



# Habituation and ecological salience: insights into the foraging ecology of the fringed-lipped bat, *Trachops cirrhosus*

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## Abstract

Animals are often confronted with more sensory stimuli than they can attend to, and so should pay attention to stimuli that are relevant to them and habituate to those that are not. We investigated attention in the fringe-lipped bat, *Trachops cirrhosus*, by playing repeated prey sounds to bats in a habituation-discrimination paradigm. We measured two behavioral responses: initial response and habituation rate, and also tested whether the bats discriminated between the different sounds. We found that bats habituated more quickly to sounds of unpalatable prey species, but contrary to our expectation, a bat's initial response was unrelated to prey palatability. Furthermore, discrimination was only detectable when bats became strongly habituated and they were less attracted to the habituated sound compared to the subsequently presented sound in the stimulus pair. Our results support the idea that in nature, many sounds can draw an animal's attention initially, but only sounds of ecological significance and perceptual salience maintain an animal's attention over time.

## Significance statement

Habituation is an almost ubiquitous way that animals filter environmental information, but is often overlooked in behavioral experiments. Animals may habituate faster to sounds that are unlikely to affect their lives and more slowly to ones that are associated with food or threats. We studied the predatory bat *Trachops cirrhosus* that hunts using prey sounds. We presented bats with prey and non-prey sounds and observed their responses over time. We found that although bats responded similarly to all the sounds at their onset, they paid attention longer to sounds from palatable prey and habituated quickly to sounds from inedible animals. This species initially attends to new sounds that it hears, but habituates in a way that helps it selectively attend to important stimuli.

**Keywords** Habituation · Discrimination · Bat · *Trachops cirrhosus* · Attention · Foraging

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## Introduction

Attention is a limited resource: there are too many sensory inputs for brains to interpret and attend to simultaneously (Dukas 2002). Animals should selectively attend to stimuli that help them make decisions and tune out those that are not relevant. Habituation, the process of decreased responsiveness to a repeated stimulus, assists in the filtering process and has been found in all animals studied to date, and even in protists (Tsang 2012) and plants (Abramson and Chicas-Mosier 2016). The speed of habituation can be used to assess what stimuli are important to animals, wherein those with higher ecological salience will retain an individual's attention longer (Wytenbach and Hoy 1999; Ratcliffe et al. 2011). We can also learn more about the process of habituation itself by observing how wild-caught animals habituate to stimuli with known ecological relationships to that population or species.

There are many reasons that a stimulus, such as a new sound, could initially attract or hold attention. A stimulus may be better able to elicit initial attention and hold this attention over time if it stimulates an animal's sensory system with high intensity, is complex, or has high contrast with the environment (Shettleworth 2010; Domjan 2015). A stimulus could also attract or sustain attention because it is associated with an outcome of high ecological relevance, such as danger, sex, or food (Shettleworth 2010). For example, a branch falling may attract attention simply because it is loud, but even very quiet sounds, like rustling leaves, may also hold attention if they may have come from the movement of a predator. These factors may influence initial and sustained attention to different degrees. It is also interesting to know what cues animals discriminate—what cues they treat as different from one another (Domjan 2015)—to understand how animals perceive and categorize stimuli. Here, we investigate initial attention, habituation, and discrimination of frog sounds in a predatory bat.

The fringe-lipped bat, *Trachops cirrhosus* (Phyllostomidae), is a Neotropical bat that hunts by eavesdropping on the acoustic mating signals emitted by its frog and insect prey (Page and Jones 2016). To do this, *T. cirrhosus* has to identify and selectively attend to prey calls amid the cacophony of other sounds in the forest at night (Lang et al. 2005). Selective attention to prey calls and rapid habituation to the sounds of unrewarding animals may be critical for efficient foraging. The diet and foraging strategies of *T. cirrhosus* are well known (Page and Jones 2016), making it an excellent candidate to investigate how predators vary in initial responses and habituation rates to stimuli with differing ecological valences.

We used a habituation-discrimination paradigm that measures initial attentiveness to, continued level of interest in, and ability to discriminate between stimuli to test how *T. cirrhosus* responds to the mating calls of frog species that span an ecological spectrum: (i) a frog that is palatable and relatively easy to capture, *Physalaemus* (= *Engystomops*) *pustulosus*; (ii) a frog that is palatable but difficult to capture, *Dendropsophus ebraccatus*; and (iii) a frog that is toxic, *Rhinella alata*. These three sympatric frogs are common and similarly sized (Ibáñez et al. 1999). *P. pustulosus* and *D. ebraccatus* are both known components of *T. cirrhosus*' diet (Tuttle and Ryan 1981; Trillo et al. 2016). *P. pustulosus* calls from the surface of small bodies of water and is likely relatively easy for this bat to capture (Arlettaz et al. 2001; Siemers et al. 2005). *D. ebraccatus* calls from cluttered vegetation (Ibáñez et al. 1999) and thus is likely more difficult to acoustically localize and physically capture. *R. alata* is a toad that secretes toxins that make it unpalatable to bats (Page et al. 2012). We predicted that *T. cirrhosus* would initially respond more strongly, and subsequently habituate more slowly, to the calls of the two palatable species than to the calls of *R. alata*, because sustained attention to the palatable calls may help bats localize

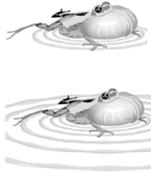
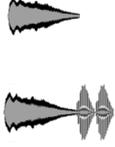
a meal. Furthermore, we predicted that *T. cirrhosus* would habituate more slowly to *P. pustulosus* than to *D. ebraccatus* because of the probable difference in the likelihood that a response to these calls would result in a successful capture.

