The effect of white noise on behavioral and flight responses of blue-tailed skinks

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Supplementary Materials

Materials and Methods

FID

We used flight initiation distance (FID), the distance between a predator and prey when the prey flees (Ydenberg and Dill 1986) as a measure of risk. As risk of mortality increases (correlated chiefly with predator approach velocity, as well as predator size), prey flight distance increases (Ydenberg and Dill, 1986), allowing us to estimate an individuals' perception of risk. To mimic anthropogenic noise, we conducted playback trials with 0 – 10 kHz white noise, which had broad spectrum masking properties, as well as the potential to distract subjects by disrupting auditory cues. The white noise was broadcasted at 95±1 dB (measured 1 m from the speaker), blocking potential information from other environmental sounds. We predicted that if masking blocks biologically important information about predation risk, then individuals exposed to white noise will increase their antipredator vigilance and FIDs (look more, escape earlier). If, however, noise acts as a distractor, then animals would unknowingly allow closer approach (shorter FID) while still exhibiting vigilant behavior, as a result of an inability to focus their attention on an approaching threat (look more, escape later).

Blue-tailed Skinks

Blue-tailed skinks are widely abundant across the island of Mo'orea, French Polynesia. Prior work has demonstrated that other closely related species of skinks use hearing for risk assessment (Fuong et al. 2014; Yan et al. 2019) and that they react to alarm calls of the non-predatory red-vented bulbul (*Pycnonotus cafer*; Fuong et al. 2014), indicating an association between auditory signals and anti-predator behavior. Because the frequency range of the red-vented bulbul call is approximately 2-4 kHz, we used 0-10 kHz white noise to potentially mask the complete auditory range of the blue-tailed skinks (Fuong et al. 2014) or distract them.

Study Sites

We conducted our experiment on blue tailed skinks in Mo'orea, French Polynesia. All experiments were conducted between 20 January and 1 February 2020 between the hours of 08:00 to 15:30 (times of peak skink activity; Williams et al. 2019 and personal observation). We studied skinks at four different sites described in McGowan, Patel, Stroh, & Blumstein (2014). Our sites were the University of California Berkeley Richard Gump Research Station (17°29'25"S 149°49'35"W), the area surrounding the Manutea–Tahiti Rotui Juice Factory and Distillery (17°29'23"S 149°49'43"W), an unpaved road connecting Cook's Bay to Opunohu Bay (17°31'25"S 149°49'53"W), and an unpaved trail around the perimeter of a pineapple plantation (17°31'21"S 149°49'50"W). Although not formally quantified, these four sites differed in the amount of human activity they experienced. The Juice Factory had higher anthropogenic noise levels from heavy vehicular traffic from shipments and tourist visits. The Gump Research Station was primarily open habitat with mid-level anthropogenic noise from a busy road that bisected the station. Additionally, both of these sites likely had higher predation risk to skinks because chickens (*Gallus gallus domesticus*), dogs (*C. lupus familiaris*), and cats (*Felis catus*), all known predators of skinks, freely wandered through these areas. Both the unpaved road and trail were largely surrounded by trees and were away from main areas of activity on the island and were thus less travelled; they had relatively little anthropogenic noise. The unpaved road connecting the two bays had more vehicular traffic from the nearby agricultural college and tourist ATVs, while vehicular traffic for the plantation trail was mainly from farm workers. A total of 41 trials were conducted at Gump, 22 were conducted at the juice factory, 27 were conducted on the road connecting the two bays, and 29 were conducted around the pineapple plantation. To control for these site differences and to ensure equal sampling of each treatment type, we alternated between white noise and silent control trials.

White Noise Playbacks

For our experimental treatment, we broadcast 0 –10 kHz white noise. Our white noise track began with 30 s of silence (played to obtain an individual baseline response to the observer), followed by up to 5 min of 0–10 kHz frequency white noise; noise was broadcast during focal behavior observations, as well as while the observer approached the focal subject. Our white noise track was created in Audacity 2.3.3 (Audacity Team 2019) and was calibrated to 95±1 dB 1 m from the speaker using a RadioShack (33–2055) Digital Level Sound Meter (weighting A, measured 1 m from speaker). The noise was broadcast from MP3 players through Tivoli Audio iPal speakers (Tivoli Audio LLC, Boston, MA, U.S.A.).

