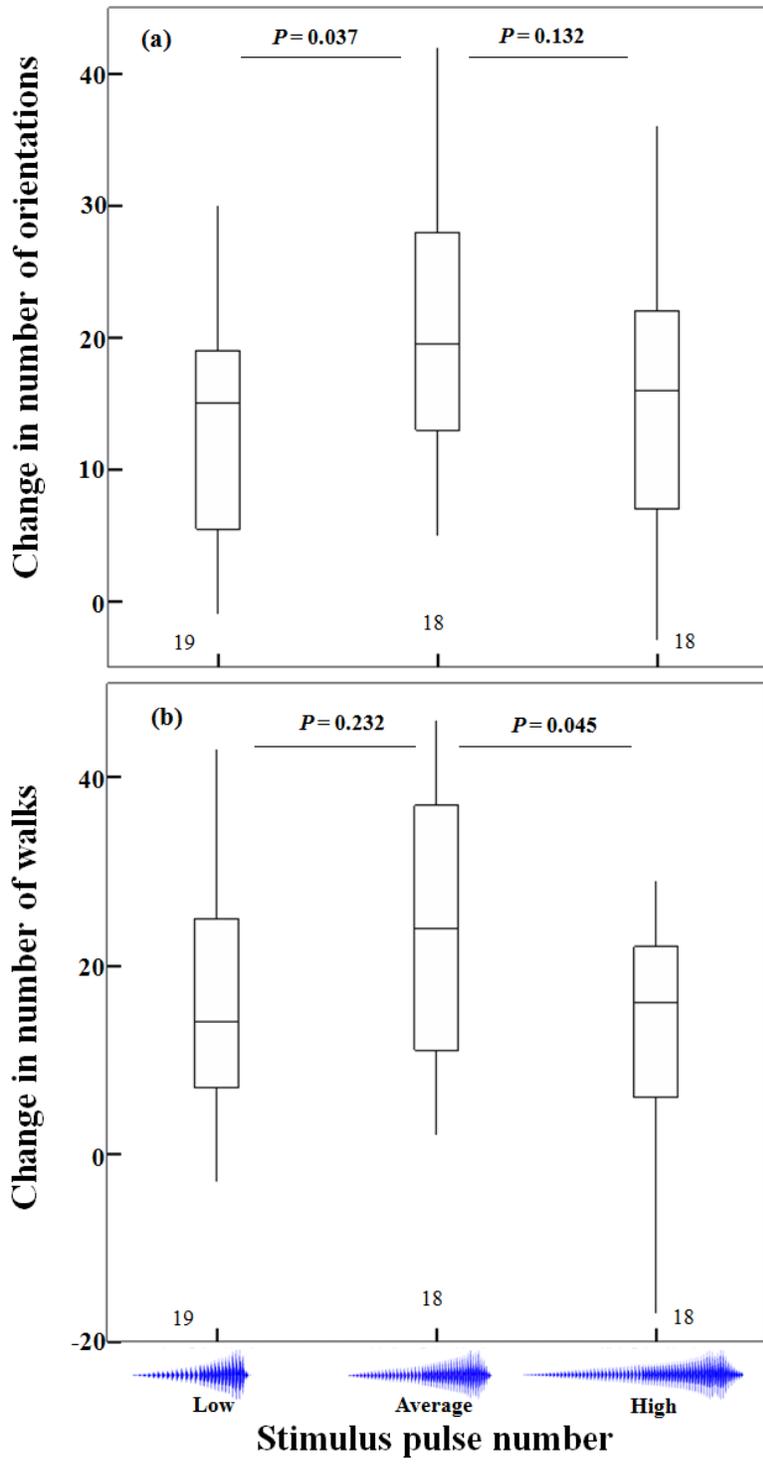| Intercall Interval	Number of pulses	call rate
H (0.210 s)	H (68) (0.241 s)	2.217
H (0.210 s)	A (48) (0.171 s)	2.625
H (0.210 s)	L (28) (0.103 s)	3.226
