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Invading the soundscape: the impacts of vocal invasive species

Thesis submitted by

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Spotted Dove (*Streptopelia chinensis*) photographed by Jaimie Hopkins

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All research was carried out with approval from James Cook University's Animal Ethics Committee. For Chapters 3, 4 and 5, research activities were approved under permit #A2547. For Appendix S1, research activities were approved under permit #A2208.

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Abstract

Acoustic signalling is an important mode of intraspecific communication in several animal groups. Co-occurring species are thought to evolve different acoustic signals to avoid miscommunication. If a new sound enters a landscape, it may overlap species' acoustic signals, masking their signals and rendering them difficult or impossible to hear. Thus, animal signallers sometimes use vocal plasticity, or adjust properties of their vocalisations in response to novel noise, presumably to reduce masking. Most of the research examining the effects of novel noise has focussed on anthropogenic sources. Far fewer studies have examined the potential effects of novel vocalisations made by invasive species. Those that have examine anuran study systems, and demonstrate that some, but not all, native anurans display vocal plasticity in response to the calls of invasive anurans. More work is needed to understand the effects of soundscape invasion on native species.

I examined the effect of invasive species' vocalisations on the vocal behaviour of native species. I used several different study systems to answer four main questions: (1) Do native species alter their behaviour in response to the calls of invasive species? (2) Do native species respond similarly to the calls of invasive species and other noise with similar properties? (3) Which noise properties elicit behavioural changes in native species? (4) Is vocal plasticity in signalling native species a reliable indicator that masking by invasive species' calls is occurring?

Birds vocalise for a variety of reasons, including maintaining contact and group cohesion. Novel anthropogenic noise can mask conspecific vocalisations and elicit vocal plasticity in several bird species, which can reduce the level and quality of communication among individuals. It is not known whether novel invasive bird calls, which have a different spectral profile to anthropogenic noise, cause similar effects. In Chapter 3, I found that native Australian Black-throated Finches (*Poephila cincta cincta*) decreased contact (distance) calling activity when exposed to playback of invasive Common Myna (*Acridotheres tristis*) calls and synthetic noise, compared to control levels. In contrast, distance calling activity did not decrease when Black-throated Finches were exposed to playback of invasive Nutmeg Mannikin (*Lonchura punctulata*) calls. Noises that are high in energy at the frequency in which a signal occurs have a high masking effect. Common Myna calls and pink noise overlapped much of the frequency range of Black-throated Finch calls, whereas Nutmeg Mannikin calls were higher in frequency. It is possible that Black-throated Finch calls were masked by Common Myna calls and pink noise, preventing individuals from hearing and responding to one another. A reduction in distance calling may impact fitness, as Black-throated Finches are a gregarious species and this call is important for maintaining contact among flock members.

Exposure to novel noise, including the calls of invasive species, can elicit vocal plasticity in signallers. It is generally assumed that signallers use vocal plasticity to prevent their calls from being masked by noise, however, it is possible that signallers respond this way for reasons unrelated to masking. In Chapter 4, I exposed native Australian floodplain toadlets (*Uperoleia inundata*) to invasive cane toad (*Rhinella marina*) calls, which are lower frequency than floodplain toadlet calls, and four tones of different combinations of frequency and amplitude, none of which overlapped in frequency with the floodplain toadlet's call. I assessed vocal plasticity in floodplain toadlets and found that they increased call effort as a general response to noise, regardless of noise properties. They also significantly lowered dominant frequency in response to loud tones, but not soft tones or the toad call. Spectral overlap between a signal and noise is a major predictor of masking. The playback noises elicited vocal plasticity in floodplain toadlets, despite the complete lack of spectral overlap with floodplain toadlet calls. It is possible that vocal plasticity was used to increase call attractiveness primarily, and call audibility secondarily. Increased call effort is energetically expensive and may impact the fitness of signallers if used long-term to compete with the vocalisations of invasive species.

When signallers increase call amplitude or effort, or alter spectral properties, these adjustments are thought to increase signal audibility amidst noise. When signallers reduce call effort, they may save energy by avoiding calls unlikely to reach receivers. However, vocal plasticity in signallers may not reliably indicate masking in a communication system, because (i) vocal plasticity may be used for reasons unrelated to avoiding masking, and (ii) masking is confirmed if the receiver fails to hear the call, not if the signaller changes their call. In Chapter 5, I examined both signaller and receiver responses in native Australian Peaceful Doves (*Geopelia placida*) exposed to conspecific calls while the calls of invasive Spotted Doves (*Streptopelia chinensis*) or a pure tone were played back. I found that Peaceful Dove signallers produced shorter calls, with longer intercall intervals, when exposed to Spotted Dove calls and the pure tone. Peaceful Dove receivers continued to respond to conspecific calls during playback of both noises, indicating that Peaceful Dove calls were not masked by either noise. Because their calls were not masked, the use of vocal plasticity

becomes difficult to explain in terms of masking. Peaceful Dove signallers may have used vocal plasticity because they were unable to judge if masking might occur, or because they were distracted or fearful. Studies examining masking and vocal plasticity should use both signaller and receiver behaviour to understand whether vocal plasticity is used to mitigate masking.

My findings demonstrate that native species sometimes alter vocal behaviour when exposed to the calls of invasive species. Native species responded similarly to the calls of invasive species and synthetic noises, suggesting that the observed changes to vocal behaviour were general responses to noise. They also displayed vocal plasticity when presented with noise that did not overlap in frequency with their calls. Because frequency overlap influences the severity of masking, it is possible that signallers sometimes use vocal plasticity for reasons other than avoiding masking. The findings also highlight the importance of examining both signaller and receiver behaviour when assessing masking and vocal plasticity in a communication system. More research is needed to understand the costs and benefits associated with adjustments to vocal behaviour in signallers exposed to the calls of invasive species. Additionally, future research is required to understand the effects of soundscape invasion on native species more generally.

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Chapter 1

Introduction

Ecology and evolution of acoustic signalling

Animals hear a variety of sounds in their habitats, some of which convey important signals that affect behaviour (Fay and Popper 2000). Individuals may hear vocal or movement-related sounds produced by heterospecifics, which, when recognised, elicit appropriate behavioural responses. For example, heterospecific alarm calls (Magrath et al. 2015) or sound made by predators (Magrath et al. 2007; ter Hofstede and Ratcliffe 2016) may initiate predator-avoidance behaviours. Similarly, sounds made by prey may help predatory individuals locate prey (Goerlitz et al. 2008).

In many species, acoustic signalling functions in intraspecific communication. These signals are used, for example, to maintain contact among conspecific group members when visual contact is lost, or in parent-offspring communication (Marler 2004). Some acoustic signals are sexually selected, allowing individuals to assess conspecific rivals and find mates (Gerhardt and Huber 2002; Collins 2004; Bradbury and Vehrencamp 2011). Because it informs decisions on behaviour, acoustic signalling is an important form of intraspecific communication in the species that use it.

For intraspecific acoustic signalling to function effectively, the intended receivers of acoustic signals must hear and distinguish those signals (Bradbury and Vehrencamp 2011). The physical attributes of habitats can affect the transmission distance and quality of acoustic signals and, as such, have selected for signal traits that transmit well in certain habitats (Morton 1975). Therefore, species that occur in the same habitat may evolve acoustic signals with similar traits (Dubois and Martens 1984). As well as the physical attributes of the habitat, the composition of the acoustic community applies selective pressures to species' acoustic signals. For signals to function effectively, individuals must be able to distinguish between conspecific and heterospecific calls (Wilkins et al. 2013). Co-occurring species may, therefore, have co-evolved to partition the total acoustic space into individual acoustic niches with reduced overlap. Species may, for example, vocalise within a certain frequency, at a certain time of day or year and from a certain location in the habitat (Krause 1987, 1993). Acoustic niche divergence among species is one mechanism allowing individuals to identify conspecific calls (Wilkins et al. 2013), so that acoustic signals can serve their intended functions.

Signalling amidst novel noise

New and unfamiliar noises are becoming increasingly common in ecosystems worldwide. Noise generated by human activity in particular is becoming prevalent as urban and industrial areas and transportation networks expand (Barber et al. 2009; Pijanowski et al. 2011; Shannon et al. 2016). The soundscape, i.e., the composition of sound in a landscape, can be drastically altered by the presence of these noises (Pijanowski et al. 2011). Wildlife can be adversely affected by new and unfamiliar noise, so it is considered a form of habitat disturbance (Barber et al. 2009).

