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# Population and seasonal variation in response to prey calls by an eavesdropping bat

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**Abstract** The fringe-lipped bat, *Trachops cirrhosus*, is an eavesdropping predator that hunts frogs and katydids by approaching these preys' sexual advertisement calls. In captivity, bats can rapidly learn to associate novel acoustic stimuli with food rewards. It is unknown how this learning ability is related to foraging behavior in the wild where prey and the calls that identify them vary over space and time. In two bat populations that differ in available prey species (Soberanía, Panama, and La Selva, Costa Rica), we presented wild-caught bats with frog calls, katydid calls, and control stimuli. Bats in Soberanía were significantly more responsive to complex calls and choruses of the túngara frog, *Physalaemus pustulosus*, than were bats in La Selva. La Selva bats were significantly more responsive to katydid calls (Steirodon sp.) than Soberanía bats. We also examined seasonal variation in bat response to prey cues. Bats were captured in Soberanía in dry and wet seasons and presented with the calls of a dry season breeding frog (*Smilisca sila*), a wet season breeding frog (*P. pustulosus*), and four katydid species. Bats captured in the dry season were significantly more responsive to the calls of *S. sila* than bats captured in the wet season, but there were no seasonal differences in response to the calls of *P. pustulosus* or the katydid calls. We demonstrate plasticity in the foraging behavior of this eavesdropping predator but also show that response to prey cues is not predicted solely by prey availability.

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## Introduction

Learning is thought to be an adaptation for variable environments (Dukas 1998; Shettleworth 1998; Dukas 2004). Learning abilities tested in the laboratory can have positive effects on proxies for fitness. For example, the opportunity to learn associations between sensory cues and nutritive food increases the growth rate of grasshoppers in the laboratory (Dukas and Bernays 2000), and in bumblebees, learning capabilities tested in the laboratory predict foraging success in the wild (Raine and Chittka 2008). For many animals, however, it is unknown how laboratory learning abilities are related to behavior in the wild. We examined whether a bat predator whose learning abilities have been demonstrated in the laboratory exhibits flexibility in response to natural spatial and temporal variation in prey availability.

Learning may be particularly important for eavesdropping predators that locate prey using the species-specific calls that prey produce to attract mates (Zuk and Kolluru 1998; Peake 2005). Prey availability to eavesdropping predators is affected by prey signaling behavior as well as prey abundance. The prey that are available to eavesdroppers vary between populations with different prey species as well as within a population if there is seasonal variation in prey calling behavior. This variation in the availability of prey cues might favor the ability of eavesdroppers to learn to recognize the calls of novel prey and to increase or decrease their responsiveness to prey cues as prey availability changes over time.

The Neotropical fringe-lipped bat, *Trachops cirrhosus*, is an eavesdropping predator on frogs (Tuttle and Ryan 1981) and katydids (Tuttle et al. 1985). In captivity, bats are very

flexible in their foraging behavior and can rapidly learn to ignore the calls of palatable prey and approach the calls previously associated with poisonous prey (Page and Ryan 2005). After exposure to a trained conspecific tutor, naïve individuals can also learn from experienced bats to approach cues previously associated with poisonous prey (Page and Ryan 2006) or completely novel stimuli such as cell phone ringtones (Jones et al. 2013).

It is unknown how the learning observed in this species in captivity is related to foraging behavior in the wild. We hypothesized that the learning abilities of *T. cirrhosus* enable individuals to flexibly shift their prey preferences with prey availability. The range of *T. cirrhosus* extends from southern Mexico through Brazil (Cramer et al. 2001) and encompasses a variety of frog and insect communities. Bats in the area near the Panamá Canal (Soberanía National Park and Barro Colorado Island) approach speakers broadcasting the calls of a number of frog species (Tuttle and Ryan 1981; Ryan and Tuttle 1983). They also approach the calls of Phaneropterine katydids (Orthoptera: Tettigoniidae) but preferentially approach frog calls over katydid calls (Tuttle et al. 1985).

We examined the responses of wild-caught adult bats to prey calls in two populations that differ in available prey: Soberanía National Park, Panamá, and the La Selva Biological Station, Costa Rica, with the prediction that bat responsiveness would correspond with prey availability. Two frog species with calls to which *T. cirrhosus* in Soberanía are very responsive, the túngara frog, *Physalaemus* (=*Engystomops*) *pustulosus*, and the pug-nosed treefrog, *Smilisca sila*, are absent from La Selva. We presented bats from both populations with the calls of *P. pustulosus* and *S. sila* to examine how La Selva bats respond to the calls of these allopatric frog species. The eavesdropping behavior of *T. cirrhosus* in La Selva has not been documented, and the diet of bats in this population is unknown. We also presented bats with calls from other frog species that are present in both populations and with katydid calls to determine which prey species the La Selva bats are likely to be eating and if there are population differences in bat response to the calls of prey that are available in both populations.

The absence of the túngara frog, *P. pustulosus*, from La Selva also provided an opportunity to investigate the role of signal complexity in bat preference for prey cues. Male *P. pustulosus* can produce simple calls: a frequency-modulated ~350-ms sweep ("whine") and complex calls: a whine followed by 1–7 broadband ~40–80-ms harmonic bursts ("chucks") (Ryan 1980). Bats, like female frogs, preferentially approach speakers broadcasting complex calls over speakers broadcasting simple calls (Ryan 1980; Tuttle and Ryan 1981). Several hypotheses have been posited for why bats prefer complex calls, including increased localization acuity, higher prey density, and sensory bias. Flight cage experiments show that complex calls are easier for bats to

localize under certain conditions (Page and Ryan 2008). Field observations demonstrate that frogs are more likely to make complex calls when there are other frogs calling nearby (Bernal et al. 2007), making complex calls indicative of higher prey densities. A recent field experiment from Ecuador suggests that *T. cirrhosus* could have a sensory bias for complex calls (Trillo et al. 2013). The sister taxa to *P. pustulosus*, *Physalaemus petersi*, have populations in Ecuador where frogs only produce simple calls and other populations in which frogs can produce complex calls (Boul et al. 2004). Bats in populations where the frogs do not make complex calls still preferentially approach speakers broadcasting complex *P. petersi* calls (Trillo et al. 2013). We included simple calls, complex calls, and choruses of *P. pustulosus* as stimuli in our population comparison to examine whether bats in La Selva that have never been exposed to *P. pustulosus* calls exhibit a similar preference for call complexity.

