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## Letter

### An endangered bird calls less when invasive birds are calling

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Novel noises can affect various animal behaviours, and changes to vocal behaviour are some of the most documented. The calls of invasive species are an important source of novel noise, yet their effects on native species are poorly understood. We examined the effects of invasive bird calls on the vocal activity of an endangered Australian finch to investigate whether: 1) native finch calling behaviour was affected by novel invasive bird calls, and 2) the calls of the finches overlapped in frequency with those of invasive birds. We exposed a wild population of black-throated finch southern subspecies *Poephila cincta cincta* to the vocalisations of two invasive birds, nutmeg mannikins *Lonchura punctulata* and common mynas *Acridotheres tristis*, a synthetic 'pink' noise, and a silent control. To determine whether the amount of black-throated finch calling differed in response to treatments, we recorded and quantified black-throated finch vocalisations, and assessed the amount of calling using a generalised linear mixed model followed by pairwise comparisons. We also measured, for both black-throated finches and the stimulus noises: dominant, minimum and maximum frequency, and assessed the degree of frequency overlap between black-throated finch calls and stimulus noises. Compared to silent controls, black-throated finches called less when exposed to common myna calls and pink noise, but not to nutmeg mannikin calls. We also found that pink noise overlapped most in frequency with black-throated finch calls. Common myna calls also somewhat overlapped the frequency range of black-throated finch calls, whereas nutmeg mannikin calls overlapped the least. It is possible that masking interference is the mechanism behind the reduction in calling in response to common myna calls and pink noise, but more work is needed to resolve this. Regardless, these results indicate that the calls of invasive species can affect the behaviour of native species, and future research should aim to understand the scope and severity of this issue.

Keywords: acoustic communication, bioacoustics, invasive species, masking interference, noise

#### Introduction

Biological invasions are one of the greatest threats to biodiversity on the planet (Secretariat of the Convention on Biological Diversity 2014). A large amount of research has targeted influences of invasive species on native species, and such studies have shown that invaders can modify habitats, or consume, compete with,



hybridise with or spread disease or parasites to native species (Goodenough 2010). Recently, the vocalisations of invasive species have been identified as potentially harmful to native species, as they can elicit changes in functionally important vocal behaviour (Both and Grant 2012, Bleach et al. 2015, Tennessen et al. 2016, Medeiros et al. 2017). The auditory systems of animals have evolved to identify ecologically relevant sounds in their habitats, such as sounds made by mates, predators or prey (Fay and Popper 2000), and the vocalisations of invasive species are, in theory, novel to native species. Therefore, the response of native species to the introduction of these noises to their habitats is unknown.

To date, few studies have examined the responses of native species exposed to the vocalisations of invasive species. All have examined the vocal behaviour of native species as the response, and have used anurans as the focal taxa (Both and Grant 2012, Bleach et al. 2015, Tennessen et al. 2016, Medeiros et al. 2017). In the presence of invasive species, properties of native species' calls can be altered in a variety of ways. Anurans may change dominant frequency, amplitude, call duration or call rate in response to the calls of invasive species. These adjustments may improve signal transmission or conserve energy amidst the calls of invasive species, indicating that invasive species' calls may mask unmodified native anuran calls (Both and Grant 2012, Bleach et al. 2015, Tennessen et al. 2016, Medeiros et al. 2017). Masking prevents individuals from receiving, processing and acting on acoustic signals (Klump 1996). In general, noises that are loud, long or overlap frequency components of acoustic signals are those most likely to cause masking (Klump 1996, Francis and Barber 2013, Francis 2015).

It remains unclear whether the calls of invasive species affect groups other than anurans, and how these animals may be affected. For example, animals exposed to novel, anthropogenic noise can experience elevated stress levels (Blickley et al. 2012b, Rolland et al. 2012), potentially caused by inability to communicate effectively amidst the noise (i.e. masking). Alternatively, increased stress levels could indicate that an animal is afraid of the noise, or unable to hear other important acoustic signals, such as predators, amidst the noise (Blickley et al. 2012b). More work is needed to understand how non-anuran taxa respond to the calls of invasive species, and eventually, how fitness is affected.