Novel noises can affect animals in several ways. For example, they may startle or scare animals, cause hearing loss or increase physiological stress (Francis and Barber 2013; Shannon et al. 2016). Novel noise can also affect acoustic communication. If a noise infringes on the acoustic niche of a species, that species' acoustic signal may not function as effectively as in the absence of novel noise (Francis and Barber 2013; Shannon et al. 2016). Noise can sometimes mask acoustic signals, preventing receivers from hearing or discriminating signals (Klump 1996; Brumm and Slabbekoorn 2005; Gelfand 2009). When masking occurs, the critical detection threshold of a signal (i.e., the minimum amplitude at which a signal can be detected) is raised by the presence of noise (Dooling 2004; Gelfand 2009). In other words, an acoustic signal needs to be louder than usual to be heard amidst noise. Several features of noise and signals influence the potential for masking. Two major features identified from controlled lab studies are noise amplitude and frequency overlap (i.e., in Hz). The masking potential of noise increases with noise amplitude relative to a signal (i.e., signal-to-noise ratio) and increased overlap in frequency with the signal (Klump 1996; Dooling et al. 2000; Dooling 2004). Novel anthropogenic noise is generally loud relative to the acoustic signals of animals (Warren et al. 2006), and covers a wide range of frequencies (Gill et al. 2015), so it has high masking potential.

Over recent decades, a variety of studies have assessed the effects of anthropogenic noise on animal acoustic communication (Shannon et al. 2016). Acoustic signalling serves several important functions, including territory defence and sexual advertisement, so any reduction in signal reception or recognition could affect fitness (Barber et al. 2009). Studies aiming to determine whether acoustic signals are masked by anthropogenic noise have generally assessed vocal plasticity in signallers (Shannon et al. 2016). Vocal plasticity is the adjustment of one or more acoustic signal properties, so that the signal differs from its stereotypical state. It is thought that signallers hear noise, recognise its masking potential, and respond by altering one or several properties of their call (i.e., vocal plasticity) (Brumm and Slabbekoorn 2005). Signallers may alter call properties in ways thought to improve call audibility, such as increasing amplitude, calling more frequently or altering call spectral parameters so that overlap in frequency with the noise is reduced. Such adjustments may increase the likelihood that calls will reach and convey intended signal information to receivers amidst the noise (Brumm and Slabbekoorn 2005). Conversely, signallers may alter call properties in ways that reduce energy expenditure, such as calling less. Adjustments such as these may be applied to prevent signallers from wasting energy on signals unlikely to reach receivers amidst noise (Costello and Symes 2014).

The use of vocal plasticity in response to anthropogenic noise has been observed in a wide range of taxa; including, orthopteran insects, anurans, birds and mammals (Shannon et al. 2016), indicating that it is a strategy widely used by signallers in response to anthropogenic noise. Many studies have concluded that signallers use vocal plasticity to mitigate the effects of masking. While this conclusion is reasonable, masking cannot be conclusively shown using studies of signaller behaviour. One reason that signaller behaviour should not be used to identify masking is that signallers may adjust calls in response to noise for reasons other than avoiding masking. Anurans, for example, are thought to use noise levels as a measurement of the degree of competition with other males, so that high levels of noise are considered a highly completive environment with many males calling (Schwartz and Bee 2013). In response to increased noise, they may try to sound more attractive, and increase call rates to convey high stamina (Schwartz 1986; Gerhardt 1991; Gerhardt and Huber 2002; Forsman and Hagman 2006), or lower frequency parameters to convey large body size (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002). Therefore, when individuals display vocal plasticity in response to noise, it may not necessarily be because they have perceived the noise as masking their own, stereotypical acoustic signals. Studies of signaller behaviour should consider this prospect and explore alternative explanations of vocal plasticity in signallers exposed to noise.

Another reason that signaller behaviour should not be used to identify masking is because unmodified calls may still reach and be identified by receivers (Costello and Symes 2014). In such instances, masking does not occur, so the use of vocal plasticity clearly does not improve signal reception amidst noise. Conversely, modified calls may not necessarily reach and be identified by receivers (Templeton et al. 2016). In light of these considerations, receiver responses are more reliable indicators of masking. To conclusively determine whether an acoustic signal is masked by a noise, receiver behaviour must be examined. If a receiver responds normally/appropriately to an acoustic signal amidst noise, the acoustic signal is clearly not masked by the noise. This does not negate the need for studies of signaller behaviour. Studies of signaller behaviour are still important, as they identify whether adjustments occur to an important form of communication, which could have fitness-related implications. Rather, such studies should recognise that drawing conclusions about masking based only on signaller behaviour is limited and, where possible, should be coupled with studies on receiver behaviour.

Invading the soundscape

The calls of invasive species are becoming more common in many ecosystems around the world. Novel noises produced by invasive species may significantly alter the soundscape (Farina et al. 2013), potentially affecting the behaviour of resident species in ways similar to anthropogenic noise. The effects of invasive species' calls on native species have received little research attention relative to the effects of anthropogenic noise and thus it is uncertain how these effects will be expressed. A number of conflicting possibilities exist. Like anthropogenic noise, the calls of invasive species are novel to the native species that hear them. That is, native species' acoustic signals have not co-evolved with invasive species' acoustic signals, thus providing no opportunities for native and invasive species to partition the acoustic space. Because invasive species calls are novel to native species, they may have similar effects to those caused by anthropogenic noise. Alternatively, they may have less of an effect because they have different noise properties from anthropogenic noise. Anthropogenic noise generally covers a wide frequency range, with most energy contained in lower frequencies (Gill et al. 2015), whereas animal calls, including those from invasive species, usually have narrower frequency ranges. As noises with more overlap in frequency with acoustic signal are greater maskers than noise with less overlap (Klump 1996; Dooling et al. 2000; Dooling 2004), it is possible that, due to its wide frequency range (Gill et al.

2015), anthropogenic noise has higher potential to mask native species' calls than do the calls of invasive species.

Conversely, it is possible that the calls of invasive species have greater impacts on native species than anthropogenic noise. Animals show hearing sensitivities to particular frequencies (Klump 1996; Dooling et al. 2000; Dooling 2004). If an invader's call contains a lot of energy within a species' sensitive hearing range, that species will likely be more affected by the invader's call than anthropogenic noise that contains less energy in that range. Native species may be particularly sensitive to the calls of invasive species that have similar ecology or shared ancestors. Invaders may have calls similar to native species due to convergence of vocal traits, or retention of ancestral vocal traits. Species' hearing sensitivities are often correlated with their acoustic signal properties (Ryan and Wilczynski 1988; Manley and Kraus 2010), so a native species may be quite sensitive to the calls of invasive species that sound like their own calls. Invasive species' calls can also be loud and frequent. For example, the Pekin Robin (*Leiothrix lutea*) produces a loud vocalisation that occurs year round and is the most common of any bird species in an Italian forest where it is invasive (Farina et al. 2013). Loud calls produced by invasive species, especially when occurring frequently, have potential to mask the calls of native species, or distract or intimidate them.

To date, very few studies have examined the effects of invasive species' calls on native species. Each of these studies has examined an anuran system and have focussed exclusively on vocal plasticity in the signaller as a response to noise. In response to the calls of invasive anurans, some native anurans display vocal plasticity (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). Like signaller responses to anthropogenic noise, vocal plasticity may be used by native anurans to mitigate masking by invasive anuran calls, and again, this may take a variety of forms involving amplitude, frequency and/or call rate. For example, native green tree frogs (*Hyla cinerea*) called louder in the presence of invasive Cuban tree frogs (*Osteopilus septentrionalis*), a tactic which may increase signal-to-noise ratio and reduce masking (Tennessen et al. 2016). Conversely, native white-edged tree frogs (*Hypsiboas albomarginatus*) (Both and Grant 2012) and snouted tree frogs (*Scinax perereca*) (Medeiros et al. 2017) altered frequency parameters when exposed to invasive species' calls, reducing the amount of spectral overlap between calls. This tactic may have also been used to reduce masking by the invaders' calls (Tennessen et al. 2016; Medeiros et al. 2017). Call rate was reduced in native marbled frogs (*Limnodynastes convexiusculus*) exposed to invasive cane toad (*Rhinella marina*) calls (Bleach et al. 2015) and note duration decreased in native snouted frogs (*Scinax perereca*) and fine-lined tree frogs (*Hypsiboas leptolineatus*) exposed to invasive American bullfrog (*Lithobates catesbeianus*) calls (Medeiros et al. 2017), a tactic which may have allowed individuals to conserve energy, rather than expend it on signals likely to go unheard. Native anurans clearly display vocal plasticity when exposed to invasive anuran calls, but more work is needed to determine whether vocal plasticity is used to mitigate the effects of masking or for other reasons.

Animal acoustic signals are diverse, varying in amplitude and frequency among species (Wilkins et al. 2013). In an ecosystem, invasive species will likely produce calls that overlap in frequency with the calls of some native species, while having minimal overlap with others. We expect that invasive species' calls that are loud and high in energy at the frequency in which a native species' acoustic signal occurs will decrease the audibility of that signal due to masking. It has been demonstrated several times that native species may display vocal plasticity when exposed to noise (Shannon et al. 2016) (including the calls of invasive species; see Chapter 2) that overlaps at least somewhat in frequency with their own call. However, it is unclear whether native species display vocal plasticity when exposed to invasive species' calls that contain little to no frequency overlap with their own call. If they do display vocal plasticity, it is possible that the vocal changes are unrelated to masking. It is important to understand which noise properties elicit vocal plasticity, to understand why animals display this behavioural adjustment when exposed to noise.