We also examined if bat responsiveness shifts over time as prey calling behavior changes seasonally within Soberanía. *P. pustulosus* breeds and calls primarily in the wet season (ca. May to November); *S. sila*, in contrast, breeds during the dry season (Ibáñez et al. 1999a). We predicted that bats would be more responsive to *P. pustulosus* calls in the wet season and more responsive to *S. sila* calls during the dry season. Bats in Soberanía also eavesdrop on katydid calls (Tuttle et al. 1985). It is unclear how much seasonal variation is there in katydid abundance in Panamá (Wolda 1978; Richards and Coley 2007; Richards and Windsor 2007). To our knowledge, no study has examined seasonal variation in katydid calling behavior, but there is evidence for fluctuations in katydid calling behavior with the lunar cycle (Lang et al. 2006). We included the calls of four katydid species in our stimuli to examine whether there were seasonal differences in bat response to katydid calls. Together, these studies provide a unique opportunity to examine how foraging flexibility demonstrated in captivity might function in the wild.

## Methods

### Study animals

A total of 43 *T. cirrhosus* were tested in this study. Bats were captured in mist nets placed at night across streams and trails in the forest. For the population comparison, bats were captured at La Selva Biological Station, Costa Rica (*N*=5 bats tested from April to May 2010), and at Soberanía National Park, Panamá (*N*=5 bats tested June 2010). The seasonal comparison was conducted in Soberanía in two dry seasons (*N*=4 bats tested from January to March 2010 and *N*=10 bats tested from January to March 2012) and two wet seasons (*N*=5 bats tested from June to July 2010 and *N*=14 bats tested from June to November 2011). All bats were either adult

males or adult nonreproductive females; no bats were used in both experiments or tested in both seasons. For each bat (with the exceptions of one bat from La Selva and two bats from Soberanía), we divided measurements of capture mass by forearm length to obtain a body condition score (Kelly et al. 2008). For the population comparison, bats were immediately released into an outdoor flight cage with ambient light, humidity, and temperature ($6 \times 4 \times 2.5$ m in La Selva and $5 \times 5 \times 2$ m in Soberanía). Bats were tested the night following capture (approximately 24 h post-capture). In the intervening time, bats were provided with four to six katydids to forage on within the cage. This was consistent at both sites. For the seasonal comparison, bats were maintained in a small ($142 \times 127 \times 203$ cm) mesh tent where they were hand-fed baitfish for the first 36 h and then released into the $5 \times 5 \times 2$ m flight cage. Testing began the following evening, approximately 48 h after capture. At the completion of the testing, bats were individually marked using Passive Integrated Transponders (PIT tags, Trovan Ltd., UK) and released at their capture sites.

Experimental protocol

Bats in the population comparison received 11 stimuli (Fig. 1). The first La Selva bat received 5 of the 11 stimuli, and the second La Selva bat received 10; all other bats received the complete set of stimuli. The stimuli consisted of calls of two frog species present only in Soberanía (*P. pustulosus* and *S. sila*) and calls of four potentially palatable prey species present in both locations, three frog species (*Dendropsophus ebraccatus*, *Dendropsophus phlebodes*, and *Smilisca phaeota*) and Phaneropterine katydid in the genus *Steirodon* (due to difficulties in identification, the species is either *Steirodon rufolineatum* or *Steirodon sandrae*). To examine the effect of signal complexity on the responses of naïve bats, we used simple calls, complex calls, and choruses of *P. pustulosus* as separate stimuli. We presented bats with the calls of the poisonous cane toad *Rhinella marina* as a natural control to assess general responsiveness to acoustic stimuli. We also included a 900-Hz tone that tested if the two populations were equally responsive to novel acoustic cues in the sonic frequency range. Our final control was a time-reversed *P. pustulosus* call, thus an upward frequency sweep rather than the normal downward sweep. This stimulus was included because it has the same frequency and energy content as the natural, simple *P. pustulosus* call, but it sounds quite different to human ears.

Bats in the seasonal comparison were presented with nine stimuli (Fig. 1): simple calls, complex calls, and choruses of *P. pustulosus*; calls of *S. sila*; calls of the katydids *Balboana tibialis* (Pseudophyllinae), *Ischnomela gracilis* (Pseudophyllinae), *Neoconocephalis affinis* (Copiphorinae), and *Steirodon* sp. (Phaneropterinae); and the 900-Hz tone as the control for seasonal differences in response to acoustic stimuli. Six of

the bats in the seasonal comparison had one stimulus for which the video recording was not of high-enough quality to analyze the bats' response and therefore was excluded from analysis (recording from a dry season bat, *I. gracilis*, and the following recordings from wet season bats: *Steirodon* sp., two tone presentations, a *P. pustulosus* simple call, and a *P. pustulosus* chorus).