Birds are a strong study system in which to explore the effects of invasive species' vocalisation on natives. Birds vocalise frequently and for a variety of reasons, including: maintaining group cohesion, sexual advertisement, territory defence and parent-offspring communication (Kroodsma and Miller 1996, Marler 2004). Therefore, if birds change vocalisation rate in response to the calls of invasive species, the response documented in anurans (Bleach et al. 2015, Medeiros et al. 2017), they could potentially suffer reduced fitness, for example if interindividual communication is reduced. Furthermore, there are a range of invasive bird species in many native communities (Blackburn et al. 2009). One example of a highly vocal invasive bird is the Pekin robin *Leiothrix lutea*. This Asian native has invaded parts of Italy,

and during a 2011 survey, its song was heard year-round and constituted 37% of all bird vocalisations, the highest of any bird species in the community (Farina et al. 2013). Similarly, invasive passerines in New Zealand, such as common blackbirds *Turdus merula*, song thrushes *Turdus philomelos*, dunlocks *Prunella modularis*, chaffinches *Fringilla coelebs* and common greenfinches *Chloris chloris* produced vocalisations that overlapped several spectral and temporal vocalisation properties of native birds (Azar and Bell 2016). Noises that overlap in frequency range are generally strong maskers of such signals (Klump 1996, Lohr et al. 2003, Dooling 2004), so there is potential for these invasive bird vocalisations to mask native bird vocalisations. Thus, it is important to understand how invasive species' vocalisations influence the vocal behaviour of native birds.

The black-throated finch southern subspecies *Poephila cincta cincta* is an endemic Australian estrildid finch (Immelmann 1982). It is gregarious and non-territorial (Zann 1977, Immelmann 1982), and sometimes forms part of mixed-species flocks (Vanderduys et al. 2012). Unlike other passerines, black-throated finches do not produce a loud song for territorial defence or sexual advertisement. The only songs in its repertoire are close-contact signals used prior to mating (Zann 1976a, Immelmann 1982) that are accompanied by visual displays (Zann 1976b, 1977). These songs are inaudible to humans in the field, travelling distances of less than ten metres (Zann 1976a). The black-throated finch's distance call is a loud, single-note vocalisation used to maintain auditory contact among flocking individuals, including mates and family members (Zann 1975). The distance call is the main vocalisation described for black-throated finches in field guides, because it is loud and conspicuous. Because black-throated finches are highly gregarious, the distance call is functionally important (Zann 1975), and failure to hear it may reduce group cohesion and decrease fitness. In this study, we examined changes in the amount of black-throated finch distance calling in response to the novel vocalisations of two invasive bird species and a synthetic noise. We aimed to determine whether 1) the calling behaviour of black-throated finches was affected by novel invasive bird calls, 2) they produced a general response to different invasive bird calls, 3) they produced a general response to any novel noise (biotic or not) and 4) any changes may have been related to frequency overlap and masking. Call rates sometimes decrease in anurans exposed to invasive species' calls and other noises (Bleach et al. 2015, Medeiros et al. 2017), similar to song rates in some birds exposed to anthropogenic noise (Fuller et al. 2007, Halfwerk and Slabbekoorn 2009) or the calls of other birds (Brumm 2006). Therefore, we expected black-throated finches to decrease call activity when exposed to invasive bird vocalisations and control noise. The black-throated finch southern subspecies is listed as endangered at both federal (Dept of Agriculture, Water and Environment 2019) and state levels (Dept of Agriculture, Water and Environment 2019). Identifying factors that affect their behaviour and site occupancy is, therefore, important for conservation, as changes to these factors may reduce the viability of already small populations.

## Methods

### Study area and detection methods

The study was conducted in a dry, open woodland near Townsville, Australia (−19.462, 146.712) from August to October 2018, where black-throated finch southern subspecies (hereby referred to as the black-throated finch) were common. As the finch is endangered, large populations were difficult to locate. We therefore focused our study on an area where black-throated finches were abundant, rather than using several sites where finch occupancy could have been low. We used distance calls as a measure of species local presence and activity. Distance calls occur year-round and can be detected over 100 m away (Zann 1975), and call counts perform as well as visual point counts as a method of bird detection (Celis-Murillo et al. 2012). We placed four bioacoustic audio recorders (BARs, manufactured by Frontier Labs) in a square, 200 m apart, and recorded daily between 0730 and 0930, at a sampling rate of 96 kHz with 40 dB gain.