Studies on signaller behaviour provide important insight into behavioural changes of native species in response to noise, including the calls of invasive species. However, receiver behaviour must be examined to conclusively determine whether invasive species' calls mask native species calls. Receivers often respond to conspecific calls upon hearing and discriminating them. For example, territorial birds will call back to or approach territorial calls of conspecifics (Collins 2004), and receptive female anurans will orient or move towards male sexual advertisement calls (Gerhardt and Huber 2002). If an individual does not respond to a conspecific call when also exposed to an invasive species' call, it is possible the conspecific call is masked or the receiver is somehow affected by the invasive species' call (e.g., distracted by, or afraid of it). However, if it continues to respond normally, the conspecific call is clearly not masked. Examining receiver behaviour is the most reliable method to assess whether the calls of native species are masked by the calls of invasive species. Additionally, examining both signaller and receiver behaviour would provide insight into whether vocal plasticity is used to mitigate the effects of masking. If vocal plasticity is used by signallers in response to masking noise, non-masking noise should not elicit vocal plasticity. Therefore, if receivers continue to respond to conspecific calls amidst noise (i.e., calls are not masked) and signallers of that species display vocal plasticity in response to that noise, it is likely vocal plasticity is used for reasons unrelated to masking (Costello and Symes 2014). More research is needed to understand the relationship between signaller and receiver responses to noise in relation to masking, in order to determine whether vocal plasticity is a reliable indicator of masking.

The study system

Birds and anurans are ideal for studying the effects of noise on vocal behaviour. Species from both groups rely heavily on vocalisations to communicate. As such, birds, followed by anurans, have been the focus of most studies examining the effects of anthropogenic noise on animal acoustic signals (Shannon et al. 2016) and both have been shown to display vocal plasticity (Halfwerk and Slabbekoorn 2009; Cunnington and Fahrig 2010). Anurans have also been the focus of the only studies examining the effects of invasive species' calls on native species (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). Examining bird and anuran responses would be useful in determining whether native species display vocal plasticity in response to the calls of invasive species as a general trend or whether this response is unique to anurans. These groups can also be used to assess the importance of certain noise properties in eliciting vocal plasticity. Additionally, because birds are responsive to conspecific calls (Marler 2004; Collins 2004), they are useful for examining receiver responses to the calls of invasive species.

In this thesis, I aim to determine the effects of soundscape invasion on native species. I have done this by conducting individual studies in three different systems. By examining several different study systems, stronger conclusions can be drawn about generality of the effects of the calls of invasive species on the vocal behaviour of native species. I have four main research questions: (1.) Do native species alter behaviour in response to the calls of invasive species? (2.) Do native species respond similarly to the calls of invasive species and other noise with similar properties? (3.) Which noise properties are important in eliciting behavioural changes in native species? (4.) Is vocal plasticity in signalling native species a reliable indicator that masking by invasive species' calls is occurring?

In Chapter 2, I review the literature examining the effects of novel noise on the acoustic signalling behaviour of animals. I describe the function and evolution of hearing and acoustic signalling in animals and go on to explain how novel noise can affect acoustic signalling function. I include studies of invasive species' calls, and anthropogenic and synthetic noise in the review, so that I am able to assess how animals respond to novel noise in general and the calls of invasive species specifically. In the second half of the review, I suggest avenues of future research on the topic of soundscape invasion.

In Chapter 3, I assess calling behaviour in the native Black-throated Finch southern subspecies (*Poephila cincta cincta*) exposed to noise, to determine whether call activity changed from control (i.e., no noise exposure) levels. Black-throated Finches were played invasive Nutmeg Mannikin (*Lonchura punctulata*) calls, invasive Common Myna (*Acridotheres tristis*) calls and synthetic broadband noise through a speaker, and their distance calls, which are used to maintain contact among flock members, were passively recorded. I subsequently quantified the amount of Black-throated Finch distance calling, which I then used as the response in a generalised linear mixed effects model. I also examined the amount of frequency overlap between Black-throated Finch calls and each of the noises, which allows me to comment on whether masking could explain the results.

In Chapter 4, I examine the call properties of signalling native floodplain toadlets (*Uperoleia inundata*) exposed to noise, to determine whether toadlets display vocal plasticity in response to certain noises. Toadlets were exposed to invasive cane toad (*Rhinella marina*) calls, and four synthetic pure tones in different combinations of frequency and amplitude. Floodplain toadlet calls do not overlap in frequency with the cane toad call or any of the tones, so I assess the importance of properties other than frequency overlap in eliciting vocal plasticity in a signaller. I measured several toadlet call properties before, during and after noise exposure, and analysed them with a principal component analysis. I used the principal component analysis to assess shifts in call properties in response to invasive cane toad calls and each of the pure tones, to determine which noise attributes elicit vocal plasticity in floodplain toadlets.

In Chapter 5, I examine both signaller and receiver behaviour in native Peaceful Doves (*Geopelia placida*) exposed to invasive Spotted Dove (*Streptopelia chinensis*) calls and a synthetic pure tone. I aim to determine whether signalling Peaceful Doves displayed vocal plasticity, and whether receiving Peaceful Doves responded normally to target signals, when exposed to the noises. I presented Peaceful Doves with conspecific calls (i.e., signal) before and during exposure to Spotted Dove calls or the tone (i.e., noise). I recorded Peaceful Dove calls, and subsequently measured several call properties and counted the number of call back responses to target signals. Each call property was analysed in separate linear mixed effect models to determine whether doves altered any call properties in response to hearing invasive dove calls or the pure tone. The number of calls was also examined with a generalised linear mixed effect model to assess the responsiveness of doves to conspecific signals under different noise playback conditions.

In addition to the main thesis chapters, I conducted a fourth study in which I describe the vocal behaviour of a widespread invasive species, the Asian house gecko (*Hemidactylus frenatus*). As this study does not examine the responses of a native species exposed to calls of an invader, it is appended to this thesis rather than included in the main body (Appendix S1).

Implications

The calls of invasive species have potential to impact the vocal behaviour of native species. It is established that native anurans display vocal plasticity when exposed to the calls of invasive anurans (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017), and birds, similarly, display vocal plasticity when exposed to novel anthropogenic noise (Slabbekoorn 2013; Francis and Barber 2013; Shannon et al. 2016). Because calling is functionally important in many groups, changes to calling behaviour could impact fitness. The first step in understanding the extent of impact of invasive species' calls on native species is to understand why native animals display vocal plasticity in response to these noises and which noises are likely to cause vocal plasticity. This thesis addresses some of those knowledge gaps, providing the groundwork for future research to investigate the problem of soundscape invasion.

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Chapter 2

Invading the soundscape: exploring the potential effects of invasive species’ calls on acoustic signals of native wildlife

Under review as: Hopkins, J. M., Edwards, W., and Schwarzkopf, L. Invading the soundscape: exploring the effects of invasive species’ calls on acoustic signals of native wildlife. Biological invasions.

Abstract

Wild animals are becoming increasingly exposed to new and unfamiliar noises, such as the vocalisations of invasive species, which can affect the ways in which they function and behave. In this review, we examined the literature on the effects of novel noise on animals to assess how novel noise, including the calls of invasive species, affects animals, especially how acoustic communication is affected. We also proposed avenues of future research that would help to provide a greater understanding of the effects of novel invasive species’ vocalisations on native species. The transmission and reception of sound, both between conspecifics and among individuals of different species, play a crucial role in individual fitness. This is because correct interpretation of meaning encoded in acoustic signals enables important context-appropriate behaviours, such as predator avoidance, foraging, and mate location and identification. Novel noise introduced into a soundscape can disrupt the processes of receiving and recognising sounds. When species persist in the presence of novel noise, the noise may mask the production and reception of sounds important to fitness. This can reduce population size, species richness, or relative abundances, and thus influence community structure. In the past, most investigations into the effects of novel noise have focussed on noises generated by anthropogenic sources. The few studies that have explored the effects of calls from invasive species suggest native species alter behaviours (particularly their vocal behaviour) in the presence of noise generated by invasive species. These effects may differ from responses to anthropogenic noises, because noises made by invasive species are biotic in origin, and may therefore be more spectrally similar to the calls of native species and occur at similar times. Thus, in some cases, negative fitness consequences for native species associated with noises generated by invasive species may constitute interspecific competition. Possible negative consequences of invasive species calls represent an overlooked, and underappreciated, class of competitive interactions. We are far from understanding the full extent of the effects of invasive species on native ones. The goal of any future research on this topic should be to understand what these effects are, what characteristics of invasive species and their calls affect the behaviours and fitness of native individuals and what characteristics of native species influence their susceptibility to the effects of invasive species' calls. Further investigation of the contribution of noise interference to native species' decline in the presence of invasive species will significantly increase our understanding of an important class of interactions between invasive and native species.