We also played reversed *P. pustulosus* and *R. alata* stimuli. Reversed acoustic stimuli retain many of the acoustic properties of the original stimuli (e.g., frequency, intensity, call rate), but differ in the temporal presentation of these acoustic components. Frog calls with similar acoustic properties may be more likely to have similar ecological relationships to the bats (i.e., signifying food or danger). Indeed, frog-eating bats are known to generalize from known acoustic stimuli to unknown ones, and respond with approach and attack to novel stimuli that sound to human ears similar to stimuli that bats know to signal prey (Ryan and Tuttle 1983; Jones et al. 2013b). But we do not know which acoustic parameters the bats attend to when making these prey categorization decisions. By presenting bats with reversed calls, we can ask whether temporal presentation is critical to bat categorization of prey sounds, or if it is sufficient to have the correct frequencies, intensity, and call rate present, regardless of their temporal order.

*P. pustulosus* can vary the complexity of their calls: males of this species can add 1–7 “chucks” as an ornament to the end of their calls (Fig. 1a). The addition of chucks makes the calls more attractive to female frogs (Ryan 1985) and easier to localize for bats (Page and Ryan 2008). We know that the bats recognize both simple and complex calls as indicating the presence of a potential meal (Page and Jones 2016) and can discriminate between them (Akre et al. 2011). Given a choice, bats prefer complex calls (Ryan et al. 1982). However, we do not know whether bats habituate at the same rate to the two types of calls. Since bats prefer complex calls and find them easier to localize, we predicted that they would initially respond more to the complex calls and habituate to them more slowly than the simple calls. Alternatively, since both call types signal the same prey item, it is possible that both calls would attract and hold a bat's attention equally. Since we know that bats can discriminate these call types, comparing them in a habituation-discrimination paradigm also serves as a control to check whether our discrimination tests are sensitive.

In this study, we tested the following hypotheses. One, that if initial attention and habituation both serve to help animals filter out irrelevant stimuli and attend to important ones, then *T. cirrhosus* should initially react more strongly and subsequently habituate more slowly to positively associated sounds, such as complex *P. pustulosus* calls, than they would to neutral or negatively associated sounds, like reversed calls or *R. alata* calls. Two, that attentional filtering takes place after animals initially perceive and respond to stimuli. In this case, only initial response should be unrelated to the ecological relevance of a sound, but habituation should still be slower with more ecologically relevant sounds. Finally, that neither initial response nor habituation are related to ecological relevance.

**Fig. 1** Images (left), waveforms (center), and spectrograms (right) of the six acoustic stimulus categories presented to *T. cirrhosus*

	Species	Image	Call Waveform	Call Spectrogram
a	<i>Physalaemus pustulosus</i>			
b	<i>Dendropsophus ebraccatus</i>			
c	<i>Rhinella alata</i>			
d	Reversed <i>P. pustulosus</i>			
e	Reversed <i>R. alata</i>			

## Methods

### Experimental animals and arena

We tested *T. cirrhosus* ( $n = 13$ , two non-reproductive females and 11 males) from February through June 2014. All applicable international, national, and institutional guidelines for the use of animals were followed. All bats were adults, and while it is not currently possible to age bats precisely once they have reached adulthood, *T. cirrhosus* in the area have their pups in and around June, meaning all individuals tested had experienced at least one wet and dry season. Since all the frogs we used are common in the area, and make loud choruses when they are breeding, all of the bats we used should have been familiar with these frogs' calls prior to experimentation. Bats were captured in Soberanía National Park near Gamboa, Panamá, using mist nets and housed individually in a large open-air flight cage (5 m  $\times$  5 m  $\times$  2.5 m). They were fed bait-fish and provided with water ad libitum. No bats were kept for more than two nights. At the end of testing, bats were individually marked with a passive integrated transponder tag and released at site of capture.