### Stimuli

To determine the responses of black-throated finches to invasive bird species' calls, we presented the wild population with four treatments: a silent control, nutmeg mannikin *Lonchura punctulata* calls, common myna *Acridotheres tristis* calls and pink noise. Nutmeg mannikins are an invasive species found in urban areas of eastern Australia. Like black-throated finches, they are gregarious, non-territorial estrildid finches (Immelmann 1982). Nutmeg mannikins produce a chipping call when in flocks, and a single-note distance call (Immelmann 1982, Morcombe 2000, Pizzey and Knight 2012). Common mynas are another urban-associated invasive species in eastern Australia (Morcombe 2000, Pizzey and Knight 2012). They are aggressive, often chasing other species from nest-hollows (Pell and Tidemann 1997, Grarock et al. 2012, Markula et al. 2016). Common mynas have a large vocal repertoire and produce a diverse array of sounds (Feare and Craig 1999). Although nutmeg mannikins and common mynas are common in the Townsville area, neither had been previously recorded in the study area, nor did we detect any throughout the duration of this experiment. Thus, we expect that both call types would be novel experiences for our test subjects. Nevertheless, black-throated finches have home ranges of approximately 51 ha (Rechetelo et al. 2016), and our study site occurred in a peri-urban area, so we cannot rule out the possibility that the individuals we studied had encountered nutmeg mannikins or common mynas in surrounding areas. To determine whether responses to invasive species' calls were due to a general response to noise, rather than invasive species' calls specifically, we included synthetic pink noise as a further, control treatment. Pink noise is similar to white noise, except it contains most energy in the lower extremes, rather than equally distributed across the frequency spectrum (Halley 1996).

We recorded the vocalisations of wild nutmeg mannikins and common mynas using a BAR with a sampling rate of

96 kHz. The calls of a flock of nutmeg mannikins (approximately ten birds) and five common mynas were recorded for stimuli. We generated WAV sound clips containing ten minutes of nutmeg mannikin or common myna vocalisations, followed by ten minutes of silence using Audacity software (Audacity Team 2019). We also created ten minutes of pink noise, followed by 10 min of silence, in Audacity.

### Playback methods

Estimation of black-throated finch behavioural responses to noise was based on playback. We placed a speaker (JBL™ 'Go Portable' Bluetooth speaker) five metres from BARs at two of the four recording locations on any given day. These were designated noise playback locations, and the remaining two locations designated silent controls. Each day, one stimulus noise type was played at the two playback locations. We systematically rotated through stimulus types each day to ensure they were spread evenly across the sampling period (i.e. nutmeg mannikin calls on day one, common myna calls on day two, pink noise on day three, nutmeg mannikin calls on day four, etc.) to prevent confounding by temporal factors such as variability in temperature and humidity. Several sampling days had to be discarded because wind rendered black-throated finch calls inaudible on recordings. Ultimately, we used five days of recordings per stimulus type, for a total of 15 sampling days. At playback locations, we played the stimuli from the speaker at a volume of 53–77 dB (at one metre) using an iPhone (version five) connected to the speaker via Bluetooth (Supporting information). The volume was chosen based on live sound-level measurements (Lutron Sound Level Meter SL-4013) taken at one meter from the focal invasive bird calls. We wanted playback to be as realistic as possible, which is why amplitude varied in stimulus noises, particularly the common myna calls. The stimuli were played for ten minutes on, ten minutes off, continuously throughout the 2-h recording period. We chose to provide silent periods to ensure the treatments would resemble actual bird vocal activity as most birds do not call constantly for two hours. Locations where treatments (playback including pink noise) and control (silence) were assigned were systematically rotated each day to ensure each of the four locations received each treatment equally.

### Sound and statistical analyses

Black-throated finch calling activity was quantified using Raven Pro V.1.5 (Bioacoustics Research Program 2014). We measured the duration of each black-throated finch calling event by visualising calls as spectrograms and listening to the corresponding audio. Black-throated finch calls were visible on spectrograms during playback of the interfering noises, they were not obscured by the noises. A single distance call, or several consecutive distance calls within ten seconds of each other, were considered an event. Event durations were summed to generate a single measure of total black-throated finch call duration per location per day in seconds. One



second was added to each summed duration to ensure all values were positive.