A (0.163 s)	H (68) (0.241 s)	2.475
A (0.163 s)	A (48) (0.171 s)	2.994
A (0.163 s)	L (28) (0.103 s)	3.759
L (0.114 s)	H (68) (0.241 s)	2.809
L (0.114 s)	A (48) (0.171 s)	3.509
L (0.114 s)	L (28) (0.103 s)	4.673
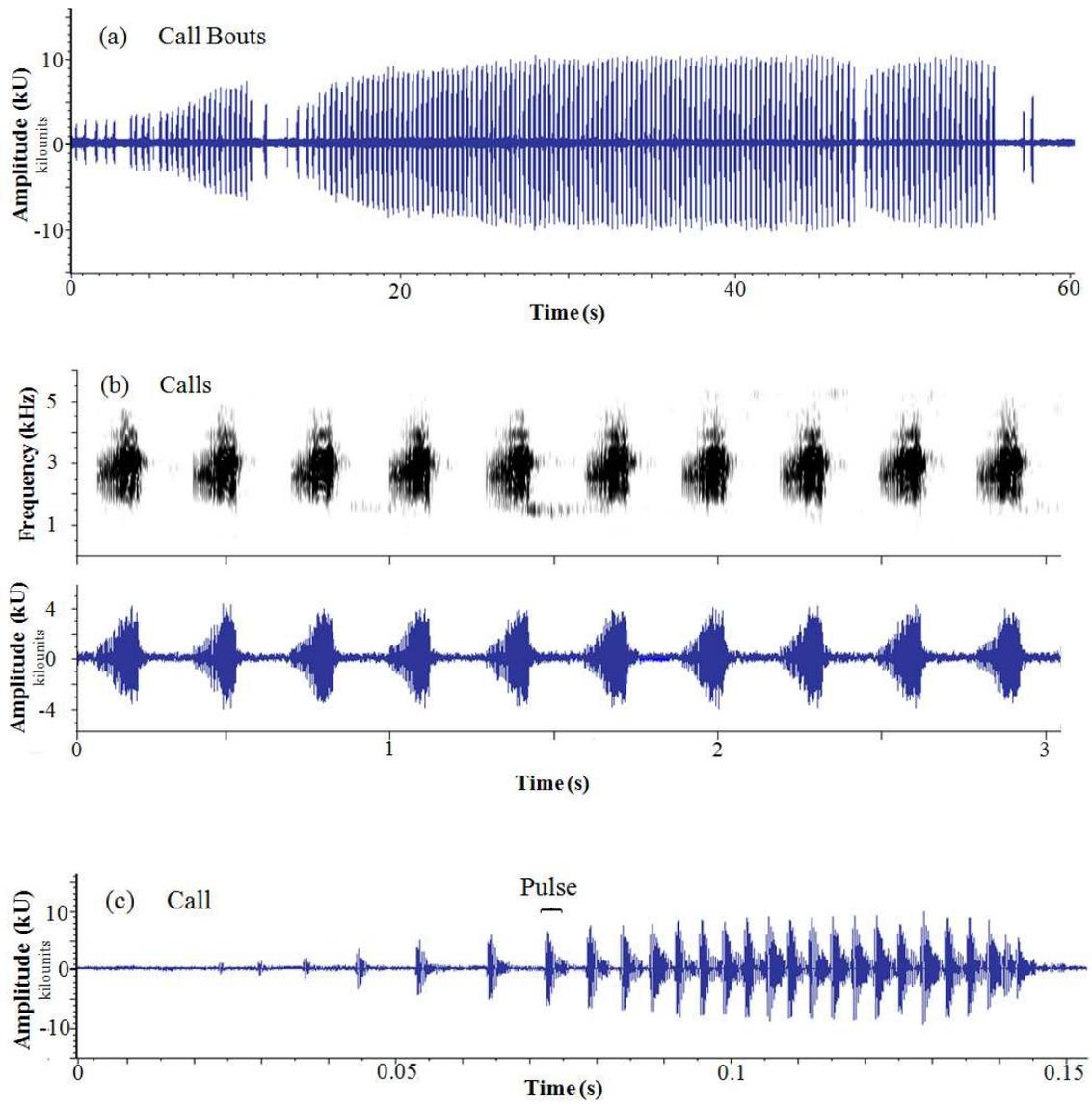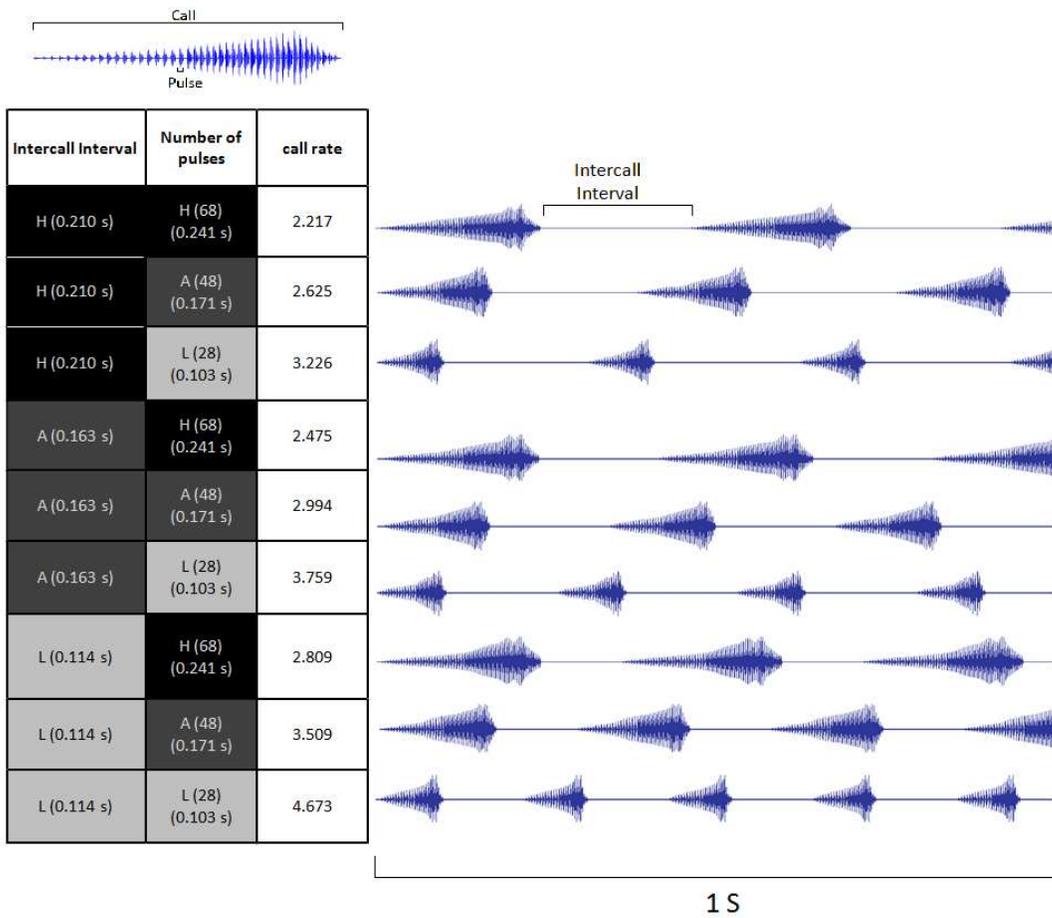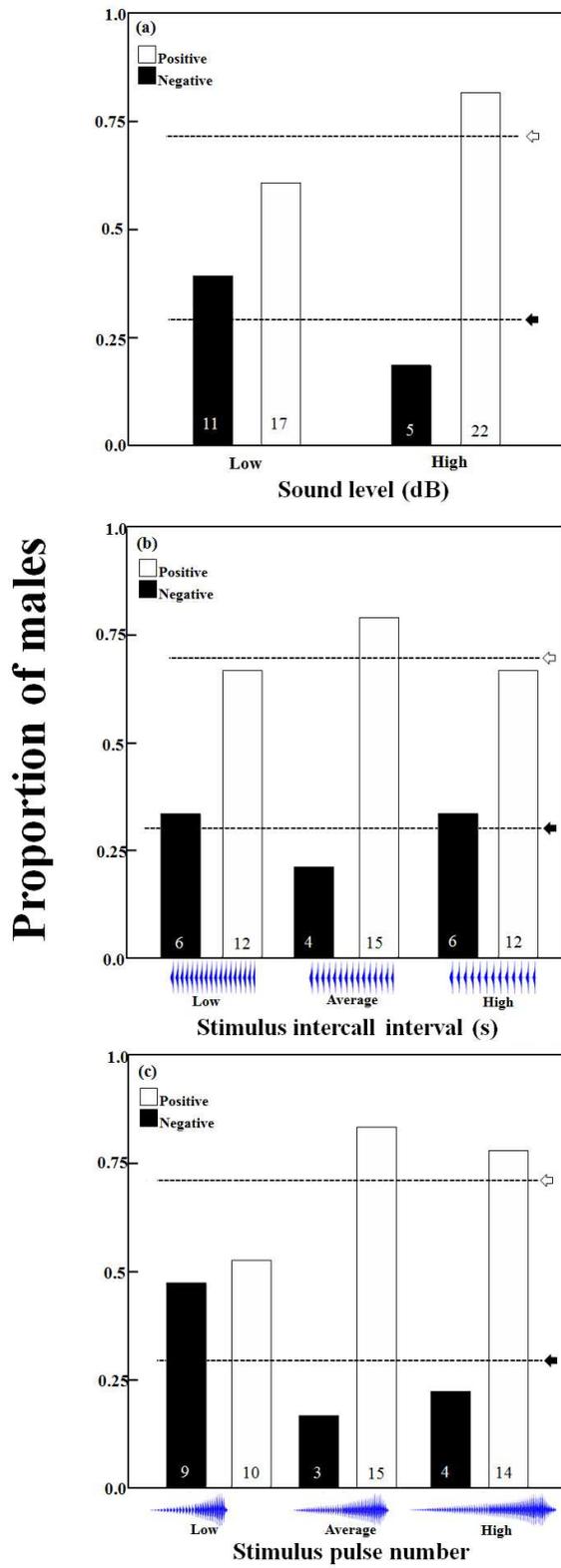
Figure2.