Stimulus playback was conducted through one of two speakers placed on the floor in the same corner of the cage connected to a Lenovo ThinkPad T500 laptop. Frog calls and control stimuli were broadcast with a RadioShack FE-103 40-1197 4-W RMS/8-W MAX speaker through a Realistic SA-150 Integrated Stereo Amplifier. Due to their high frequencies, the katydid calls were played with an Avisoft Scanspeak Ultrasound speaker through an Avisoft UltraSoundGate Player 116 (Avisoft Bioacoustics, Berlin, Germany). A wire mesh screen ($1.5 \times 1.5$ m) covered in leaf litter was placed over the two speakers to conceal them, and each speaker was moved between stimulus presentations, although they always remained approximately 1 m apart. Small pieces of baitfish were placed on top of the screen over the speakers as rewards to maintain the bats' motivation throughout the night. Rewards were placed on the speaker broadcasting the stimulus as well as the silent speaker. Bats never landed on the silent speaker, indicating that they were responding to the acoustic stimulus and not to the cues associated with the reward.

Stimuli

Katydid calls were recorded by Hannah ter Hofstede from wild-caught individuals on Barro Colorado Island (see ter Hofstede et al. 2010 for details). Frog calls were recorded by Ximena E. Bernal, Roberto Ibáñez, Patricia L. Jones, Rachel A. Page, A. Stanley Rand, Michael Reichert, and Michael J. Ryan. Frog calls were recorded with a Nagra reel-to-reel tape recorder, a Sony cassette recorder, or a Marantz PMD661 digital recorder and a Sennheiser microphone. All recordings were made in the Soberanía area (Ibáñez et al. 1999b). The number of individuals from which we have recordings for each species is listed in Fig. 1.

The peak amplitude of each stimulus was normalized using Adobe Audition version 3.0. To approximate natural amplitude levels, frog calls and other sonic stimuli were broadcast 1 m from the speaker at 70-dB sound pressure level (SPL, re. 20 μPa) and katydid calls at 93 dB SPL. We attempted to present all stimuli at their natural duty cycle. The stimuli were broadcast for 10 calls or until the bat landed on the speaker, whichever came first. Three of the stimuli (*P. pustulosus* chorus, the toad *R. marina*, and the katydid *N. affinis*) were continuous and could not be partitioned into discrete calls; therefore, they were broadcast for 30 s or until the bat landed on the speaker. Each bat received each stimulus only once, and the order of presentation was randomized.
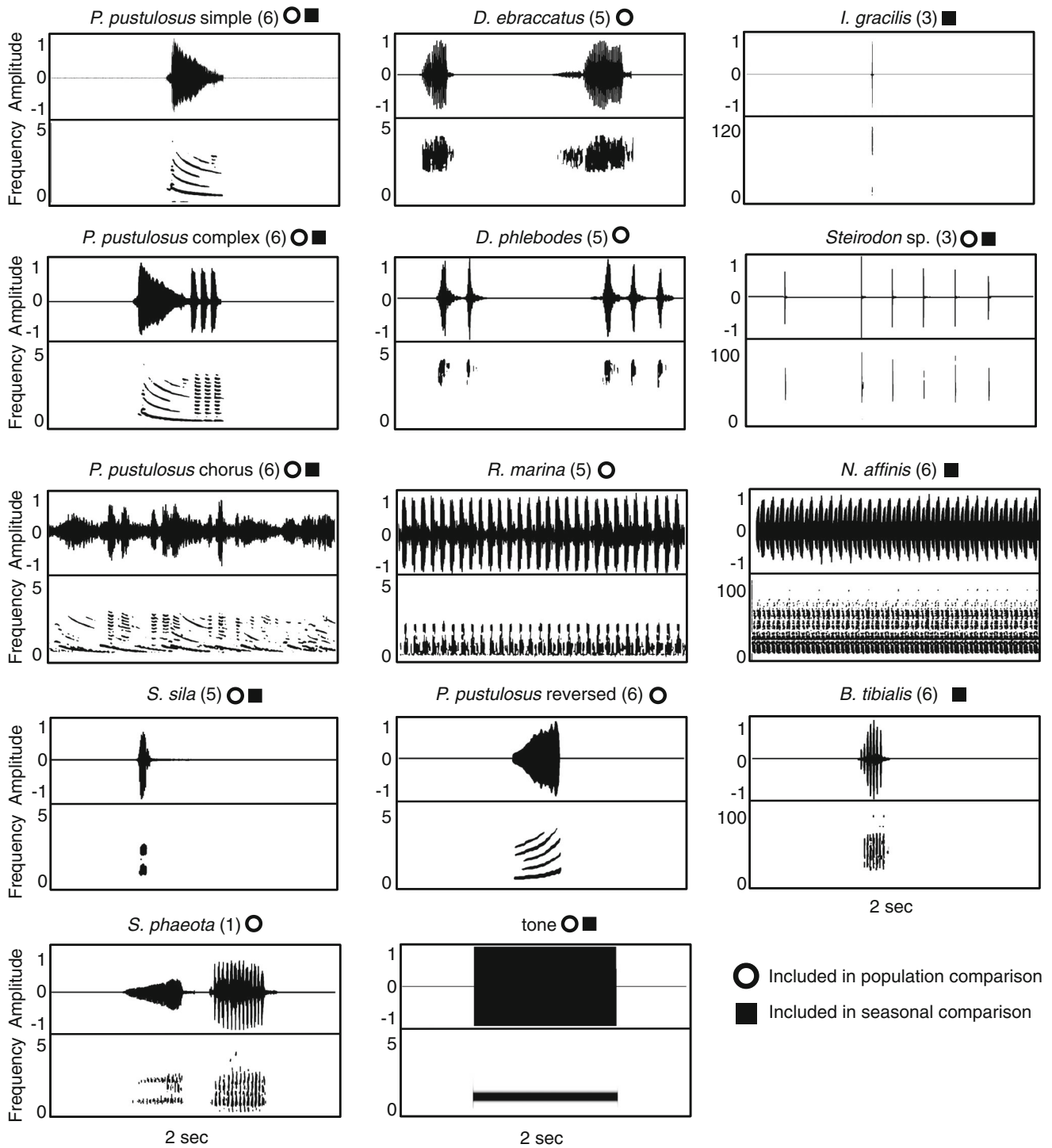
**Fig. 1** Waveforms and spectrograms (in kilohertz) of the stimuli used in the two experiments. Spectrogram scales are not the same across all the panels. The *number in parentheses* indicates the number of different individuals from which recordings were made