We investigated differences in the duration of black-throated finch calling among treatments using a generalised linear mixed model with a Gamma family and log-link function. The response variable was duration of calling in seconds, with treatment (i.e. silent control, nutmeg mannikin calls, common myna calls or pink noise) as the fixed effect, and location as a random effect. The model was generated in R Studio (RStudio Team 2015, <www.r-project.org>) using the *lme4* package (Bates et al. 2015). We examined the significance of treatment by comparing the model to a reduced model without treatment via a parametric bootstrap analysis (1000 simulations) using the *pbkrtest* package (Halekoh and Højsgaard 2014). To assess pairwise differences between treatments, we used asymptotic Wald tests using the *emmeans* package (Lenth 2019).

We also measured the spectral properties of black-throated finch calls, and the three stimulus noises, to examine the degree of spectral overlap between the call and the noises. Audio files obtained during this study that contained clear black-throated finch calls were selected for spectrographic analysis. These files contained calls that were unobstructed by other sounds, such as bird calls, insect chirps or wind, and were high in energy, indicating that finches were close to the microphone. The stimulus noise files that were used in noise playback were also used for spectrographic analysis. Noises were not measured from playback files directly, however. Instead, the noises were played from the same speaker that was used in experimental trials, and re-recorded with a BAR at a distance of one metre. We chose to take spectrographic measurements from re-recorded stimulus noises, rather than the raw files directly, in case sound properties from the stimulus noise were altered during playback through the speaker. We wanted to ensure that we were measuring the same noises to which black-throated finches were exposed. For spectrographic analysis, audio files were imported into Raven Pro, and spectrograms with a window size of 512 were generated (Fig. 1). We measured a total of 154 black-throated finch

calls, 148 nutmeg mannikin calls, 116 common myna vocalisations and seven samples of pink noise. Using the selection tool, we selected the sections of sound to be measured (i.e. individual bird calls or song elements, or segments of pink noise), and measured dominant frequency (i.e. peak frequency), maximum frequency (i.e. frequency 95%; the frequency at which the summed energy exceeds 95% of the total energy) and minimum frequency (i.e. frequency 5%; the frequency at which the summed energy exceeds 5% of the total energy) in Hz. Because minimum frequency measurements can be affected by background noise (Zollinger et al. 2012, Ríos-Chelén et al. 2017), we also measured minimum frequency with the threshold method. This method involves using a power spectrum to identify the amplitude at the dominant frequency of a call, and subtracting from that amplitude a pre-selected threshold (in decibels) to determine the minimum frequency at which that threshold occurs (Podos 1997, Ríos-Chelén et al. 2017, Billings 2018). Means and standard deviations were generated for each sound, and were graphed to compare the degree of spectral overlap between black-throated finch calls and each stimulus noise.

## Results

Black-throated finches called at all locations and in all treatments. Treatment was significant in predicting the amount of black-throated finch calling in our study ( $LRT_3 = 8.176$ ,  $p = 0.04$ ). Post-hoc tests revealed that black-throated finches called most when exposed to nutmeg mannikin call treatments, but this was not significantly more than in the controls ( $z = -1.412$ ,  $p = 0.16$ ). They called marginally less when exposed to common myna call treatments than the controls ( $z = 1.876$ ,  $p = 0.06$ ) and significantly less in pink noise treatments than in controls ( $z = 2.133$ ,  $p = 0.03$ ) (Fig. 2).

There was variation in the spectral overlap of black-throated finch calls and the different stimulus noises. All three noises had higher maximum frequencies than black-throated finch calls, so the degree of overlap was based on