Recording Behavior and FID

Three observers independently observed one of the sites on each observation day. We walked quietly along the road or path next to the brush scanning the area visually for skinks. When found, skinks were usually stationary or walking slowly. Once a focal individual was identified, the observer first confirmed whether it was *E. impar,* which is commonly confused with another closely related species on the island, *E. cyanura* (*E. impar* have brighter blue tails). Observers practiced making species distinctions together using both photographs and live animals in the field before starting the experiments.

After properly identifying a blue-tailed skink, we positioned ourselves 2–5 m away from the focal subject and began setting up the speaker apparatus. Starting distance was constrained by the topography of where the speaker could be placed, leading to a range of starting distances. Each speaker was placed on a tripod 60 cm high and directed toward the skink. Although we attempted to point the speakers directly toward the skink, this was occasionally limited by factors such as terrain and skink movement. After finishing equipment setup, we started a trial by starting a playback and quietly dictating all observed behavioral transitions as voice memos into Apple iPhones (Apple Inc., Cupertino, CA, U.S.A.). All behavioral measures followed an ethogram used in a prior skink behavioral study (Fuong et al. 2014). Playbacks started with 30-s of silence before onset of either white noise or silent control, to allow skinks to adjust and acclimate to our presence.

We quantified skink behavior for 30-s before the onset of the playback and 30-s after the onset of the playback. Focals occasionally ended before 60-s when an individual prematurely fled, defined as the moment the skink left the immediate area and could no longer be seen by the observer. After the focal, we stopped recording voice memos and began to approach the skink in order to measure FID. At the beginning of our approach, we dropped a flag at our starting position and began approaching the skink at a trained constant speed of 0.5 m/s (average ± standard deviation = 0.49 ± 0.06 m/s, n = 13). We dropped flags at our position when the skink fled from us (flight initiation distance, FID) and at a visual estimate of the final position of the skink when they fled. We then measured the distance between each flag to the nearest cm. Starting distance was the distance from the observers' starting position to the final position of the skink before fleeing. FID was the distance between the observers' position when the skink fled to the final position of the skink when it fled.

For each trial, we also recorded a visual estimate of percent foliage cover, wind speed (Beaufort scale), the initial location of skinks (rock, log, leaf, litter, branch, ground, or coconut shell), skink size (estimated visually by experimenters after training with pre-measured sticks placed among foliage), number of conspecifics within 1 m (visually counted during trial) and the tail color intensity (scored on a scale of 1–dull blue, 2–light blue, and 3–bright blue; observers agreed upon these categories before collecting data). After each trial, we moved 50 m before searching for another skink in order to avoid carryover effects from the loud white noise trial. To avoid repeated sampling, observers would not return to previously sampled areas within a site on the same day. It is unknown whether blue-tailed skinks are territorial, but given the abundance of blue-tailed skinks on the island, it was unlikely that the same skink would be resampled on subsequent observation sessions. No trials were carried out on rainy days or days of high winds (wind intensity > 2 on the Beaufort Scale).

Behavioral recordings were then categorized as either looking or locomotion. We focused on looking and locomotion because they have been used to evaluate responses in skinks in previous studies (Fuong et al. 2014; McGowan et al. 2014). Looking occurred when an individual was still with its head fixed in one position (each distinct turn of the head was scored as another looking event). Locomotion was a broad term which included walking (movement using all four legs), running (rapid movement with all legs), and hopping. A skink was noted as out of sight if it temporarily was out of view for any reason during the focal recording (e.g., darting behind a rock). All observers scored their behavioral recordings with JWatcher v. 1.0 (Blumstein and Daniel 2007) (trained to inter-observer reliability > 0.95).

Observers attempted to eliminate differences between them by wearing light blue/gray long sleeve shirts and using standardized approach methods. However, personal differences such as counting of rates (despite coordination/training prior to the start of the experiment), or different body postures adopted by observers (such as standing or crouching beside speakers) during observations may have systematically affected skink behavior.

Statistical Analysis

We used JWatcher v. 1.0 (Blumstein and Daniel 2007) to analyze our recorded focals. We calculated rates (N/s) of looking and locomotion by dividing the number of occurrences of the behavior during the time period by the total time a subject was in sight (i.e., visible to the observer) for the 30-s before the onset of the treatment and for the 30-s following the onset of the treatment, but finding no difference we then divided the 30-s following the onset of the treatment into two 15-s time periods. This allowed us to more closely examine changes in behavior post-treatment. To detect whether behavior changed in response to the treatment and whether skinks responded to the white noise playback, we calculated the difference in rates between each of the 15-s post-treatment time periods and the 30-s pre-treatment baseline period. The response to treatment was stronger in the first 15-s post-treatment than the second 15-s post-treatment, so we focused our analysis on the first 15-s post-treatment. A positive difference between the pre-treatment 30-s baseline period and first 15-s post-treatment time period corresponds to an increase in the behavior during the treatment while a negative difference in rates corresponds to a decrease in the behavior during the treatment.