Introduction

The sound profiles of many landscapes are changing (Barber et al. 2009; Shannon et al. 2016b). The soundscape, i.e., the composition of sound in a landscape, typically consists of biotic sounds, such as animal vocalisations, and abiotic sounds, such as wind and rain, but worldwide, soundscapes are becoming dominated by novel noises (Pijanowski et al. 2011). In particular, anthropogenic noises have changed environmental sound profiles significantly, because of an increase in both the volume, and spatial and temporal variation, of noise (Warren et al. 2006).

For wildlife, these changes present new challenges. Novel noises can mask acoustic signals energetically, when the noise and the signal occur at the same time, so signals must be louder for the receiver to hear them amidst the noise (Klump 1996; Brumm and Slabbekoorn 2005; Gelfand 2009). Loud, long, or similar-frequency noises are those most likely to energetically mask acoustic signals of native species (Barber et al. 2009; Shannon et al. 2016b). Even noises that are not especially similar to native species calls can cause masking. Energetic masking occurs peripherally in the cochlea when a signal is physically masked by noise. Another type of masking, information masking, occurs in the auditory system's central processor when noise perceptually interferes with a signal (Gelfand, 2009). Signals that should be audible in the cochlea, and are not energetically masked (e.g., signals that are spectrally separated from noise) can still be informationally masked by noise, because it is difficult to identify signals amidst noise (for more detail, see Chapter 10 of Gelfand, 2009).

Vital information encoded in acoustic signals can be masked, affecting individual survival and reproductive success, leading to population declines and changes in community composition (Stone 2000; Habib et al. 2007; Slabbekoorn and Ripmeester 2008; Halfwerk et al. 2011b).

The vocalisations of invasive species are potential sources of novel noise detrimental to native wildlife (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). Compared to anthropogenic noise, the calls of invasive species have received little research attention, limiting our knowledge of the responses of native species to invasive species' calls. The effect of invasive species calls warrants further investigation for two main reasons. First, studies of anthropogenic noise show that the reproductive success and population sizes of species that rely on detecting auditory signals can decline in response to novel noises (Stone 2000; Habib et al. 2007; Slabbekoorn and Ripmeester 2008; Halfwerk et al. 2011b). Just like anthropogenic noise, the calls of invasive species are novel to native species, but their biological origin means they have different sound properties. Thus, responses of native species to invasive species' calls may differ from their responses to anthropogenic noise. Second, biological invasions are increasing globally (Secretariat of the Convention on Biological Diversity 2014). Invasive species often outcompete native species for essential resources, which may lead to population decline in native species (Davis 2003). Studies have focused on competitive interactions between invasive and native species, over, for example, shelters (Downes and Bauwens 2002), or food (McGee et al. 2015). Although largely overlooked as a negative effect, invasive species' calls represent a significant potential additional avenue for competition among species, especially given the importance of call competition within and among native species (Gerhardt and Huber 2002; Burt and Vehrencamp 2005; Otter and Ratcliffe 2005). Thus, given the possible severity of impact, the effect of invasive species' vocalisations on native species needs to be assessed.

In this review, we examine the threat to native species of vocalisations from invasive species, and identify areas in need of further research. Although the calls of invasive species could affect native species in many ways, for example by sounding threatening or attractive, this review will focus on masking of acoustic signals by invasive species' calls. We used Google Scholar (https://scholar.google.com/) to search for manuscripts using the keywords 'acoustic adaptation', 'acoustic communication', 'acoustic competition', 'acoustic interference', 'acoustic niche', 'acoustic signalling', 'anthropogenic noise', 'auditory masking', 'invasive species', 'noise pollution', 'signal change' 'signal plasticity', 'vocal plasticity'. We examined the abstracts to determine the suitability of manuscripts and only included manuscripts that examined the responses of animals to noise. First, we briefly review the function and evolution of hearing and acoustic signalling in animals. Then, using the research on anthropogenic noise as a foundation, we review the effect of noisy habitats on the ability of animals to receive and process sound, and translate signals into appropriate behavioural responses. Then, to assess the potential impact of invasive species' calls on native species, we identify similarities and differences between anthropogenic noise and invasive species' calls, and describe their likely impacts on native species. To support our conclusions, we review the small number of studies that have examined the effects of invasive species' vocalisations. Finally, we recommend areas of future research required to quantify the nature and magnitude of the effects of invasive species' calls on native species.

Function and evolution of hearing and acoustic signalling

Many vertebrates can receive, recognise and respond to sounds in their native soundscapes. The evolution of hearing predates vocalisation in vertebrates, and thus, animals can often hear a wider frequency range of sounds than they can produce (Fay and Popper 2000; Barber et al. 2009). Many species recognise vocalisations and movement-related sounds produced by heterospecifics (Bradbury and Vehrencamp 1998; Goerlitz et al. 2008). For example, individuals can avoid the sound of a predator's footsteps (Magrath et al. 2007; Haff and Magrath 2010) or calls (ter Hofstede and Ratcliffe 2016), or heed the warning calls of other species (Magrath et al. 2015).

Although individuals hear and react to sounds made by heterospecifics, vocalisations have evolved mainly for intraspecific communication. Conspecifics can be differentiated from heterospecifics and other noises using vocalisations (Wilkins et al. 2013). For example, banded wood frogs (*Batrachyla taeniata*) respond more strongly to the calls of conspecifics than those of sympatric congeners, using differences in pulse rate (Penna 1997; Penna and Velásquez 2011). Individuals are typically more sensitive to signals produced by conspecifics, because there are often tight correlations between a species' sound production and its reception capabilities (Ryan and Wilczynski 1988; Manley and Kraus 2010). Acoustic signals may also contain individual-level information that inform conspecifics about, for example, a caller's size, sex, reproductive status, or lineage (Wilkins et al. 2013). Females of many frog species, for example, prefer low frequency conspecific calls, a call trait that often indicates larger body size (McLean et al. 2012; Gingras et al. 2013).

Habitat plays a key role in the evolution of acoustic signals. Selection should favour sounds that propagate effectively in specific habitats (Morton 1975). Attenuation, or the loss of signal intensity, and sound degradation, or the loss of signal form, increase with structural complexity of habitats, and with atmospheric turbulence, caused by wind and thermal effects (Morton 1975; Bradbury and Vehrencamp 1998; Wilkins et al. 2013). For example, densely vegetated, closed forests have many reflective surfaces, reducing signal transmission distance. Longer, and lower frequency sounds travel further than higher frequency sounds, and are favoured in closed forests, as they have a greater chance of reaching the intended receiver (Ey and Fischer 2009). Convergence of signal properties in acoustic communities occupying the same habitat may occur (Morton 1975). For example, multiple *Nanorana* frog species in the Himalayas produce short duration calls within a narrow frequency band that propagate well in their noisy, stream habitat (Dubois and Martens 1984). In contrast to signal convergence within habitats, signals may diverge in populations of the same species occupying different habitats. For example, Satin Bower Birds (*Ptilonorhynchus violaceus*) living in rainforest produce lower frequency calls than conspecifics in open forests (Nicholls and Goldizen 2006). These examples demonstrate that acoustic adaptation to habitat can drive acoustic signal evolution (Wilkins et al. 2013).

Convergence in signal design may create problems. Producing a vocalisation adapted to propagate achieves efficient signal transmission, but if multiple species produce similar signals, receivers may have difficulty identifying conspecifics. Species within a community should, therefore, evolve vocalisations that partition the acoustic space. For example, *Geospiza* finches are endemic to the Galapagos Islands and share a common ancestor. Medium Ground Finches (*Geospiza fortis*) and Cactus Finches (*G. scandens*) faced acoustic competition with the Large Ground Finch (*Geospiza magnirostris*) after it flew to from a neighbouring island and established on Daphne Major Island. As a result of acoustic competition with the Large Ground Finch, sons of the Medium Ground Finch and Cactus Finch sang faster-trilled songs than their fathers, and by doing so, reduced call similarity with the Large Ground Finch (Grant and Grant 2010). Overlap in call traits should be avoided because the production of species-specific vocalisations allows receivers to identify conspecifics amidst the noise of the acoustic community, avoiding masking and costly errors, for example mistaking heterospecifics for conspecifics (Krause 1987, 1993). Acoustic niche partitioning, like acoustic adaptation to the habitat, is an important process thought to shape species' calls (Wilkins et al. 2013).

Acoustic signal evolution is also constrained by traits of the signalling species. Phylogenetic history may influence the sounds made by individuals (Wilkins et al. 2013). For example, morphological constraints on the signaller, such as body and beak size (Podos 2001) or neurophysiological constraints on the receiver, such as the sensitivity of hearing structures (Römer 1993) may affect the evolution of acoustic signals. Multiple pressures and constraints, including all those outlined, influence the evolution of species-specific acoustic signals, producing signals that increase fitness in specific physical and biotic environments (Boncoraglio and Saino 2007; Wilkins et al. 2013). Thus, we expect that changes in the physical or biotic environment, such as the introduction of a novel invasive species' call, could alter the effective transmission of acoustic cues in that environment.