The flight cage was set up with a cloth roost in one corner, and the experimenters in the opposite corner. We broadcast acoustic stimuli at 80 dB SPL (re. 20  $\mu$ P) at 1 m, the

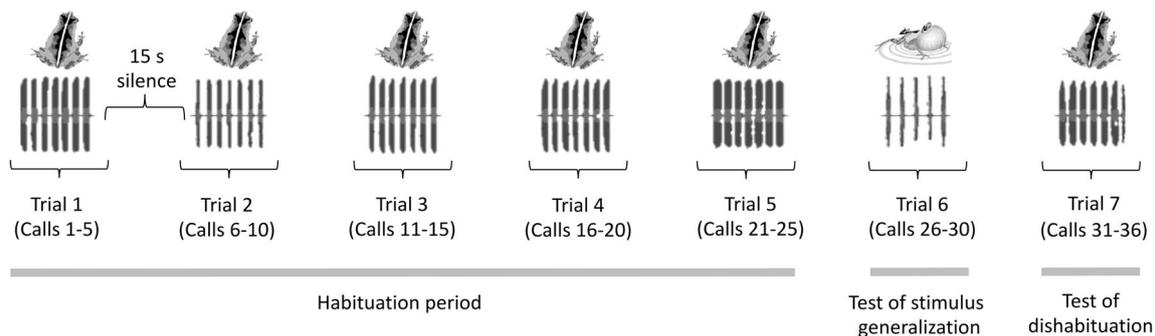
approximate call intensity of *P. pustulosus* in the wild (Ryan 1985), through a Fostex FE103En speaker placed in the center of the flight cage, connected to a Pyle Pro PTA2 amplifier and a laptop. Trials were recorded with a Sony DCR-SR45 Handycam camcorder and illuminated with a 25-W red light-bulb and four CMvision infrared lights.

Each bat was tested the night after their capture. At 1815 h, we fed each bat 2 g of fish, and then left it undisturbed for an hour before starting experiments at approximately 1930 h. We provided no food during testing. While we attempted to standardize motivation by controlling food intake and time of testing, at times individual bats became generally unresponsive. We tested for general arousal level before each 160-s test (described below) by presenting the bat with a loud, broadband click made by depressing a metal lid and allowing it to pop out. If the bat did not ear-twitch to this sudden sound, we reasoned that it was in a state of low arousal. In such instances, we paused the experiment, fed the bat 2 g more fish, and then left it undisturbed for an hour before resuming the experiment. If a bat had already been given an hour break, we continued tests even if the bat did not respond to the noise but noted the bat's response. One male bat was removed from the dataset because it did not respond to any of the stimuli, thus reducing our effective sample size to 12.

### General paradigm

To test each bat’s (i) initial attentiveness to, (ii) continued level of interest in, and (iii) ability to discriminate between our acoustic stimuli, we used a fixed trial, integrated habituation/discrimination paradigm. This paradigm relies on the strongly supported assumption that habituation is stimulus specific and will be disrupted if an animal is presented with a strong second stimulus that it categorizes as distinct from the first (Thompson and Spencer 1966; Rankin et al. 2009). In our version (Fig. 2), the subject was repeatedly presented with a given stimulus over five 10-s trials, each trial was separated by 15 s of silence (*habituation period*). The stimulus was then changed in the sixth trial (*test of stimulus generalization*). If the subject categorized this second stimulus as different, the animal should not have been habituated to it and may have responded more strongly (indicating a lack of stimulus generalization and thus discrimination). Following this, in the seventh trial, we presented the animal with the original stimulus again (*test of dishabituation*), to further test discrimination (Thompson and Spencer 1966; Wytenbach and Hoy 1999; Rankin et al. 2009; Ratcliffe et al. 2011). If the individual’s response to the habituating stimulus (trial 7) *increased* compared to the trial at the end of the habituation period (trial 5), it can be said that the subject has discriminated between stimuli. While this integrated design allows two means of determining stimulus discrimination (Wytenbach and Hoy 1999), we did not include a control condition where the sixth trial was silent (see Rankin et al. 2009). This makes the “test of dishabituation” the weaker test as an increased response could indicate spontaneous recovery rather than dishabituation.

For each comparison, we used both stimuli as habituating and test of generalization stimulus (i.e., AAAAABA and BBBBAB). Each subject was tested in 10 different 160-s habituation-discrimination tests in a row, with 2 min between each test. To control for potential ordering effects, test order was pseudo-randomized for each bat by following the rule that successive tests could not have the same habituating stimulus. To avoid pseudo-replication, the sound files were drawn at random for each trial from a bank of calls from 10 individual frogs of each species.



**Fig. 2** Schematic of experimental design. During the habituation period, we presented one stimulus category for 10 s, followed by 15 s of silence. We repeated this for five trials. On the sixth trial, we tested for stimulus

### Interspecific experiment

In the interspecific experiment, we tested bat responses to different frog species. We used calls from *P. pustulosus*, *R. alata*, and *D. ebraccatus*. Trials 1 through 5 and 7 each consisted of calls from a different individual of the same species, and trial 6 was from a random individual from a different species. To approximate natural call rates, *P. pustulosus* and *D. ebraccatus* calls were broadcast at a rate of one call every 2 s; *R. alata* was played at a rate of one call every 1.5 s (Ibáñez et al. 1999). We also played the bats reversed *P. pustulosus* and *R. alata* calls; reversed calls were broadcast at the call rates listed above for each species. We did not reverse all of the calls because we did not want to present bats with too many tests, such that they would habituate to the overall experimental paradigm.