## Response variables

Audio–video recording of all trials were made for later analysis with a Sony Handycam DCR-SR45 using the "nightshot" setting and an additional Sony HVL-ILM Battery IR Light (DC 7.2 V, 2 W/1.5 V×2). The audio track allowed the viewer

to determine the onset and offset of stimuli. We recorded three behavioral measures to assess the bats' responses to each stimulus: (1) proportion of the stimulus (time for continuous stimuli or number of calls for discrete stimuli) to which the bat moved its ears, (2) proportion of the stimulus to which the bat's head was oriented to the corner of the cage where the

speakers were placed, and (3) whether or not the bat flew toward the speakers. The screen covering the two speakers and the video recording equipment were always placed in opposite corners of the flight cage. These fixed locations allowed us to assess orientation and flight direction when analyzing the video. Each of these behaviors is a different requisite stage in the chain of events between the detection of a stimulus and the decision whether to attack. The analysis of each of these behaviors allowed us to catalog different levels of bat responsiveness.

## Statistical analyses

Analyses were conducted using R version 3.2 (R Core Team 2013). Condition score and forearm length were compared between populations, and condition score between seasons using Wilcoxon rank sum tests. We analyzed each of the three different behavioral responses separately using mixed-effects models with the lme4 package in R (Bates et al. 2013) with individual bat as a random effect. For flight to the speaker, we used a mixed model with a binomial distribution. For each stimulus and each behavior, we then conducted planned comparisons of between seasons or between populations using the glht function from the R multcomp package (Hothorn et al. 2008).

## Results

### Bat size and body condition

We found no significant difference between bat sizes (mean forearm length±SD, median forearm length) in La Selva (58.17±1.10 mm, 58.4 mm, $N=4$) and Soberanía (57.84± 0.71 mm, 57.7 mm, $N=5$, Wilcoxon rank sum test $W=7$, $p=$ 0.556). There was also no difference in condition scores (mean±SD, median) between the La Selva bats we tested (0.56±0.046 g/mm, 0.59 g/mm) and the Soberanía bats (0.53±0.025 g/mm, 0.53 g/mm, Wilcoxon rank sum test $W=$ 8, $p=0.730$). Our sample of bats therefore did not provide evidence for a role for size or body condition in population differences in foraging behavior. We also found no significant difference in condition between bats captured in Soberanía during the dry season (mean±SD=0.54±0.051 g/mm, $N=13$) and the wet season (mean±SD=0.55±0.047 g/mm, $N=18$, Wilcoxon rank sum test $W=104$, $p=0.622$). Our data provide no evidence that the observed seasonal differences in bat response to prey cues are products of differences in body condition.

### Population comparison

Analysis of ear motions in response to the experimental stimuli indicated a marginally significant effect of population ($X^2$ (1, $N=10$)=3.51, $p=0.061$), a significant effect of stimulus ($X^2$ (10, $N=10$)=31.71, $p<0.001$), and a significant interaction effect ($X^2$ (10, $N=10$)=30.60, $p<0.001$) (Fig. 2a). When we analyzed the orientation to the speakers, we found no significant effect of population ($X^2$ (1, $N=10$)=0.049, $p=$ 0.825) or of stimulus ($X^2$ (10, $N=10$)=8.047, $p=0.624$) but a significant interaction effect ($X^2$ (10, $N=10$)=32.33, $p<0.001$) (Fig. 2b). The bats we tested from the two populations, therefore, did not differ statistically in responsiveness, but there were population differences in bat response to specific stimuli. We also analyzed flight to the speaker and found no significant effect of stimulus ($X^2$ (10, $N=10$)=8.17, $p=0.612$) or population ($X^2$ (1, $N=10$)=0.00, $p=0.999$), or interaction effect ($X^2$ (10, $N=10$)=0.00, $p=1.000$) (Fig. 2c).

From our planned comparisons, La Selva bats exhibited significantly more ear motions and orientation to the speakers than Soberanía bats to the calls of the katydid *Steirodon* sp. (ear motions $z=-2.41$, $p=0.016$; orient $z=-2.80$, $p=0.005$). Two bats from La Selva flew to the speaker in response to the *Steirodon* sp. call whereas no bats from Soberanía approached the stimulus, but this difference was not statistically significant ($z=-0.0020$, $p=0.999$). La Selva bats also moved their ears and oriented to a significantly higher proportion of the playback of the *D. phlebodes* treefrog than Soberanía bats (ear motions $z=-2.59$, $p=0.010$; orient $z=-1.969$, $p=0.049$), and one La Selva bat flew to the speaker ($z=-0.001$, $p=0.999$). La Selva bats also moved their ears to significantly more of the toxic toad, *R. marina*, call than Soberanía bats ($z=2.71$, $p=0.007$), but there was no significant difference in orientation ($z=1.252$, $p=0.211$), and no bats in either population flew to the speaker broadcasting the toxic toad calls ($z=0.00$, $p=1.000$).

Soberanía bats oriented toward a significantly greater proportion of the *P. pustulosus* complex calls and *P. pustulosus* choruses than La Selva bats (complex $z=$ 2.34, $p=0.019$; chorus $z=1.97$, $p=0.049$). Soberanía bats also moved their ears to a significantly higher proportion of the chorus ($z=1.99$, $p=0.046$), and there was a trend yet no statistically significant difference in ear motions for the complex call ($z=1.81$, $p=0.070$). We also found no significant population difference in flight to the speaker for either stimulus (complex $z=0.0020$, $p=0.999$; chorus $z=0.002$, $p=0.999$); four Soberanía bats flew to the *P. pustulosus* chorus and three flew to the *P. pustulosus* complex call, whereas no bats from La Selva flew to either stimulus.