The problem of acoustic masking by novel noise

Anthropogenic noise is a severe form of habitat disturbance, and has been the focus of many studies (Shannon et al. 2016b), providing an understanding of animal responses to novel noises in general. Typically, anthropogenic noise masks movement-related or vocal sounds produced by other wildlife (Francis and Barber 2013; Shannon et al. 2016b). Anthropogenic noise masks signals used by mammals (Siemers and Schaub 2010), birds (Huet des Aunay et al. 2014), amphibians (Bee and Swanson 2007), fish (Codarin et al. 2009), and insects (Bent et al. 2018). When masking happens, communication, movement, vigilance, mating and foraging can be negatively affected (Shannon et al. 2016b) (Fig. 2.1).

Regardless if the source is anthropogenic, if a noise is loud enough, any acoustic signal can be masked. However, short sounds are particularly easily masked, as are low frequency sounds, because ambient noise typically has higher energy in lower frequencies (Okanoya and Dooling 1990; Lohr and Dooling 1998; Brumm and Slabbekoorn 2005; Gelfand 2009). Additionally, noise occurring at the same frequency as an acoustic signal will have a significant masking effect (Klump 1996; Lohr et al. 2003; Brumm and Slabbekoorn 2005; Gelfand 2009). Anthropogenic noise is typically loud, low-frequency (Warren et al. 2006), and may be constant and long-term or chronic, making it a potent masker of (particularly low frequency) acoustic signals, potentially leading to a lack of, or atypical responses, to important cues (Francis and Barber 2013). Although long-term or chronic exposure can lead

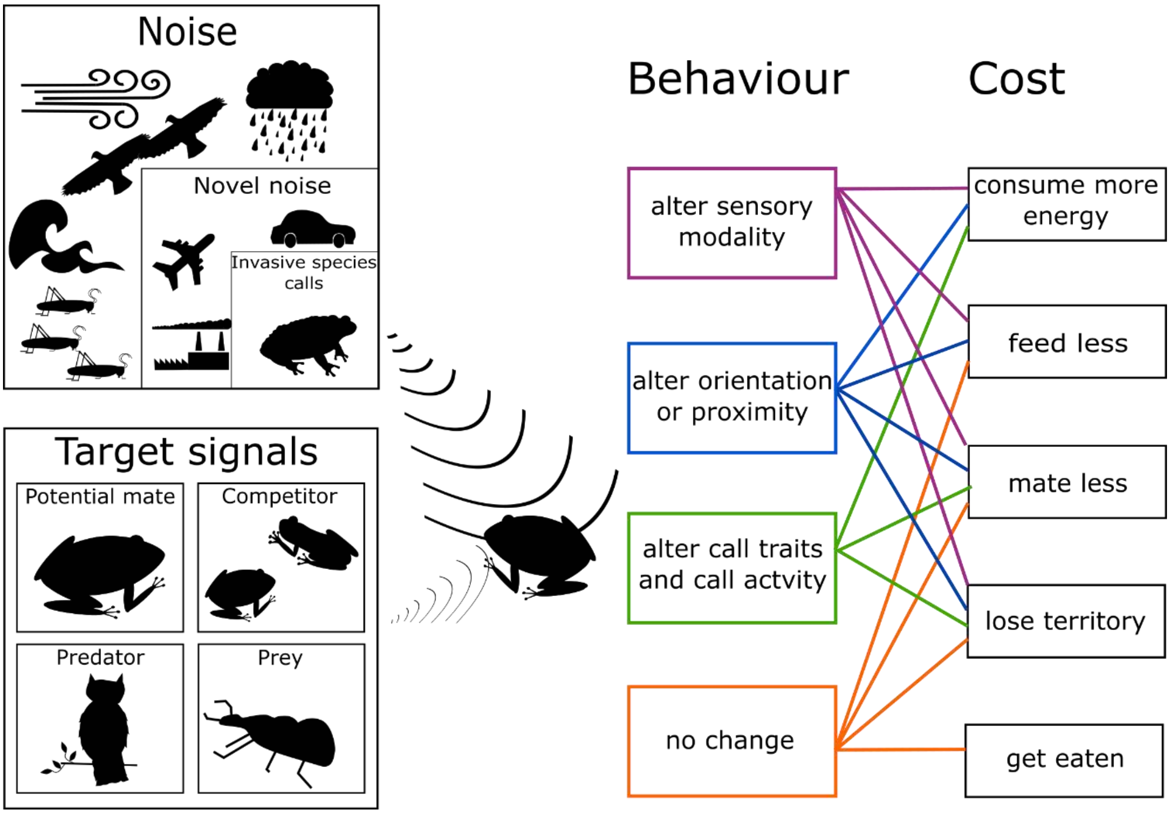

Fig. 2.1 Behaviours that could occur due to masking of target signals by the calls of invasive species and other noise. The costs of each behaviour are indicated by coloured lines that correspond with the behaviour.

to habituation or accommodation in some cases (e.g., Smith et al. 2004; Ditmer et al. 2018), there is evidence that it can have negative effects in nature. For example, foraging success of greater mouse-eared bats (*Myotis myotis*) is reduced near noisy roads, because it masks the rustling of prey that they use to forage (Siemers and Schaub 2010).

Much of the research examining the effects of masking by anthropogenic noise has focused on masking of intraspecific communication (Shannon et al. 2016b). Males of many species, especially birds and anurans, call to attract females (Bradbury and Vehrencamp 1998) and calls may be broadcast to fewer potential mates amidst anthropogenic noise (Barber et al. 2009). For example, female canaries (*Serinus canaria*) presented with urban noise have reduced responsiveness to lower-frequency male calls (Huet des Aunay et al. 2014). Vocal plasticity in the sender, however, allows some species to avoid masking. Vocal plasticity occurs when it is possible for a sender to alter spectral or temporal properties of their calls, or produce louder calls (Fuller et al. 2007; Slabbekoorn 2013; Templeton et al. 2016). For example, high frequency bird calls typically elicit greater responses from conspecific receivers amongst anthropogenic noise (which is typically low frequency) than do unmodified calls (Halfwerk et al. 2011a; Pohl et al. 2012; Huet des Aunay et al. 2014; LaZerte et al. 2017). However, these adjustments can come at a cost. When species' vocalisations have evolved to function as signals of fitness (notably male quality), altering vocalisations may reduce signal quality (Slabbekoorn and Ripmeester 2008). Typically, females prefer low-frequency songs, so males with higher-frequency songs have lower reproductive success than those with lower-frequency songs (e.g., in Great Tits, *Parus major*, Halfwerk et al., 2011a). Because of female preferences, singing at higher frequencies may not necessarily improve reproductive success, despite improving signal transmission (Slabbekoorn and Ripmeester 2008; Halfwerk et al. 2011a).

The inability to hear important acoustic signals amidst novel noise may cause an increase in stress levels or behavioural changes in individuals. Elevated stress levels have been observed in several animals exposed to novel anthropogenic noise (Shannon et al. 2016b), which may occur because individuals struggle to detect, for example, the sounds of predators, competitors, prey or mates amidst masking noise or because the noise itself is perceived as a threat (Blickley et al. 2012b). Some individuals do appear to adjust their behaviour to counter the masking effect of anthropogenic noise. They may, for example, increase vigilance for predators, initiating predator avoidance behaviours, such as flight, earlier in the presence of anthropogenic noise (Meillère et al. 2015; Shannon et al. 2016a). Although this tactic allows individuals to react appropriately to cues, taking flight and sacrificing foraging opportunities is energetically costly (Preisser et al. 2005; Shannon et al. 2016a). Therefore, the masking effects of novel noise can impact an individual's physical fitness through behavioural adjustment costs and elevated stress levels.

Invasive species vocalisations vs. anthropogenic noise

Studies of anthropogenic noise have examined the masking effects of novel noise on wildlife extensively. They have identified the noise properties most likely to mask wildlife sounds, and the effects of masking. Their findings can be generalised to predict the responses of native species to the calls of invasive species. Like anthropogenic noise, the calls of invasive species are often loud, sometimes louder than those of native species. For example, cane toads (*Rhinella marina*) can call at a volume of 85 dB at 1 m, which is as loud as a standard household vacuum cleaner, and louder than many native frogs in invaded regions of Australia (Bleach et al. 2015). Similarly, invasive Cuban treefrogs (*Osteopilus septentrionalis*) (Olson et al. 2012) and American bullfrogs (*Lithobates catesbianus)* (personal observation) can dominate the soundscape where they occur. Also like anthropogenic noise, invasive species' calls can be frequent, or of very long duration, or both. For example, over the course of one year, invasive Pekin Robins' (*Leiothrix lutea*) calls made up 37% of all bird songs in their invaded range in Europe (Farina et al. 2013). Likewise, the calls of invasive birds such as Common Mynas (*Acridotheres tristis*), Rock Doves (*Columba livia*) and European Starlings (*Sturnus vulgaris*) seem to dominate the urban environments they have invaded (personal observation). So, loudness and high rate of occurrence are characteristics of the calls of many invasive species, that, like some anthropogenic noises, can have negative effects on native species. In response to loud, persistent noise, individuals are likely to experience similar negative impacts to those described previously.