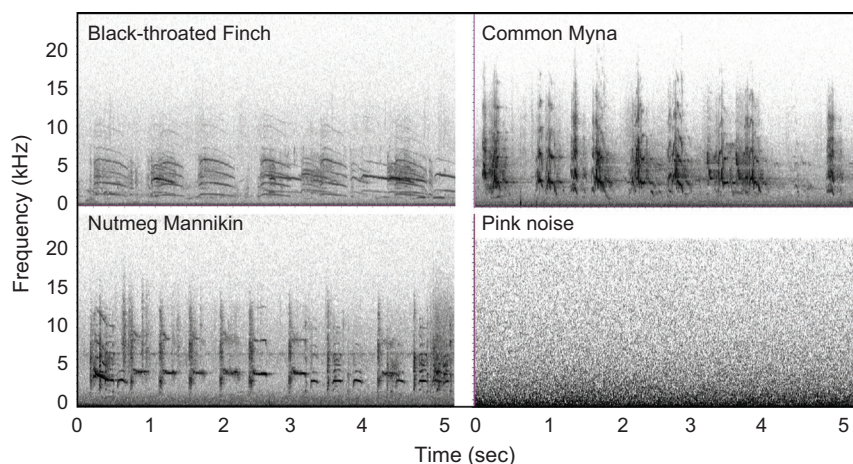


Figure 1. Examples from spectrograms of black-throated finch calls and each of the stimulus noises.

minimum frequencies of the noises (Fig. 3). At one extreme, the pink noise completely overlapped the frequency range of black-throated finch calls, with a much lower minimum frequency than the calls. Common myna calls overlapped much of the black-throated finch calls, but had a higher minimum frequency. Nutmeg mannikin calls overlapped black-throated finch calls the least of all the stimulus noises, with a much higher minimum frequency than black-throated finch calls. Dominant frequency also varied among black-throated finch calls and the stimulus noises. The pink noise had a much lower dominant frequency, and nutmeg mannikin calls a much higher dominant frequency than black-throated finch calls. Common myna calls, on average, had a higher dominant frequency than black-throated finch calls. However, common myna call dominant frequency was highly variable, such that some calls were of similar dominant frequency to black-throated finch calls (Fig. 3).

## Discussion

The black-throated finches examined in this study displayed some sensitivity to novel noises. Their calling activity was reduced when exposed to the common myna call and pink noise treatments, but black-throated finches continued to call when exposed to the calls of nutmeg mannikins.

The results of this study indicate that the responses of black-throated finches to different novel invasive bird calls are not uniform. If finches were affected by the introduction of such noises simply because they were novel and biotic in origin, they should have responded similarly to the nutmeg mannikin and common myna calls. Instead, the opposite occurred. black-throated finches called less than control levels when exposed to common myna calls, but slightly (although not significantly) more than control levels when exposed to

nutmeg mannikin calls. Therefore, it is likely one or more sound properties of the invasive species' calls affected black-throated finch responses.

The responses of the black-throated finches to the pink noise treatment reveal that novel, non-biological noises also affect calling behaviour. Whether or not the noise was made by an invasive species proved unimportant as black-throated finches were as strongly affected by pink noise as they were by common myna calls. The reduction in calling activity in response to these two noises, but not the nutmeg mannikin calls, indicates that some shared noise property elicits the response observed in black-throated finches.

We suggest that masking may have been the mechanism driving the reduction in calling activity of black-throated finches exposed to common myna calls and pink noise. As calling is energetically costly (Taigen and Wells 1985, Oberweger and Goller 2001, Ophir et al. 2010), individuals sometimes reduce calling activity to avoid wasting energy expended in producing a signal that is unlikely to reach its intended receiver amidst noise (Sun and Narins 2005, Brumm 2006, Costello and Symes 2014, Orzi et al. 2016). Individuals may also call less amidst noise because they cannot hear conspecific calls to which they would normally respond (Templeton et al. 2016). It is possible that black-throated finches reduced their call rate, or left the area, to avoid wasting energy on calls that could not be heard amidst the common myna calls or pink noise. Alternatively, individuals may have failed to hear conspecific calls amidst those noises, and were, therefore, unable to call back. Black-throated finches often respond to conspecific calls (Zann 1975), so it was likely that, overall, individual call rates would be lower when conspecific calls were masked.