We found no significant population differences in the bats' responses to *P. pustulosus* simple calls (ear motions $z=-0.63$, $p=0.530$; orient $z=0.78$, $p=0.437$; flight $z=0.00$, $p=1.000$) or calls of *S. sila* (ear motions $z=1.36$, $p=0.170$; orient $z=0.87$, $p=0.385$; flight: $z=-0.001$, $p=0.999$). Both of these are the calls of prey only present in Soberanía. There were also no significant population differences in bat response to the calls of the treefrogs *S. phaeota* (ear motions $z=-0.42$, $p=0.670$;
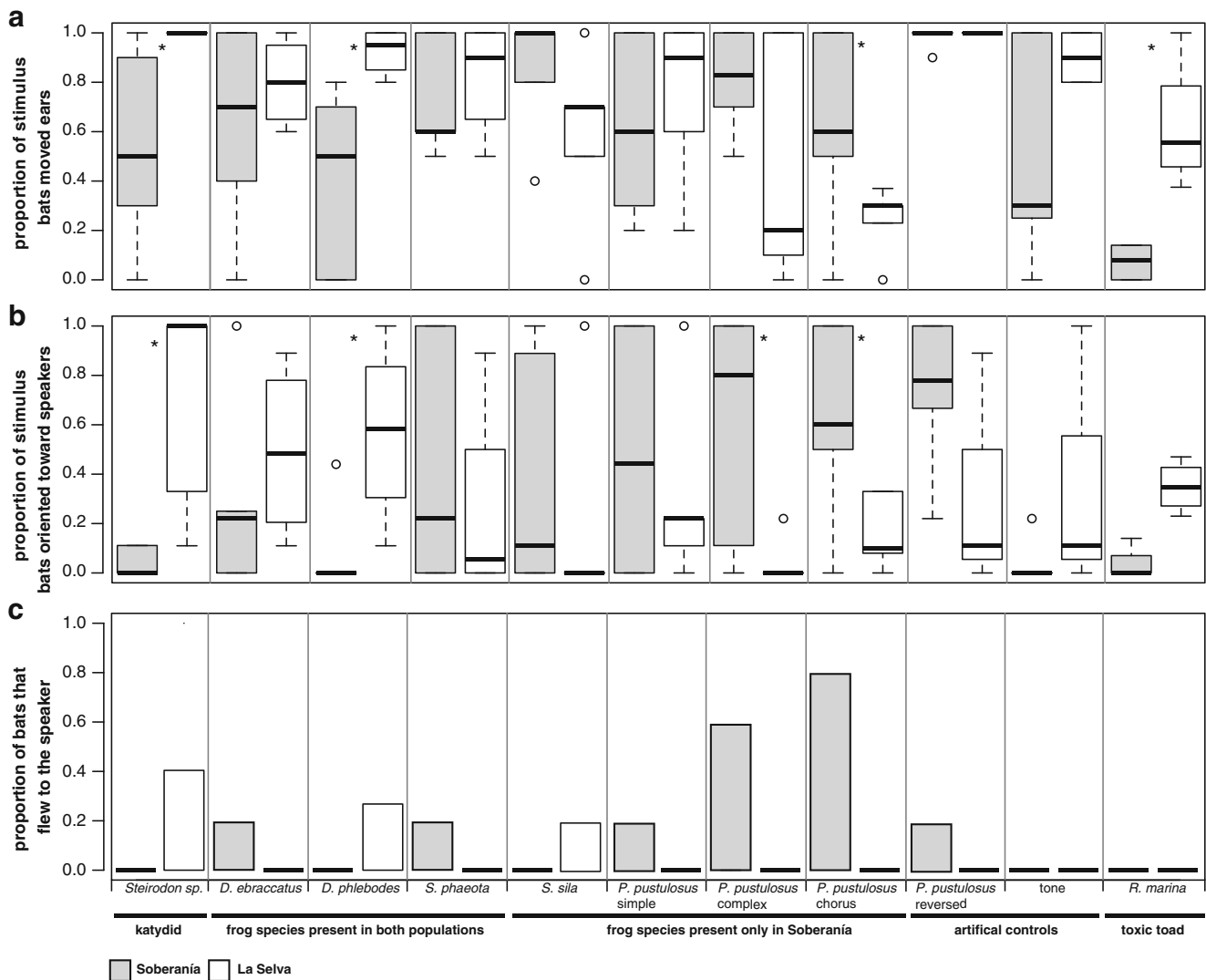
**Fig. 2** Population differences in bats' response to each of the experimental stimuli. Data from bats captured in Soberanía are in *gray*, and bats captured in La Selva are in *white*. **a** Boxplot of the proportion of the stimulus that bats captured in La Selva and Soberanía moved their ears. The *bold lines* indicate the median, and the *edges of the boxes* indicate the first and third quartiles. Whiskers extend to the lowest datum that is 1.5 times the interquartile range from the lower quartile and the highest datum within

1.5 times the interquartile range from the upper quartile. *Open circles* indicate outliers. *Asterisks* indicate significant differences (*p*<0.05) between the two populations for that stimulus. **b** Boxplot of the proportion of the stimulus that bats oriented their heads toward the corner of the cage containing the speakers. **c** Barplot of the proportion of bats that flew in the direction of the speaker

orient $z=0.81$, $p=0.420$; flight $z=0.002$, $p=0.999$) and *D. ebraccatus* (ear motions $z=-0.89$, $p=0.370$; orient $z=-0.81$, $p=0.422$; flight $z=0.0020$, $p=0.999$), which are present in both populations. Additionally, we found no statistically significant population differences in ear motions or flight in response to the reversed *P. pustulosus* simple call (ear motions $z=-0.10$, $p=0.920$; flight: $z=0.002$, $p=0.999$). For orientation to the speaker, there was a trend toward Soberanía bats orienting to a higher proportion of the reversed *P. pustulosus* call, but the difference was not statistically significant (orient $z=1.88$, $p=0.060$). There was also a trend toward La Selva bats moving their ears to a larger proportion of the playback of the artificial tone, but this was not statistically

significant ($z=-1.93$, $p=0.054$). There was no population difference in orientation or flight to the tone (orient $z=-1.06$, $p=0.289$; flight $z=0.00$, $p=1.000$).