Although invasive species' calls and anthropogenic noises share some features, these noises are also different in important ways. Thus, we expect the effect of invasive species calls to have other effects, not observed in response to anthropogenic noise. The distribution of sound energy constitutes a major difference between invasive species' calls and anthropogenic noises (Fig. 2.2). Typically, sound energy in animal vocalisations is concentrated in particular frequencies, i.e., they have harmonics, (Bradbury and Vehrencamp 1998), whereas anthropogenic noise is typically broad-spectrum energy, concentrated in lower frequencies (Slabbekoorn and Peet 2003; Slabbekoorn and Ripmeester 2008). Animals also call in notes (Bradbury and Vehrencamp 1998), whereas anthropogenic noises may sound constantly for long or unpredictable periods of time, for example traffic or machinery (Habib et al. 2007; Francis et al. 2009). Also, frequency modulation, the distribution of energy across frequencies over time, is more variable in the calls of animals than in anthropogenic noise. Finally, animal call components are typically of different lengths and frequencies, whereas many types of anthropogenic noises are often monotonous and unmodulated (Fig. 2.2).

Another important difference between the calls of invasive species and anthropogenic noise is that one may be reactive to the environment while the other is not. Being abiotic, the characteristics of anthropogenic noise will not change in response to changes or fluctuations in the environment. However, invasive species will likely react to aspects of their habitats, such as background noise or the presence of native species, which could potentially affect their vocal behaviour. Changes to invasive species' vocal behaviour could be beneficial or

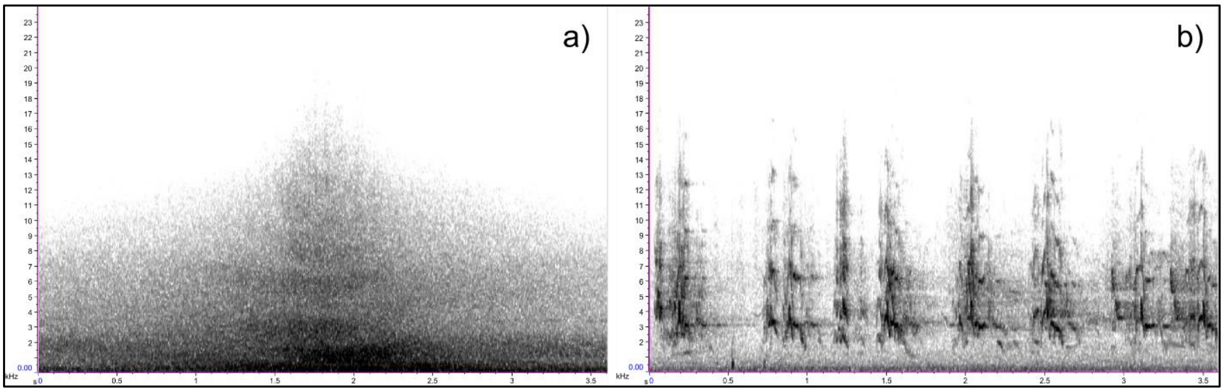

Fig. 2.2 Spectrogram (created in Raven Pro 1.5; Bioacoustics Research Program 2014) of: a) a passing car; b) a Common Myna bird (*Acridotheres tristis*) call, showing the spectrographic differences between anthropogenic noise and animal calls.

detrimental to native species. For example, if an invader makes an alarm call in response to a common predator which native species recognise, eavesdropping on the invasive species' alarm call could allow native species to initiate predator avoidance behaviours. Conversely, if an invasive species increases call effort or amplitude in response to hearing the calls of native species, native species will face increased levels of competition with the invader.

Because of differences between invasive species' calls and anthropogenic noises, we expect the effects of invasive species calls on native species to be different. Animals may habituate to anthropogenic noise, and cease to react to it (Smith et al. 2004; Ditmer et al. 2018), but if native species can better detect, or pay more attention to the sounds of other animals than to anthropogenic noise, then invasive species' calls may have a greater effects than anthropogenic noise. For example, if an invader is related to, or has a similar ecology to species in the recipient ecosystem, its call may sound like those of native species, at least in general. Species may share call properties if they have shared ancestry, or there may have been convergence of call properties caused by similar ecological or morphological constraints (Morton 1975; McCracken and Sheldon 1997). Correlations exist between a species' vocal properties and its hearing range in many taxa (Moiseff et al. 1978; Ryan and Wilczynski 1988; Manley and Kraus 2010; Zuk et al. 2017). Native species may, therefore, be attuned to vocalisations that sound similar to conspecific calls, and if an invader's call fits into this category for the reasons outlined above, the calls of invasive species may have more of an effect on native species than does anthropogenic noise.

The timing, diurnal or seasonal, of invasive species' calls and anthropogenic noise may differ. Anthropogenic noise is often aseasonal (e.g., traffic, urban noise), whereas seasonal activities critical to fitness, such as breeding, may overlap in native and invasive species, especially when the invasive species is in the same broad taxonomic group. In the case of breeding season overlap, the likelihood of masking increases. For example, native birds and anurans chorusing may face acoustic competition with breeding invaders that chorus at the same times of day and year (Farina et al. 2013; Bleach et al. 2015).

Compared to invasive species' calls, many types of anthropogenic noise may peak at times that do not really influence native species activities. There is, for example, more traffic noise, in daylight when people are most active (Barber et al. 2009). Native animals may avoid the negative effects of anthropogenic noise at these times if they are not important activity periods, whereas noisy, ecologically similar invaders are more likely to interfere at the same activity times. An overlap in activity periods between native and noisy invasive species may elicit changes in native species' behaviour. For example, if native individuals cannot hear conspecific calls amongst the calls of invasive species, they may have fewer mating opportunities.

The effects of invasive species' calls on native vocal communication

Very few studies examining the effects of novel noise on animals have focussed on the calls of invasive species. Those that have done so have measured the vocal responses of native species to masking by the calls of invasive species. The responses from native species, even in these few studies, have been variable. For example, some native anurans altered call frequency, note duration, call rate or amplitude when exposed to invasive species vocalisations (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017, Chapter 4), while others showed no response (Bleach et al. 2015; Tennessen et al. 2016).

One study suggested that native species whose calls overlap in frequency with the invader's call were most likely to alter call traits. Tennessen et al. (2016) presented two native treefrogs with calls of invasive Cuban treefrogs (*Osteopilus septentrionalis*) and white noise with the same or higher frequency than the invader's call, and recorded native treefrogs' calls. The native treefrog (*Dryophytes cinereus*) that called at a similar frequency to the invader produced louder, shorter calls when presented with the invader's call, or white noise of the same frequency, but not white noise of higher frequency. The other native treefrog (*D. femoralis*), which called at a different frequency to the invader, did not alter its call during any of the noise treatments. These results indicate that the degree of spectral overlap between an interfering noise and the focal call can influence whether it will alter its call when signalling amidst the noise (Tennessen et al. 2016). Frequency overlap is also a predictor of signal change in birds exposed to anthropogenic noise (Hu and Cardoso 2010; Francis et al. 2011), indicating signal adjustment may be a general response to novel noise overlapping in frequency with an individual's call, and is employed to avoid masking (Slabbekoorn and Peet 2003).

Another study suggested that native species with fast call rates are those most likely to alter calls, regardless of the degree of frequency overlap. Bleach et al. (2015) found that a fast-calling native frog (*Limnodynastes convexiusculus*) reduced call rate during playback of invasive toad (*Rhinella marina*) calls, lawn mower noise, and the calls of sympatric native frogs. It also increased call rate during silent periods. The authors suggested the fast-calling frog saved energy by reducing calling rate at times when calling was likely to be masked. Another native frog, with a slower call rate (*Litoria rothii*), did not adjust call behaviour in response to any of the noise treatments, perhaps because energy savings would have been negligible (Bleach et al. 2015).

A third study suggested that both biotic and abiotic properties of noises influence vocal responses of native species. Medeiros et al. (2017) tested whether the calls of invasive species had a greater impact than sympatric native species' calls or synthetic noise on the vocal properties of native anurans' calls. Frogs changed calls in response to each noise, however, the types of changes differed among noises. In general, frogs produced similar calls when presented with the invasive bullfrog (*Lithobates castesbianus*) and a native toad call (*Rhinella icterica*), however, they produced different calls when hearing synthetic noise. Additionally, the types of vocal adjustments differed among the native species examined. The authors suggested anurans may be more attuned to anuran calls than to other sounds (Medeiros et al. 2017). Acoustic signals have specific traits, such as harmonics and frequency modulations, (Marler and Slabbekoorn 2004), which receivers can identify (Cynx et al. 1990; Vignal et al. 2008). Likely, receivers that identify such traits can distinguish between biotic and abiotic noises. In these cases, receivers should be more attuned to and, therefore, more affected by a masking biotic noise (e.g., heterospecific calls) than a masking abiotic noise (e.g., synthetic noise) (Medeiros et al. 2017).