Masking noises generally overlap in frequency components with the acoustic signals that they mask (Klump 1996, Brumm and Slabbekoorn 2005, Francis and Barber 2013,

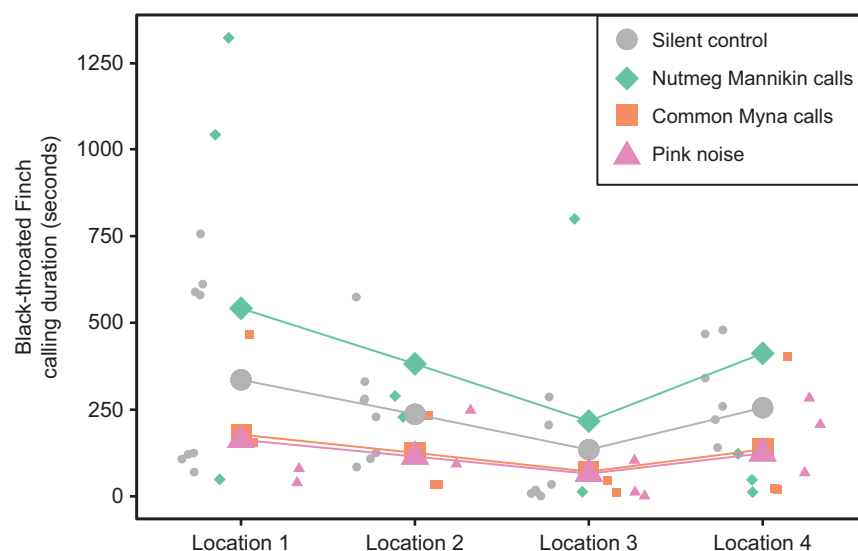


Figure 2. Raw values (small points) and predicted values derived from the model (large points) of the summed duration of black-throated finch calling over two-hour sampling periods.

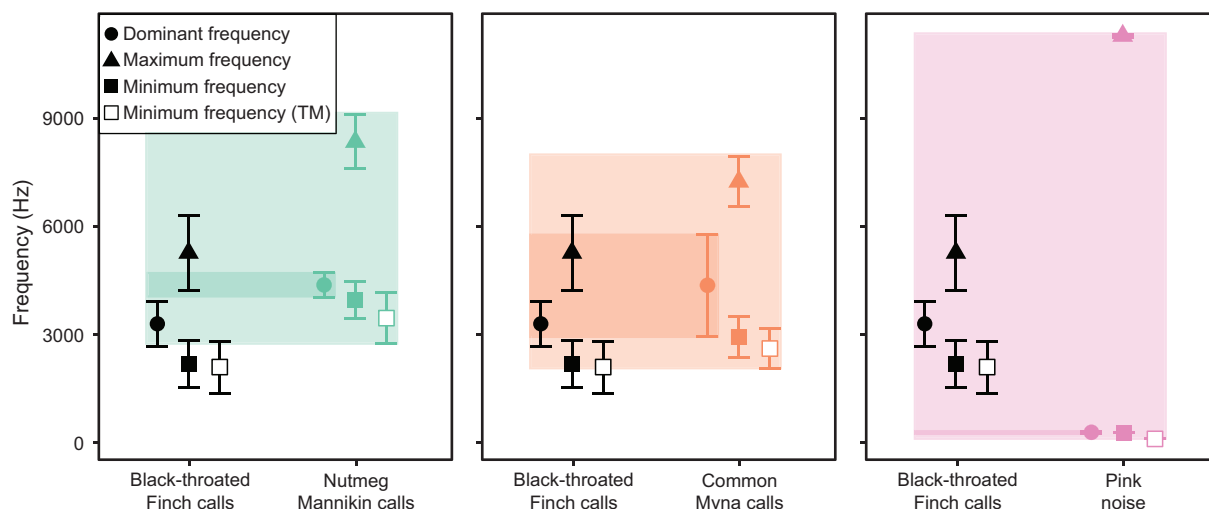


Figure 3. Frequency overlap between black-throated finch calls and stimulus noises. Points and error bars are mean values  $\pm$  standard deviation of dominant frequency, maximum frequency and minimum frequency of black-throated finch calls and the stimulus noises. Minimum frequency = frequency 5% measurement, minimum frequency (TM) = threshold method measurement. Light shading indicates mean maximum frequency + standard deviation and mean minimum frequency (TM) - standard deviation (i.e. the frequency range) of stimulus noises. Dark shading indicates mean  $\pm$  standard deviation of dominant frequency of stimulus noises.