To test for different responses between time bins, we fitted six general linear models (one set of three models each for the looking and the locomotion rates, with the three models being the difference between the first and second 15-s time bins, the difference between the first 15-s time bin and the 30-s pre-treatment time bin, and the difference between the second 15-s time bin and the 30-s pre-treatment time bin) as a function of treatment, observer, testing site, and speaker distance (distance between subject and speaker, which was the same as starting distance). Observer was removed from all models except the linear model (pre-treatment 30-s vs first post-treatment 15-s) where there was a significant observer effect. We used estimated marginal means to quantify the different responses to the treatments (function emmeans() from package emmeans Lenth 2020). We used pairwise comparisons of these to determine significance and effect size (function eff_size() from package emmeans; Lenth 2020). We $\log_{10}+1$ transformed the difference in rates of locomotion to satisfy distributional assumptions and normalize the residuals.

Lastly, to determine whether skinks modified risk assessment while hearing a white noise playback, we fitted a general linear model with the dependent variable FID and the residuals of both our looking and locomotion models, treatment, starting distance, testing site, and the interaction between starting distance and treatment as fixed effects. We included model residuals because they quantified an individual's state following playback after controlling for variation explained by treatment and speaker distance.

To determine whether any variables were confounding, we tested for the relationship between treatment and the following independent variables: starting distance, speaker distance, skink size, and number of conspecifics within 1 m using ANOVA. For categorical independent variables we used Chi Square tests to see if wind speed (Beaufort 0, 1, 2), tail color, initial location of subject, observer, and testing site co-varied with treatment. We interpreted any p-values < 0.05 as significant. None of these covaried by treatment ($p \geq 0.108$); thus, these variables could not be potentially confounding.

We evaluated assumptions of all linear models by graphically inspecting the histograms of the model residuals for normality, the residuals' quantile-quantile plots, and plots of the models' fitted values vs. residuals. For the behavior models, we calculated R values and partial R^2 values (functions rsq() and rsq.partial() from packages rsq Zhang, 2018 and MuMIn Barton, 2019). For the FID model, we calculated R values and η^2 values (functions rsq() and eta_sq() from packages rsq Zhang, 2018 and sjstats Lüdecke, 2020). All statistical analyses were conducted in R version 3.6.2 (R Foundation for Statistical Computing, 2019).

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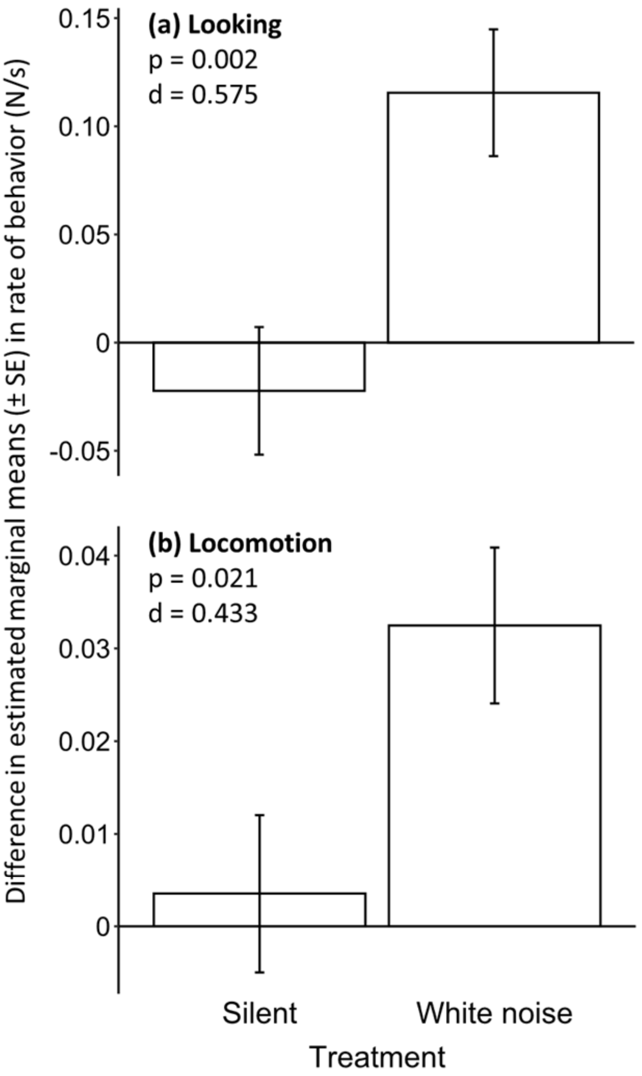
Table S1. Skink responses to playback. Results of general linear models explaining variation in the difference in rates of looking and locomotion between the first 15-s following treatment onset and the 30-s baseline period.