The theory that invasive species are more attuned to and, therefore, by affected by biotic than abiotic noise has been further examined. Native Australian floodplain toadlets (*Uperoleia inundata*), Black-throated Finches (*Poephila cincta cincta*) and Peaceful Doves (*Geopelia placida*) modified at least one call property when exposed to the calls of invasive species. These species also modified call properties when exposed to synthetic noise controls, so that calls or call rates resembled those produced when the species were exposed to invasive species' calls (Chapter 3; Chapter 4; Chapter 5). These results indicate that these species respond similarly to invasive species' calls and biologically irrelevant synthetic noise controls, in contrast to conclusions drawn by Medeiros et al. (2017). It is likely that, rather than a biotic origin *per se*, specific properties of noise influence native species' call responses in the presence of the noise.

Overall, the vocal plasticity observed in these studies indicates that native species try to counter masking by temporarily occupying a different acoustic niche. These studies demonstrate that invasive species' calls are important sources of novel noise which can cause changes in native individuals' behaviour, however, whether these changes incur fitness costs remains unexplored. This field of study is new, and more work is needed to understand the range of ways in which native animal behaviour can be affected by masking from invasive species' calls, and importantly, how populations and communities may be impacted.

New approaches to studying the effects of invasive species' calls

In this section, we propose avenues of future research examining the impacts of invasive species' vocalisations on native species. The goal of any future research on this topic should be to understand: 1) the effect of the calls of invasive species on native species; 2) what characteristics of invasive species and their calls affect the behaviours and fitness of native individuals; and 3) what characteristics of native species influence their susceptibility to the effects of invasive species' calls.

As outlined in the above section, the only studies that we are aware of to date that have examined the effects of invasive species' calls on native species have observed vocal plasticity in native species exposed to invasive species' calls (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). While reduction of masking may be the outcome, the change in behaviour may not occur for that reason. For example, several anuran species are thought to use high noise levels in general as a proxy for high competition

(Schwartz and Bee 2013), so call adjustments may occur in response to perceived higher competition, rather than masking. The only way to truly determine the importance of masking is to examine the responses of the intended receiver. For example, if a receptive female frog orients or moves towards a male conspecific call in the presence of an invasive species' call, it is likely that the male's call is not masked by the invasive species' call. Similarly, if territorial birds continue to approach conspecific intruder calls in the presence of an invasive species' call, it is also likely that the intruder's call is not masked by the invasive species. Studies examining receiver responses to conspecific calls amidst invasive species' calls will complement the existing studies examining anuran vocal plasticity, and resolve the relative role of masking versus other factors in eliciting vocal plasticity in anurans exposed to invasive species' calls.

To better predict and manage the impacts of invasive species' calls on native species, we need to understand which characteristics of an invader's call, and which ecological traits influence the severity of masking. We consider several traits as potentially important predictors of masking, and suggest that future research should be aimed at examining questions related to these traits.

Timing of calling is an important ecological trait of invasive species, which probably differs from most anthropogenic noise, and that likely has strong effects on native individuals. Vocalising invaders often have daily or seasonal peaks in calling activity that coincide with the same peaks in activity (both vocalising and other activities) in native species. If, for example, an invasive bird and a native bird call most intensely during the dawn chorus, the invader's call will occur with (and potentially mask) the native vocalisations. Native callers that are unable to effectively send their acoustic signals to conspecifics in such situations will likely suffer reduced mating success and higher energy consumption, for example if they try to counter masking effects by calling more frequently, for longer periods or at louder amplitudes. We suggest that temporal overlap in key activity periods between invasive and native species is probably an important predictor of fitness consequences in native species, and future studies should aim to examine this hypothesis. For example, a long-term acoustic monitoring study would be useful in examining the degree and consequences of temporal overlap between invasive and native species' sexual advertisement calls. If invasive and one or more native species initially call most intensely, for example, at the same time of day, and over time one or more of these native species begins to call most intensely at a slightly different time of day, it would suggest that these native species are avoiding signalling at the same time as the invader. Additionally, by concurrently monitoring reproductive output of native species, conclusions can be drawn on the influence of such changes on call timing and, therefore, reproductive success.

Sound properties of invasive species' calls will also likely influence their degree of impact on native species. When species invade a new soundscape, they may introduce noises that are loud (Farina et al. 2013; Llusia et al. 2013) or overlap in sound properties with native species' calls or other important sounds (Azar and Bell 2016). Masking could be particularly severe for native species that call alongside an invader in a similar niche, and that share call properties with the invader. We suggest examining the relative masking effect of properties of invasive species' calls on the sounds used by native species. It is well-established that masking is primarily a function of signal-to-noise ratio, but it is also influenced by target signal duration, and spectral overlap between the target signal and noise (Okanoya and Dooling 1990; Klump 1996; Dooling et al. 2000; Dooling 2004). However, invasive species' calls are markedly different from noises that have been used to examine masking in previous studies, such as anthropogenic noise or synthetic broadband noise, or pure tones. Invasive species' calls are diverse, and may consist of, for example, single tonal chirps, trilled notes, or complex songs with varying duration, amplitude, and frequency modulation. Identifying the sound properties of invasive species' calls responsible for masking could be achieved by manipulating the amplitude, frequency or temporal parameters of an invader's call and documenting a native's responses. Understanding which invasive species call traits (e.g., long, loud, tonal or broadband calls) are most likely to have the greatest impact on native species will help in prioritising prevention or management of invasions.

To complement our understanding of the ecological and vocal traits of invasive species that cause the greatest impact, we need to identify the characteristics of native species that make them most susceptible to these impacts. We suggest that behavioural plasticity in native species likely influences the level of impact of invasive species' calls. One could argue that masking by invasive species' calls is most likely to affect individuals that are unable to modify their behaviours, although it depends on the cost of modifications made by species with vocal or behavioural plasticity. Behavioural adjustment in response to hearing impairment in noisy environments occurs in animals exposed to anthropogenic, biotic, and synthetic noise (McClure et al. 2013; Huet des Aunay et al. 2014; Meillère et al. 2015; Medeiros et al. 2017), but is not universal. It is important to understand whether behavioural adjustments by signallers or receivers improve signal reception and discrimination, and the cost of such adjustments to fitness.

The studies outlined earlier that examined native species exposed to invasive species' calls, demonstrated that vocal plasticity is one type of behavioural adjustment used by individuals (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017), but that not all species appear to use it. Although not clear, the outcome of vocal adjustments is probably improved signal transmission. To complement the studies we reviewed on invasive anurans, the responses of native species to adjusted and unadjusted conspecific calls, expressed amidst the calls of invasive species, should be examined. Such studies would help to determine whether this behavioural adjustment in the signaller improves signal reception in the receiver and whether failing to adjust signals translates to lower signal reception rates. Again, using, for example, the responses of receptive female anurans or territorial birds to altered calls could reveal if adjusted calls reduce masking effects by invasive species' calls.

Receivers may also apply behavioural adjustments to improve signal reception and discrimination rates amidst the calls of invaders. For example, orienting or moving towards a target signal or away from an invasive species call may reduce the masking effect of the invader's call, as has been similarly demonstrated in female grey treefrogs (*Dryophytes chrysoscelis*) exposed to male conspecific calls played alongside chorus-shaped synthetic noise in an experimental, circular arena (Bee 2008). Alternatively, receivers may abandon attempts to hear a target signal, and instead rely on a different sensory modality, such as vision. A study of birds exposed to experimental traffic noise found that they spent more time with their heads upright in noisier treatments. This increased vigilance indicated that the birds were compensating for the masking effect of the traffic noise by using vision to obtain information (Ware et al. 2015). We suggest that examining behavioural modifications in receivers, such as those described here, would be useful to determine whether these adjustments can compensate for the effects of the calls of invasive species. Additionally, the physiological costs associated with these behavioural modifications, such as increased stress levels and energy consumption, should be quantified, to assess fitness costs associated with behavioural modification.

Conclusion

There is extensive research documenting the effect of anthropogenic noise on animals, but few studies have examined the impact of the calls of invasive species on native species. Invasive species' calls differ from anthropogenic noise, and, thus, may affect native species in different ways. The vocalisations of invasive species have potential to impact native species, particularly calls with acoustic properties that dominate the soundscape. Studies of the impact of invasive species' calls have found that these novel noises can affect the behaviour of native species that communicate vocally, expressed *via* changes in native species' calling. Little is understood about the impact of masking by invasive species on native species, and a greater degree of understanding of these interactions is urgently needed. Invasive species with loud, long calls, from species with similar ecology and phylogenetic background seem the most likely to negatively impact natives, and determining the native species most likely to be impacted, and what these impacts are likely to be, should constitute the goal of future research. Answering these questions will help us to determine the degree to which soundscape intrusion by invasive species should be considered in future conservation initiatives.

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Chapter 3

An endangered bird calls less when invasive birds are calling

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Abstract

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: (i) native finch calling behaviour was affected by novel invasive bird calls, and (ii) 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.