Seasonal comparison

In the seasonal comparison, we found a significant effect of stimulus on all of the bat's behavioral responses (ear motions $X^2$ (8, $N=33$)=291.13, $p<0.001$; orient $X^2$ (8, $N=33$)=237.25, $p<0.001$; flight $X^2$ (8, $N=33$)=38.06, $p<0.001$), but no effect of season (ear motions $X^2=1.03$, $p=0.310$; orient $X^2=0.098$, $p=0.754$; flight $X^2=0.12$, $p=0.730$) or interaction effect (ear motions $X^2=4.74$, $p=0.790$; orient $X^2=15.50$, $p=0.050$; flight

$X^2$=5.00, $p$=0.760; Fig. 3). The Soberanía bats we tested were therefore significantly more responsive to some stimuli than others, but there was no overall effect of season on their response. Planned comparisons indicated that bats tested during the dry season oriented to a significantly higher proportion to the calls of *S. sila* (dry season calling treefrog species) than bats tested during the wet season ($z$=2.46, $p$=0.014) and flew to the stimulus significantly more often ($z$=2.04, $p$=0.042). There was no seasonal difference in ear motions for *S. sila* ($z$=1.11, $p$=0.270).

*P. pustulosus* predominantly calls during the wet season, but we found no significant seasonal difference in bat response to *P. pustulosus* simple calls (ear motions $z$=1.15,

$p$=0.250; orient $z$=0.20, $p$=0.844; flight: $z$=−0.33, $p$=0.740), complex calls (ear motions $z$=−0.16, $p$=0.870; orient $z$=0.17, $p$=0.869; flight $z$=0.12, $p$=0.900), or choruses (ear motions $z$=1.62, $p$=0.110; orient $z$=1.42, $p$=0.156; flight $z$=0.1, $p$=0.890). We also found no seasonal differences in bat response to the katydid calls: *B. tibialis* (ear motions $z$=0.65, $p$=0.520; orient: $z$=−0.19, $p$=0.850; flight $z$=0.090, $p$=0.930), *I. gracilis* (ear motions $z$=−0.51, $p$=0.610; orient $z$=0.42, $p$=0.677; flight $z$=0.01, $p$=1.000), *Steirodon* sp. (ear motions $z$=−0.32, $p$=0.750; orient $z$=−0.50, $p$=0.614; flight $z$=0.44, $p$=0.660), *N. affinis* (ear motions $z$=0.090, $p$=0.930; orient $z$=0.16, $p$=0.870; flight $z$=0.010, $p$=0.990), or to the control tone (ear motions $z$=−0.28, $p$=0.780; orient $z$=1.73, $p$=0.084;
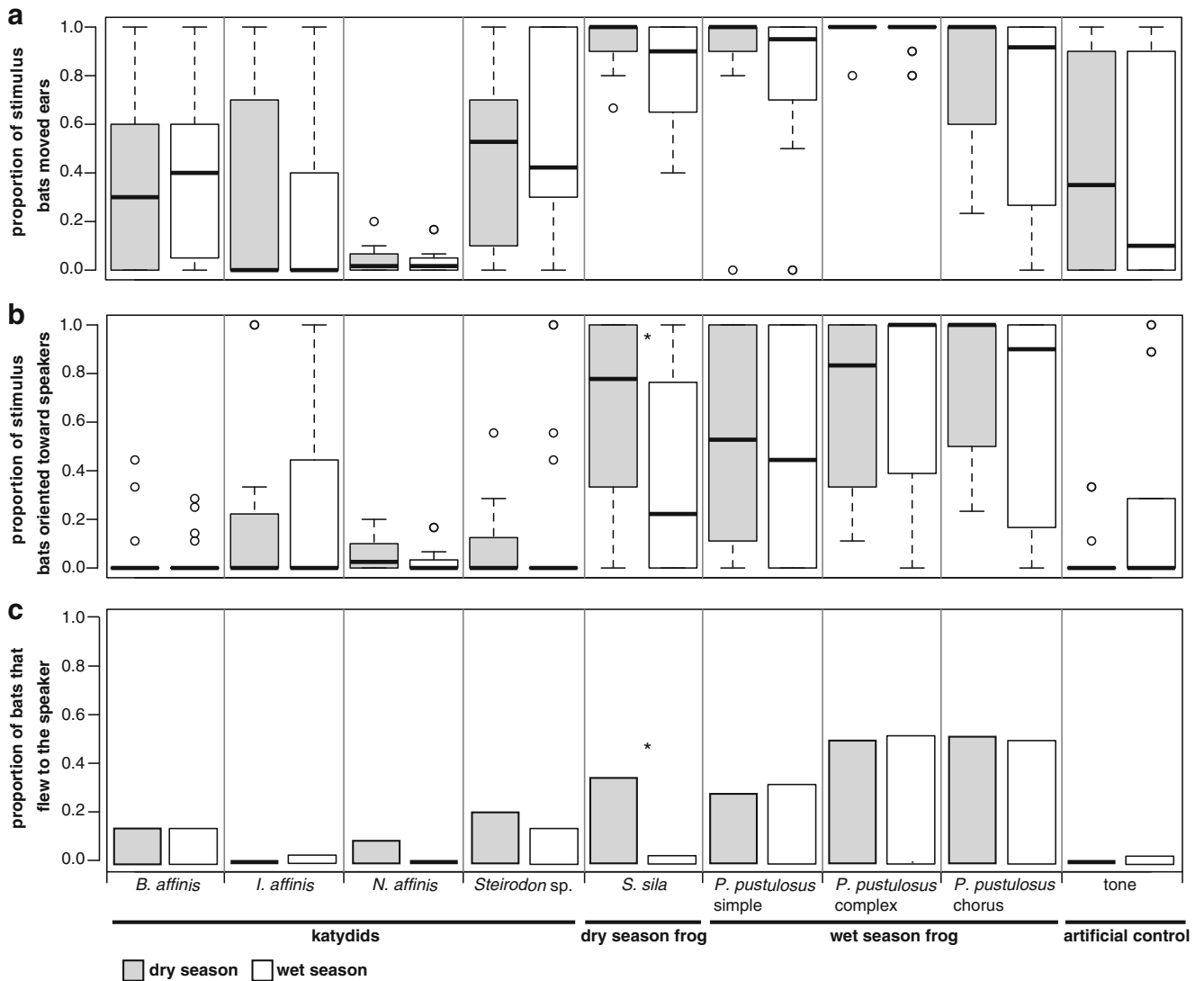