### Intraspecific *P. pustulosus* experiment

In the intraspecific *P. pustulosus* experiment, we tested if bats discriminated between calls of different complexity (zero-chuck versus two-chuck calls). Here, all the calls within a test were from the same individual male frog, and the only difference between the habituation stimulus and test of generalization stimulus was whether the calls had zero or two chucks (Fig. 1a).

### Behavioral scores

We quantified the behavioral responses using Solomon coder version beta 14.10.04. To minimize observer bias, videos were scored by individuals that were blind to the stimuli and experimental predictions. We scored bats on their highest behavioral response to the stimulus per trial on an ordinal scale: 0—no response, 1—small ear twitches in time with the sound, 2—large ear twitches in time with the sound, 3—approach to the speaker, and 4—lands on the speaker (for a video example, see Online Resource 1). *T. cirrhosus* twitches its ears in response to sounds as a way to localize them, and ear-twitching is a good indicator of interest in this species (e.g., Ryan et al.

generalization by presenting a 10-s stimulus from a different category. On the seventh trial, we tested for dishabituation by presenting a 10-s stimulus from the first category

1983; Falk et al. 2015). During small ear twitches, only the bat's ears move, whereas with large ear twitches, the bat's entire head moves. We analyzed behavior as three different categories: *initial response*, *habituation*, and *discrimination*. To determine *initial response*, we recorded the highest response to the first trial of a test. As a metric of *habituation*, we calculated the slope of the change in response over the first five trials for each stimulus category. To determine *discrimination*, we compared the response in trial 5, the last trial of the habituation period, first, to the response in trial 6 and, second, to the response in trial 7.

## Statistical analysis

We analyzed our data in R (R Core Team 2016). We used cumulative link models and mixed models (CLMs and CLMMs) (ordinal package; Christensen 2015) to model our data and used the lsmeans package (Lenth 2016) to generate estimates, confidence intervals, and significant differences for the models. CLMMs are appropriate for data that are ordinal and that include multiple observations of the same individual, such that subject is a random effect (Agresti 2010). Analyses are further described below.

### Initial response

Initial response was our response variable and was scored as the highest interest score in the first trial, the first time a bat was exposed to a given stimulus in captivity. Treatment was our predictor variable. Neither subject nor test order explained significant variation in the models as random effects (likelihood-ratio test,  $P=0.25$  and  $P=1$  respectively), so we proceeded to use a CLM. We calculated least-squared means and used Tukey honestly significant difference (Tukey HSD) post hoc tests to determine estimates and differences between stimulus categories.

### Habituation

We calculated habituation speed as the estimate for the interaction between stimulus category and trial number over the habituation period, using a CLMM. Interest score was the response variable. The predictor variable was the interaction between stimulus category and trial number. The best random effects structure was test order nested within subject (likelihood-ratio test, compared with next best model,  $P < 0.01$ ). This model tests how the change in the bats' responses over a test varied by stimulus category (i.e., did bats habituate to different stimuli at different rates?), controlling for test order and individual bat. We calculated estimates and conducted pairwise comparisons using Tukey HSD post hoc tests.

## Discrimination

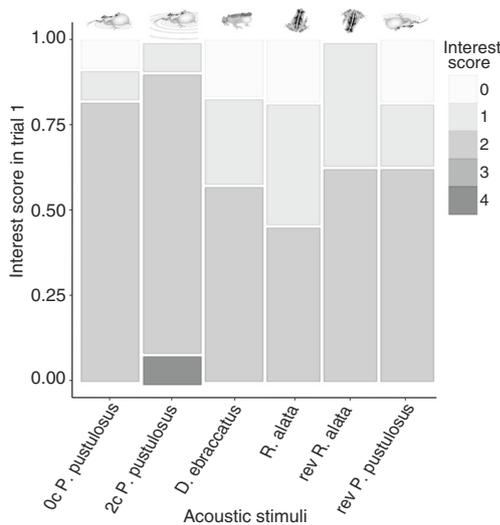
To measure if bats discriminated between stimulus categories, for each test, we compared bat interest score in the fifth trial of the habituation period to scores in the test of stimulus generalization (sixth trial) and then again to scores in the test of dishabituation (seventh trial). We used Wilcoxon matched-pairs signed-rank post hoc tests with Holm corrections for multiple comparisons to test for differences between the scores of these two groups.

## Results

### Interspecific experiment

There were no significant differences in initial response to any of the different species, including the reversed calls (Tukey method for comparing a family of six estimates:  $P \geq 0.05$ ; Fig. 3, Online Resource 2). We measured the change in interest score over the habituation period as a measure of habituation (Fig. 4, Online Resource 3). Bats habituated most steeply to the *R. alata* (95% CI for slope =  $-0.98$  to  $-0.54$ ), and this trend was not significantly different from the response to the reversed *R. alata* (95% CI =  $-0.85$  to  $-0.44$ ). Bats had intermediate responses to the zero- and two-chuck *P. pustulosus* calls (95% CIs =  $-0.50$  to  $-0.21$  and  $-0.43$  and  $-0.04$ , respectively). Bats did not habituate significantly to *D. ebraccatus* at all, as the 95% confidence interval for the slope crossed above zero (95% CI =  $-0.21$  to  $0.20$ ), although this was not significantly different from the response to the two-chuck *P. pustulosus*. Habituation rates to the reversed *R. alata* and *P. pustulosus* calls were not different from habituation rates to their respective forward calls or one another (reversed *R. alata*: 95% CI =  $-0.85$  to  $-0.44$ , and reversed *P. pustulosus*: 95% CI =  $-0.57$  to  $-0.17$ ).