Looking	Estimate	SE	*t*	*p*	Partial R^2
(Intercept)	-0.219	0.113	-1.936	0.055	
Observer (1 reference)					0.096
2	**0.165**	**0.048**	**3.418**	**<0.001**	
3	0.108	0.055	1.955	0.053	
Site (Gump reference)					0.081
Juice Factory	0.030	0.059	0.511	0.610	
Pineapple Plantation	0.085	0.056	1.525	0.130	
Dirt Road	**0.167**	**0.055**	**3.007**	**0.003**	
Speaker distance	0.0001	0.0003	0.343	0.732	0.001
Treatment (Silence reference)					0.093
White noise	**0.138**	**0.041**	**3.368**	**0.001**	
Locomotion					
(Intercept)	-0.003	0.003	-0.919	0.360	
Site (Gump Reference)					0.028
Juice Factory	0.002	0.002	1.416	0.160	
Pineapple Plantation	-0.0004	0.002	-0.315	0.753	
Dirt Road	0.001	0.002	0.924	0.358	
Speaker distance	0.00007	0.00009	0.787	0.433	0.005
Treatment (Silence reference)					0.050
White noise	**0.003**	**0.001**	**2.438**	**0.0163**	

Bold = Significant effect ($p < 0.05$)

Figure S1. Estimated marginal means (±SE) of difference in rates of (1a) looking and (1b) locomotion (N/s) between the first 15-s post-treatment and the 30-s baseline period.



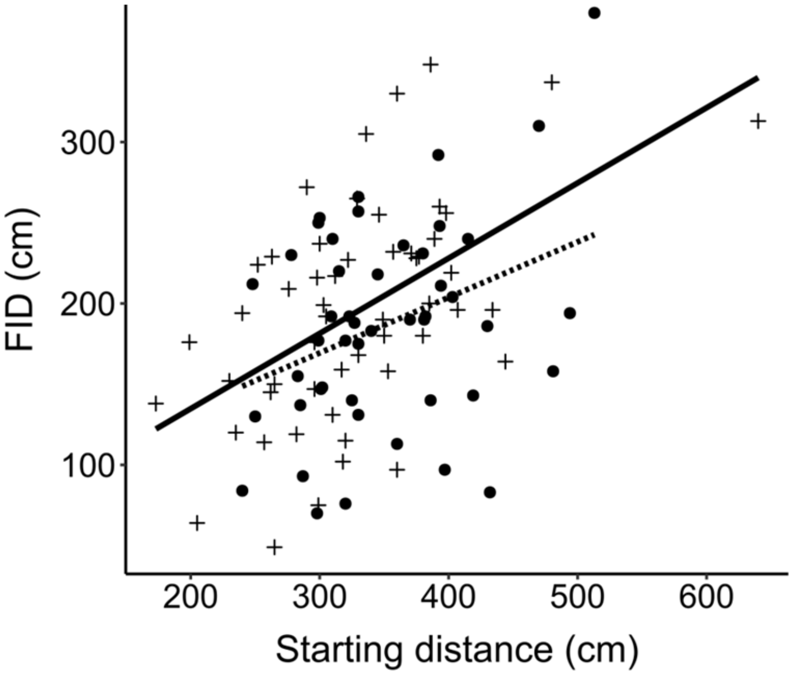


Figure S2. The relationship

between starting distance (cm) and flight initiation distance (cm) as a function of treatment, silent (+, solid line) or white noise (•, dashed line).

Figure S3. The relationship between the residuals of the behavioral model for the difference in rates of looking between the first 15-s following the treatment and the 30-s pre-treatment time period and flight initiation distance (cm). The behavioral model for looking is contained in Table S1.