Fig. 3 Seasonal differences in bats' response to each of the experimental stimuli. Data from bats captured in the dry season are in gray, and bats captured in the wet season are in white. **a** Boxplot of the proportion of the stimulus that bats captured in the dry season and in the wet season moved their ears. The *bold lines* indicate the median, and the *edges of the boxes* indicate the first and third quartiles. Whiskers extend to the lowest datum that is 1.5 times the interquartile range from the lower quartile and the highest datum within 1.5 times the interquartile range from the upper quartile. *Open circles* indicate outliers. *Asterisks* indicate significant differences ($p$<0.05) between the two seasons for that stimulus. **b** Boxplot of the proportion of the stimulus that bats oriented their heads toward the corner of the cage containing the speakers. **c** Barplot of the proportion of bats that flew in the direction of the speaker

flight $z=0.01$, $p=1.000$). Overall, bats captured in Soberanía in the dry season and the wet season responded similarly to all the stimuli presented except the calls of *S. sila*.

## Discussion

Availability of prey to a predator depends on the predator's means of locating and capturing prey (Faure and Barclay 1992). In the case of eavesdropping, prey availability is determined largely by prey signaling behavior. Different predator populations have different prey species with different signals, and within a population, there is often seasonal variation in prey signaling behavior. We examined whether the responses of eavesdropping bats to prey sexual advertisement calls differed between populations with different available prey or between seasons within a population. In our population comparison, the bats that we tested were different in their responses to a number of prey cues. The Soberanía bats exhibited more ear motions and orientation to the speaker in response to complex calls and choruses of the túngara frog, *P. pustulosus*, than the bats from La Selva where *P. pustulosus* is absent. The La Selva bats, in contrast, exhibited more ear motions and orientation to the speaker to calls of a treefrog, *D. phlebodes*, and a Phaneropterine katydid species in the genus *Steirodon* than Soberanía bats although these prey species are present in both populations. There were also differences in the numbers of bats that flew to the speaker in response to each of these stimuli, but likely due to the small sample sizes, these differences were not statistically significant. Although it is a small sample, these results point toward population specialization in the behavior of this eavesdropping predator.

Population differences in response to prey cues and foraging behavior have been demonstrated for a number of species (e.g., snakes, Arnold 1977; spiders, Hedrick and Richert 1989; bumblebees, Ings et al. 2009; and crayfish, Pintor and Sih 2009). For these species, population differences in behavior have a substantial genetic component. Given the ability of *T. cirrhosus* to rapidly learn novel associations between prey cue and prey quality in captivity (Page and Ryan 2005, 2006), it seems more likely that the population specializations that we observed in *T. cirrhosus* are learned. Learned population differences in foraging behavior are not as well documented as genetic differences, but there is evidence from primates (capuchins, Panger et al. 2002; chimpanzees, Whiten et al. 1999; and orangutans, van Schaik et al. 2003). In some of these primates, foraging behaviors are socially learned. *T. cirrhosus* is capable of learning novel prey cues socially (Page and Ryan 2006; Jones et al. 2013), but learned population differences could also arise from individual trial and error learning.

*T. cirrhosus* could also learn to prefer calls with complex components. Previous studies have demonstrated that the bats in Soberanía and on nearby Barro Colorado Island preferentially approach complex *P. pustulosus* calls over simple calls (Ryan et al. 1982; Akre et al. 2011). La Selva bats, however, exhibited lower responses (in terms of ear motions and orientation to the speaker) to complex calls and choruses of *P. pustulosus* than to simple calls. When presented with novel stimuli, therefore, the *T. cirrhosus*, we tested, were not more responsive to more complex stimuli or to stimuli that represent multiple individuals calling (choruses). Our result conflicts with research from Ecuador where bats preferentially approached speakers broadcasting complex *P. petersi* calls even in a population where frogs do not make complex calls (Trillo et al. 2013). This discrepancy could be due to a number of factors. Our sample size was small, and the study in Ecuador quantified the number of passes that the bats made over speakers in the wild and was not able to determine how many individual bats made such passes. It is also possible that the preference for call complexity in Ecuador is because bats already have experience with simple calls of that prey species, which could be a prerequisite for a preference for call complexity. Bats in Soberanía may have learned their preference for complex calls after having higher capture success in approaching complex calls than approaching simple calls. Higher capture success could arise because complex calls are easier to localize (Page and Ryan 2008) or because frogs are more likely to make complex calls when they are at higher densities (Bernal et al. 2007), thereby providing more capture opportunities for a bat approaching a complex call.