Francis 2015). Pink noise overlaps the entirety of the black-throated finch's call range, and common myna calls overlap much of it. Conversely, the minimum frequency of the nutmeg mannikin's call is approximately 500 Hz higher than the minimum frequency of the black-throated finch. Therefore, the lower frequencies of the black-throated finch call should have been audible and discernible when nutmeg mannikin calls were playing, provided that a signal occurring in those frequencies was relevant to black-throated finches. Zebra finches *Taeniopygia guttata* are close relatives of black-throated finches, much closer than nutmeg mannikins (Olsson and Alström 2020). Zebra finches are sensitive to sounds in the frequency range of 1000–6000 Hz (Hashino and Okanoya 1989), and if black-throated finches have similar hearing sensitivities, it is possible that they can hear and discriminate the low frequencies of conspecific calls amidst the slightly higher pitched nutmeg mannikin call.

Because we did not explicitly test for masking in this study, we offer some alternative explanations for the reduction in black-throated finch calling. Reduced calling activity in response to common myna calls and pink noise could have occurred because these stimuli were perceived by black-throated finches as a threat. When individuals feel threatened by novel noise, they may flee an area or freeze (Karp and Root 2009, Mueller-Blenkle et al. 2010, Blickley et al. 2012a, McClure et al. 2013, Mancera et al. 2017), behaviours which could cause a reduction in calling. We cannot rule out the possibility that black-throated finches innately recognised nutmeg mannikin calls as non-threatening estrilid finch calls, but felt threatened by common myna calls (and pink noise) and reduced calling as a result. It is also possible that hearing sensitivity explains our result. Several bird species are sensitive to certain frequencies (Dooling 2004), so if common myna calls and pink noise are high in energy

in the sensitive hearing range of black-throated finches, but nutmeg mannikin calls are not, the finches will likely pay more attention to common myna calls and pink noise than nutmeg mannikin calls. These noises could distract black-throated finches from conspecific calls, leading to a reduction in group calling activity. Given that all three stimulus noises were at least fairly novel to black-throated finches, and the nutmeg mannikin calls overlapped the least in frequency with black-throated finch calls, we suggest that masking is a stronger overall explanation of our findings. However, more work is required to determine if this is the case.

It is not possible for us to distinguish the behaviour displayed by black-throated finches when calling less. They could have left the area, or remained while reducing calling. Observational studies would, in theory, be useful in determining the precise behaviours of black-throated finches, however, due to their cryptic nature and frequent movements, such studies would be challenging. Regardless of the precise behaviours, the reduction in calling activity from control levels when exposed to some stimuli indicated a change in behaviour. Changes to call rate may reduce fitness, because distance calling is an important form of communication in the black-throated finch (Zann 1975). More work is needed to understand the fitness consequences for natives co-occurring with noisy invasive birds. For example, examining stress hormone levels or reproductive success in black-throated finches may help to determine whether the calls of invasive birds impact fitness.

To date, few studies have examined the vocal responses of native species to the calls of invasive species, and these have been undertaken exclusively in anurans. These studies found that some species use vocal plasticity to try mitigate the effects of masking (Both and Grant 2012, Bleach et al. 2015, Tennessen et al. 2016, Medeiros et al. 2017). In our study,

black-throated finches produced fewer calls when exposed to noises that completely overlapped the frequency range of their call. With habitat degradation threatening this species (Mula Laguna et al. 2019), it is important to identify which factors may render habitat unsuitable. Here, we have demonstrated that calls produced by the common myna affect vocal activity in black-throated finches. The expansion of invasive species that call in the same frequency range may impact habitat suitability for this endangered species. Urban-associated invasive species may have a particularly strong impact on endangered species, as urban areas and roads expand into natural habitat. Our findings indicate that the calls of invasive species have the potential to affect native species and this represents a novel form of competitive interaction that is potentially underappreciated in invasive species research.

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## Author contributions

**Jaimie Hopkins:** Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Methodology (equal); Project administration (lead); Visualization (lead); Writing – original draft (lead). **Will Edwards:** Formal analysis (supporting); Methodology (equal); Supervision (equal); Writing – review and editing (equal). **Juan Mula Laguna:** Conceptualization (equal); Data curation (supporting); Methodology (equal); Writing – review and editing (equal). **Lin Schwarzkopf:** Conceptualization (equal); Formal analysis (supporting); Methodology (equal); Supervision (equal); Writing – review and editing (equal).

## Transparent Peer Review

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## Data availability statement

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.x0k6djh9>> (Hopkins et al. 2020).

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