Figure3.

Figure4.

Figure5.

Figurelegends

Figure1 .(a)Soundoscilogramofarepresentative"bout"o rcallgroupproducedbymalesof
O. histrionica . Call groups usually consists of a few calls to a several hundred calls. (b)
Spectrogram (top) and oscilogram (bottom) of ten ca lls with a time expanded scale. (c)
Representationofasinglecallwhichconsistsofa variablenumberofpulses.Allrecordings
weremadeatairtemperatures23-25°Cmeasuredat theindividualcallingposition.

Figure2. Theexperimentaldesingfollowedafactorial(2x3x 3)arrangement.Thesynthetic
callsusedasstimulusfortheplaybackexperiments whereassembledvariyingthreefactors
independentlyofeachother:SPL(twolevels:high= 62dBandlow=53dB,notrepresented),
the intercall inteval and the number of pulses per call. Both temporal traits varied 1.5
standarddeviations(positiveandnegative)around thepopulationmeanvalues;lettersdenote
L= low, A= average and H= high. Call rate is a comp osite parameter (1/call duration +
Intercall interval) therefore, we unavoidably obtai ned call rates ranging between 2.22 and
4.67calls/s.

Figure 3. Proportions of males that did (□ positive response) and did not (■ negative
response)reachedtheloudspeakerwithintheexperi mentalperiod.Arrowsindicatethemean
proportionofeachphonotacticresponse.

Figure 4. The SPL of the stimulus call affected the change in the number of orientations and pulses per call performed by males of *O. histrionica* (MANOVA: $F_{4.600}$, P=0.0020; Table 3). The change in the number of (a) orientations and (b) pulses per call, differed between levels of SPL (ANOVA $F_{20.632}$, P<0.001; F $_{4.578}$, P=0.0378, respectively). Numbers at the bottom of each panel denotes the number of males tested.

Figure 5. The variation in the number of pulses of stimulus call affected the change in the number of movements of males of *O. histrionica* (MANOVA: $F_{2.332}$, P=0.0177; Table 3). The Change in the number of (a) orientations and (b) walks performed by males differed between levels of low, average and high number of pulses of the stimulus call (ANOVA: F$_{3.797}$, P=0.0299; F $_{3.413}$, P=0.0417, respectively. Numbers at the bottom of each panel denotes the number of males tested. Statistical significance between levels is highlighted after *post hoc* procedures.