If bats do learn to alter their foraging behavior to take advantage of available prey, we would also expect seasonal variation in response to prey cues as prey availability changes seasonally. In our seasonal comparison, we found that Soberanía bats oriented to a significantly higher proportion and flew significantly more often to the calls of the dry season breeding frog *S. sila* in the dry season when it was currently calling, but there was no seasonal difference in any bat response to calls of the wet season breeding frog *P. pustulosus*. We therefore demonstrate seasonal variation in bat response to some prey cues but not to others. It is clear from the bat responses in both the population and seasonal experiments that *P. pustulosus* is the preferred prey of all the stimuli offered to Soberanía bats. It is possible that this high preference for *P. pustulosus* results in a consistently high response to calls regardless of temporal availability. *S. sila*, in contrast, is less preferred than *P. pustulosus*. Bats may maintain a high response to preferred prey regardless of availability, but for less preferred prey, bat response is affected by availability.

Shifting foraging behavior with prey availability has benefits and risks. While it may enable access to novel food sources, trying novel food is always associated with the possibility that

such food could be unpalatable or otherwise dangerous to consume (many katydids have a strong bite). Whether or not to learn novel prey could therefore be viewed from the perspective of risk-prone or risk-averse foraging strategies (Stephens 1981). Risk prone is defined as a preference for high variability in food resources, whereas risk averse is a preference for low resource variability (Barnard and Brown 1985). High flexibility in foraging behavior with frequent shifts to novel prey that may or may not produce high rewards could be viewed as risk prone, whereas low flexibility could be categorized as risk averse. Whether animals engage in risk prone or risk averse foraging behavior can be affected by a number of factors, including body condition and age. Over the course of their lifetimes, individual bats may make many decisions about when to engage in risk-prone behavior and learn novel prey and when to be risk-averse and stick with prey that they have prior experience with. Current foraging success on known prey has been shown to affect social learning of novel prey (Jones et al. 2013), but this may be only one of the multiple factors that generate foraging flexibility.

There were a number of stimuli for which we found no population or seasonal difference in response. For all of these stimuli, bats exhibited a generally low overall response. In our population comparison, these included the treefrogs, *D. ebraccatus* and *S. phaeota*, and our control stimuli. These treefrogs may not be palatable, may be inaccessible, or may simply be low-preferred prey. It should also be noted that the call recordings used in this experiment were made in Soberanía. We do not know of geographic variation in the calls of these frog species, but it is possible that La Selva bats would have responded slightly differently if we used local recordings. If this was the case, we might expect lower responses in La Selva to nonlocal individual calls than in Soberanía where the calls were of local individuals, but the La Selva bats we tested were not significantly less responsive to any of these calls. It is therefore unlikely that the bat's low responses were due to the origins of the recordings. The low responses to the artificial control stimuli indicate no population or seasonal differences in response to novel acoustic cues.

Bat response to katydid calls was low across both seasons in Soberanía. Bats in La Selva, however, were very responsive to the calls of the *Steirodon* katydid, significantly more responsive in orientation and ear motions than bats in Soberanía. It is possible that the presence of *P. pustulosus* in Soberanía, in which bats in Soberanía exhibit a strong preference, may result in a decreased preference for alternative prey, such as katydids. Similarly, the presence of *P. pustulosus* in Soberanía may reduce the response to calls of the treefrog *D. phlebodes* that is present in both populations but to which bats in La Selva exhibited more ear motions and orientation toward the speakers.

In the population comparison, we found a significant difference in the proportion of the stimulus to which the bats moved their ears for the toxic toad, *R. marina*. We did not find significant differences for any of the other behavioral measures. This large, unpalatable toad species is abundant at both sites. The significant difference appears to be a product of the very low variance in response by the Soberanía bats. La Selva bats, on average, did not move their ears to a larger proportion of the toxic toad call than to the artificial tone. No bats in either population flew to approach the stimulus. This example highlights the interesting complexity that emerges from examining three different behavioral measures. Each of these behaviors indicates a different step in the range of bat responses between first detecting the stimulus and attack. High levels of ear motions appear to be required for flight to the speaker but is not necessarily predictive, as indicated by some bats that moved their ears to large proportion of the artificial tone but did not fly to the speaker. Analysis of multiple behaviors gives a more complete picture of bat response.

The foraging behavior of *T. cirrhosus* arises from auditory specializations for detecting frog calls (Bruns et al. 1989) and glandular specializations for consuming frogs (Tandler et al. 1996) most likely in combination with the honing of preferences through learning to associate particular cues with their prey. Our study does not rule out that bat populations have diverged genetically to produce the observed population differences in response to prey cues, and considerable genetic variation in at least one gene (CO1) has been demonstrated for *T. cirrhosus* over its range (Clare et al. 2011). Given the learning abilities demonstrated for *T. cirrhosus*, however, it is likely that learning plays an important role in the development of bats' associations between prey cue and prey quality. The flexibility in response to prey cues demonstrated experimentally for *T. cirrhosus* may not only enable bats to focus their attention on their preferred prey but also take advantage of novel prey as it becomes available. Whether or not individual bats learn novel prey may be affected by a number of factors including body condition and age. The circumstances under which bats undertake the risk of approaching novel prey are in need of further investigation. By demonstrating natural variation in bats' responses to some prey cues but not to others, our results indicate that prey availability is not the only factor affecting bat response to prey cues. This highlights the complexity of the foraging behavior of this eavesdropping predator.

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