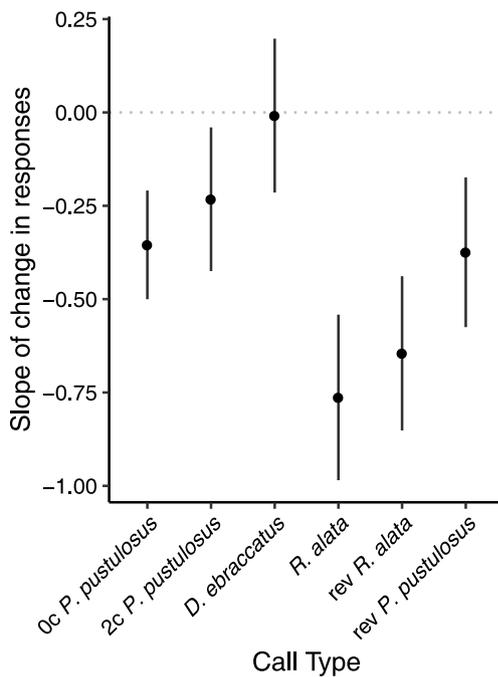
We determined that bats discriminated between stimuli if they responded significantly *less* in the last trial of the habituation period (the fifth trial) than to *either* the test of generalization (the sixth trial and other category of stimulus) *or* the test of dishabituation (the seventh trial, the original stimulus category) (Fig. 5, Table 1). We detected discrimination between *R. alata* and zero-chuck *P. pustulosus* when *R. alata* was the habituated stimulus (trial 6 > trial 5, Wilcoxon signed-rank:  $n = 11$ ,  $V = 0$ ,  $P = 0.024$ ; Table 1) and discrimination between reversed *R. alata* and reversed *P. pustulosus* when the reversed *R. alata* was the habituated stimulus (trial 6 > trial 5, Wilcoxon signed-rank:  $n = 11$ ,  $V = 4$ ,  $P = 0.029$ ; Table 1). In each of these cases, we did not detect discrimination when the other stimulus in the pair was the habituated stimulus (e.g., AAAAABA but not BBBBABB). We did not detect discrimination between the *D. ebraccatus* and *P. pustulosus* stimuli ( $P > 0.3$ ), or between any of the other interspecific comparisons (Table 1).



**Fig. 3** Initial behavioral responses of the bats to the six acoustic stimuli used in this study: túngara frog (*Physalaemus pustulosus*) calls with 0 chucks (0c) and 2 chucks (2c); hourglass tree frog (*Dendropsophus ebraccatus*) calls; leaf litter toad (*Rhinella alata*) calls; and the reversed calls of *R. alata* and *P. pustulosus*. Behavioral responses were scored on an ordinal scale: 0—no response, 1—small ear twitches, 2—large ear twitches, 3—approach to the speaker, and 4—lands on the speaker. The size of the boxes indicates the proportion of bats that had that response as their highest response to a given stimulus

**Intraspecific experiment**

There was no significant difference in initial response to the two-chuck and zero-chuck *P. pustulosus* calls (Tukey method



**Fig. 4** Estimates of the slope of the change in the interest score over the habituation period. Vertical lines represent the 95% confidence intervals for the slope, and circles indicate the estimated means. See Fig. 3 for a description of call types

for comparing a family of six estimates:  $P > 0.874$ ; Fig. 3, Online Resource 2). There was also no significant difference in the habituation rates ( $P = 0.759$ ; Fig. 4). There was no evidence of discrimination between the call types when the two-chuck call was the habituated stimulus (Wilcoxon signed-rank:  $n = 12, V = 1.5, P = 1$ ), but there was evidence of discrimination when the one-chuck call was the habituated stimulus (trial 6 > trial 5, Wilcoxon signed-rank:  $n = 12, V = 0, P = 0.029$ ).

**Sex differences**

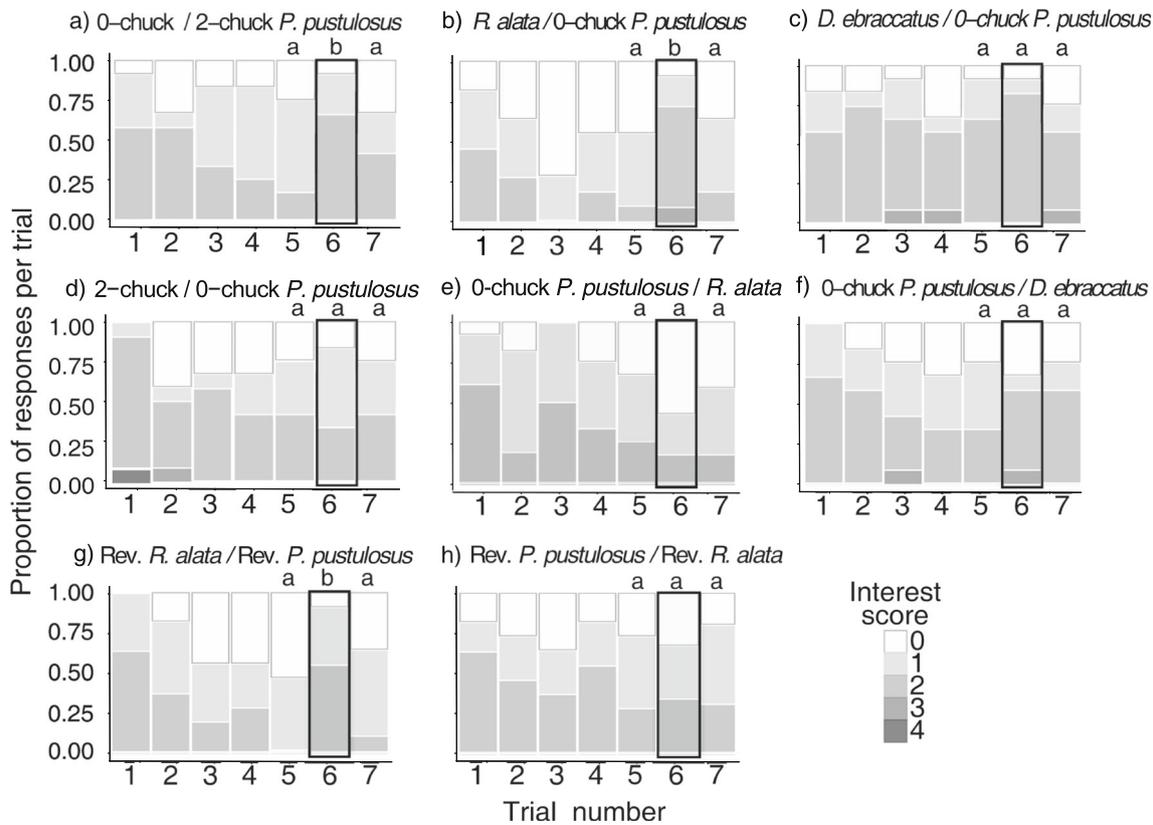
While we tested too few females ( $n = 2$ ) to perform statistical tests comparing males and females, we saw no evidence of sex-based differences in behavior. That is, the responses of the female bats did not obviously cluster together in comparison to the males. Interestingly, however, the only individual that landed on the speaker during one of the trials was female. Sex differences in initial response and in habituation to a range of acoustic stimuli would be an interesting question to pursue in a future study.

**Discussion**

Our results suggest that *T. cirrhosus* may habituate adaptively to frog sounds in its environment. Bats habituated fastest to sounds that were either ecologically unimportant or potentially dangerous to them, and slowest to those that signaled palatable prey. Below, we discuss the results of each experiment in detail, the broader utility of this paradigm in behavioral ecology, and the adaptive function of habituation more generally.

**Interspecific experiment: initial response**

We had initially predicted that the most biologically salient, edible frog stimuli would induce the strongest initial response. Thus, we predicted that bats would respond strongly to the *P. pustulosus* stimuli when they first heard them, because these common frogs call from open puddles and are relatively easy for the bats to capture in nature. We predicted bats would respond somewhat less to the calls of *D. ebraccatus*, which vocalizes from within vegetation but is edible, and least to calls of the inedible toad *R. alata*. We found, however, no strong differences in the bats' initial responses to any of these acoustic stimuli (Fig. 3). Animals may initially respond to the onset of a sound for many reasons. On a given night, any new stimulus may signal a change in the environment. It may take an animal time to evaluate this change, or to recall an association with this stimulus from memory. As such, it may be beneficial for an individual to initially attend to any new stimulus. The acoustic characteristics of a new sound may also control attention. We predicted a low initial response to *R. alata* because it is not a viable prey item, but *R. alata* has acoustic characteristics that



**Fig. 5** Proportions of bats' peak interest scores to the acoustic stimuli in each of seven trials in a test. Black rectangles indicate the test of stimulus generalization, where playback consisted of a different stimulus. We tested whether bats discriminated between stimuli by comparing responses in the last trial of the habituation period (trial 5) to those in the test of stimulus generalization (trial 6) and the test of dishabituation

(trial 7). Bats showed discrimination if their response in the sixth or seventh trial was higher than that in the fifth trial. Lettering indicates whether there were significant differences; trials with the same letter were not significantly different. See Fig. 3 for a description of acoustic stimuli

**Table 1** Wilcoxon matched-pairs signed-rank *P* values for discrimination between the interest scores in the fifth (last trial of the habituation period) and sixth (test of generalization), and fifth and seventh (test of dishabituation) trials of each acoustic stimulus; determination of discrimination on right. *P* values were adjusted with Holm corrections. Trials marked with (\*) were significant at the  $\alpha = 0.05$  level

Treatment	Wilcoxon matched-pairs signed rank contrasts		Discrimination?
	Trials	<i>P</i> value	
<i>R. alata</i> /zero-chuck <i>P. pustulosus</i>	5–6	0.024*	Yes
	5–7	0.588	
Reversed <i>R. alata</i> /reversed zero-chuck <i>P. pustulosus</i>	5–6	0.029*	Yes
	5–7	0.345	
<i>D. ebraccatus</i> /zero-chuck <i>P. pustulosus</i>	5–6	0.692	No
	5–7	0.692	
Reversed zero-chuck <i>P. pustulosus</i> /reversed <i>R. alata</i>	5–6	1	No
	5–7	1	
Zero-chuck <i>P. pustulosus</i> / <i>D. ebraccatus</i>	5–6	0.374	No
	5–7	0.298	
Zero-chuck <i>P. pustulosus</i> / <i>R. alata</i>	5–6	0.259	No
	5–7	0.586	
Zero-chuck <i>P. pustulosus</i> /two-chuck <i>P. pustulosus</i>	5–6	0.029*	Yes
	5–7	0.608	
Two-chuck <i>P. pustulosus</i> /zero-chuck <i>P. pustulosus</i>	5–6	1	No
	5–7	1	

*T. cirrhosus* is known to prefer: the calls have a long pulse duration (Fugère et al. 2015), have strong temporal modulation (a trill) (RAP, unpublished data), and are broadband (Page and Ryan 2008). Together, these results suggest that *T. cirrhosus*' initial attention to these sounds was driven by the sounds being new on a given night, rather than by previous associations with edible or inedible frogs. However, while the overall bat response to the acoustic stimuli was similar, the two-chuck *P. pustulosus* stimulus is the only one that was ever attacked in the first test of a trial, even if by only one bat. So, while the bats attended to all the stimuli, only this high-value stimulus elicited a strong response.

### Interspecific experiment: habituation

Bats habituated most rapidly to the stimulus representing unpalatable *R. alata* and to the biologically irrelevant reversed *R. alata* stimulus. They habituated least rapidly to the *D. ebraccatus* and the two-chuck *P. pustulosus*, suggesting that these stimuli best held the bats' attention. These results suggest that habituation filtered initial attention away from stimuli that are not ecologically relevant to the bats. Acoustic habituation rates appear to be an effective assay to test for species that are prey items for *T. cirrhosus* and may be a useful assay for testing prey inclusion for other gleaning bats, and perhaps other predators with less well-known diets.

There were not significant differences between the slopes of habituation to *D. ebraccatus* calls and the complex *P. pustulosus* calls (i.e., two-chuck calls) (Figs. 4 and 5c, d). Bats therefore may not prefer *P. pustulosus*, despite it being easier to capture. Field observations of bats approaching the mating calls of *D. ebraccatus* and *P. pustulosus* with one chuck found no difference in attraction rates, suggesting that bats may indeed not prefer one over the other (Trillo et al. 2016).

It is interesting that the bats habituated to the reversed stimuli (*P. pustulosus* and *R. alata*) at the same rate as the forward ones (Figs. 4 and 5g, h). We know that *T. cirrhosus* is much more permissive than female *P. pustulosus* in how much a frog call can be changed and still responded to as a *P. pustulosus* male. *T. cirrhosus* still approaches calls that fall out of "recognition space" for female frogs: female *P. pustulosus* frogs do not approach a chuck alone (a sound that almost never occurs in nature) (Ryan 1985), but *T. cirrhosus* does (Jones et al. 2013a). It makes sense for predators to have flexible acoustic templates, as prey choices can be more catholic than mate choices—a bat that flies to a novel variant of a familiar frog call may find a new food source, whereas a female frog approaching the same may waste her eggs on a heterospecific male. Accordingly, *T. cirrhosus* acoustic associations with frog calls could be for the call components in general, not necessarily in the correct temporal order. A reversed call that still has similar frequencies and structure might possess sufficient acoustic elements to attract attention.

### Interspecific experiment: stimuli discrimination

Trials were designed to test whether a habituation-discrimination paradigm is effective for determining when bats discriminate or generalize between stimuli. For the interspecific combinations tested, *D. ebraccatus*/*P. pustulosus* and *R. alata*/*P. pustulosus*, there is either research or anecdotal evidence that the bats can discriminate between them, and that they use these distinctions to make foraging decisions (Ryan and Tuttle 1983; Rhebergen et al. 2015). Therefore, if we observed evidence of discrimination between *D. ebraccatus* and *P. pustulosus* in our habituation/discrimination paradigm, we could then better trust comparisons between sounds that we do not know if bats can discriminate. For example, we could compare *D. ebraccatus* and *D. microcephalus*, a smaller (less profitable) congener that has a similar call.

Overall, we only detected discrimination in tests where (1) bats had habituated to the primary stimulus to the point that almost no bat responded at all in the fifth trial of the test (Fig. 5b, g) or (2) a less attractive stimulus was used in the habituation period, and there were large differences in the previously established attractiveness of the two stimuli (Fig. 5a, b). As such, we often only detected discrimination in one direction in a stimulus pair, such as with the *R. alata*/zero-chuck *P. pustulosus* comparison (Fig. 5b, e). As a result, our experiments may represent an overly conservative measure of these bats' ability to discriminate between acoustic stimuli.

We did not detect discrimination between *D. ebraccatus* and *P. pustulosus* calls for either the habituated *D. ebraccatus* or the habituated *P. pustulosus* stimuli. Because (i) the calls from these two frogs are quite different (Table 1), (ii) the bats should all have had experience with both these call types in the wild, and (iii) the habituation rates to the two stimuli were different; this likely reflects limitations in the experimental design rather than a biological phenomenon. Bats had similar initial responses and did not habituate much to either stimulus during the trials (8 of 12 subjects did not habituate to *D. ebraccatus* at all). This made it difficult to detect a difference in response between the last habituation trials and the test of stimulus generalization. If we had presented the habituation stimulus for longer, so that bats were more habituated to it, then we may have detected discrimination. However, we used a fixed number of presentations between experiments and stimulus categories to allow for comparison in habituation rates.

Supporting the above interpretation, we only detected discrimination between *R. alata* and *P. pustulosus* when non-preferred, unpalatable *R. alata* was the habituated stimulus (Fig. 5b). By the fifth trial, most bats were thoroughly habituated to *R. alata* with responses near zero, so the increase in response to *P. pustulosus* when the stimulus changed was detectable by our statistical tests. These calls are very different acoustically, and it is important that bats do not mistake these

two frogs, as one is edible and the other toxic. That is, this discrimination has functional consequences in nature.

### Intraspecific experiment: initial interest and habituation

We did not detect a significant difference in the initial interest or rate of habituation between the *P. pustulosus* stimuli (Figs. 3, 4, and 5d, e). This contrasts with previous studies with *T. cirrhosus*, which found both a preference and increased localization performance for complex *P. pustulosus* frog calls compared to simple ones (Tuttle and Ryan 1981; Page and Ryan 2008; Akre et al. 2011). Perhaps attention and preference are not tightly correlated: bats prefer to fly to complex calls, but they may recognize that simple or complex calls signify the same prey item. Thus, attentionally, both call types may hold bats' interest equally well.

### Intraspecific experiment: discrimination

Like the interspecific comparison, there is strong previously reported evidence that bats discriminate between *P. pustulosus* complex and simple calls and use this discrimination to make foraging decisions (Ryan et al. 1982; Page and Ryan 2008; Akre et al. 2011). We detected discrimination between the two-chuck complex *P. pustulosus* and the simple, zero-chuck *P. pustulosus* stimuli when the simple one was the habituated stimulus, but not in the other direction (Fig. 5a, d; Table 1). Bats did not habituate much to the complex stimulus, so the response to the fifth trial of the complex stimulus was still higher than the initial response to the simple stimulus. Although the bats can discriminate between these two call types, they may generalize between them under some conditions.

## Conclusions

Our experimental results suggest that, as hypothesized, habituation works adaptively: bats habituate more quickly to stimuli that do not signify food in nature or that could be risky if approached. They habituate more slowly to stimuli that signify palatable prey. The rate at which bats habituated was more reflective of these biological relationships than the bats' initial responses to the stimuli. After eliciting initial attention, we found that only stimuli with biological importance maintained the bats' interest. While initial and sustained attention are closely related and are interdependent processes (Heilman et al. 2011), their underlying mechanisms may differ. Initial attention may be a more general response to any change in the environment, whereas habituation rates may reflect secondary processes in which animals use previous associations with incoming stimuli to evaluate their importance.

There are often sudden, loud sounds in the jungle. In order to eat, and not be eaten, animals have to quickly categorize and ignore the sounds that do not matter in order to focus on and respond to the ones that do.

Our tests of discrimination were conservative. To allow for inter-condition comparison, we broadcast the stimuli for a set amount of time before we switched them. We determined the duration of this set amount of time during our pilot work. As a result, for some subjects and stimulus categories, we switched the stimulus before habituation was strong enough to detect a change in response. Future experiments could either err toward having a longer habituation period or switch the stimulus flexibly based on the animal's behavior (a technique that has other considerations, see Colombo and Mitchell 2009).

Our study provides further support that habituation is an adaptive mechanism for filtering attention toward salient stimuli. We argue that habituation should be considered as a response variable when designing experiments. Habituation rates are an effective method for elucidating the stimuli that are important to animals in foraging and in other contexts.

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**Data availability** The data generated in the current study are available on github at [https://github.com/maydixon/Attn\\_Project/blob/master/Hab\\_and\\_eco\\_sal.csv](https://github.com/maydixon/Attn_Project/blob/master/Hab_and_eco_sal.csv).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All experiments were licensed and approved by the Smithsonian Tropical Research Institute (IACUC no. 2014-0101-2017) and by the Government of Panamá (ANAM: SE/A-9-14).

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