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 (Rolland et al. 2012; Blickley et al. 2012b), 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)*, Dunnocks (*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 1976b; Immelmann 1982) that are accompanied by visual displays (Zann 1976a, 1977). These songs are inaudible to humans in the field, travelling distances of less than ten metres (Zann 1976b). 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 (i) the calling behaviour of Black-throated Finches was affected by novel invasive bird calls, (ii) they produced a general response to different invasive bird calls, (iii) they produced a general response to any novel noise (biotic or not) and (iv) 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 (Department of the Environment 2019) and state levels (Department of Environment and Science, Queensland 2017). 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 4 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 40dB 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 ten minutes of silence, in Audacity.

Playback methods

Estimation of Black-throated Finch behavioural responses to noise was based on playback. We placed a speaker (JBL^{TM} '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 (Fig. S2.1). 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 two-hour 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; R Core Team 2019) 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. 3.1). We measured a total of 154 Black-throated Finch calls, 148 Nutmeg Mannikin calls, 116 Common Myna vocalisations and 7 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

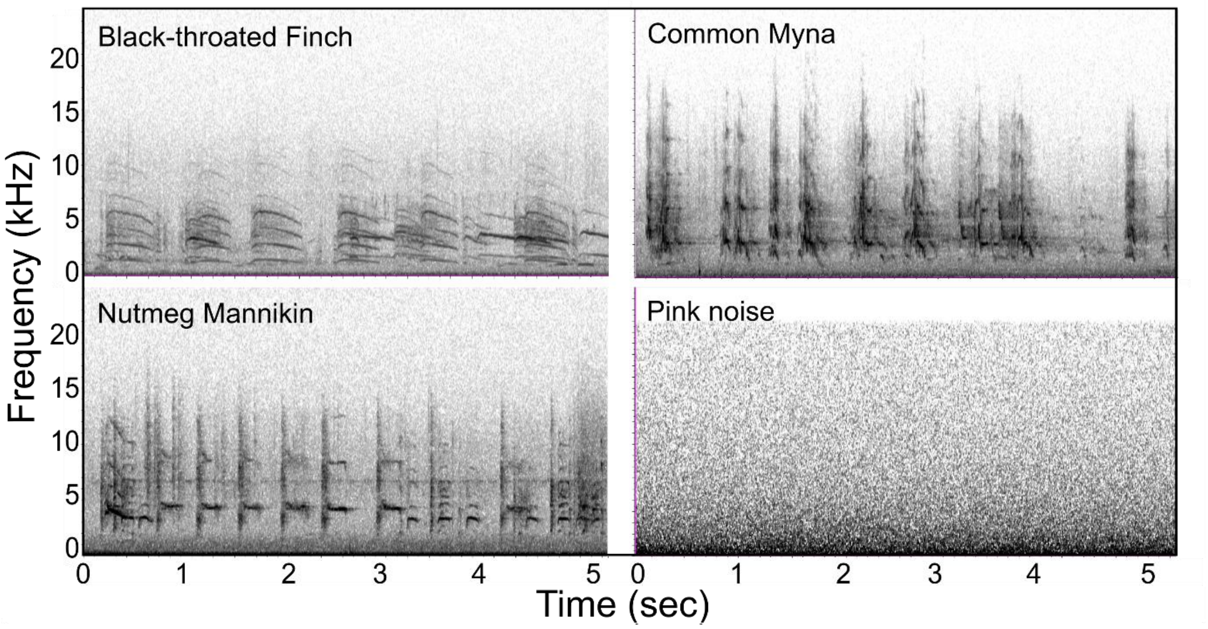

Fig. 3.1 Examples from spectrograms of Black-throated Finch calls and each of the stimulus noises.

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. 3.2).

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 minimum frequencies of the noises (Fig. 3.3). 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 3.3).

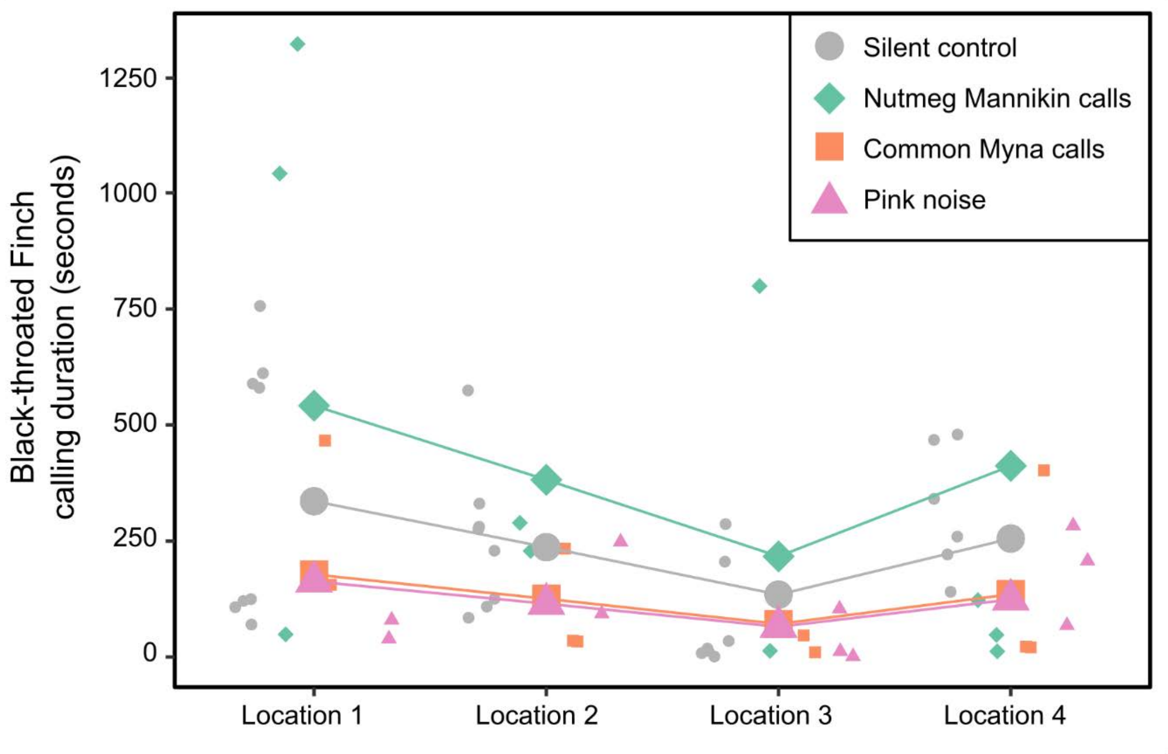

Fig. 3.2 Raw values (small points) and predicted values derived from the model (large points) of the summed duration of Black-throated Finch calling over two-hour sampling periods.

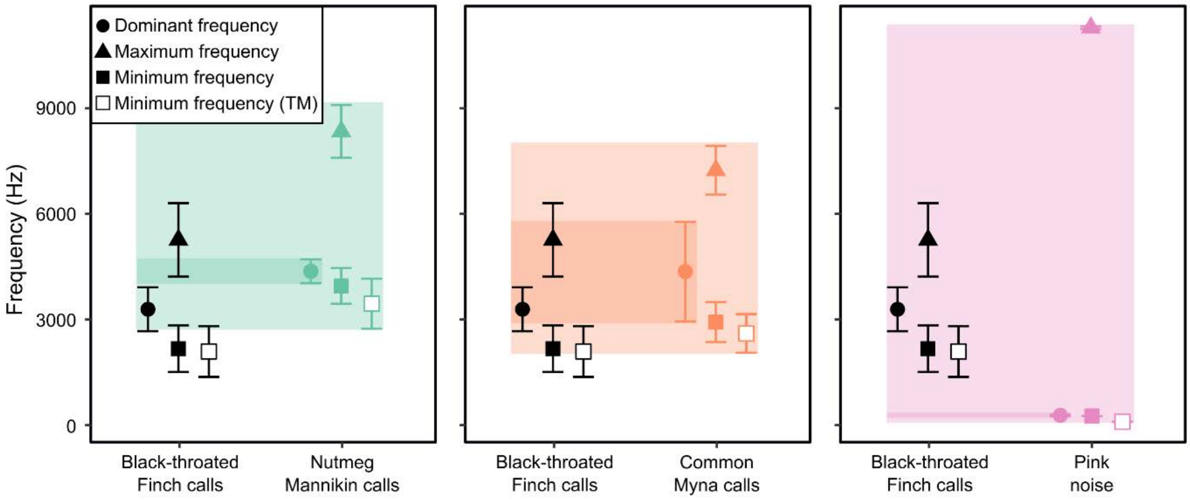

Fig. 3.3 Frequency overlap between Black-throated Finch calls and stimulus noises. Points and error bars are mean values +/– standard deviation of dominant frequency, maximum frequency and minimum frequency of Black-throated Finch calls and the stimulus noises. Minimum frequency = Frequency 5% measurement, 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 +/– standard deviation of dominant frequency of stimulus noises.

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; Orci 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; 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 to 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 estrildid 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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Chapter 4

The effect of invasive cane toad calls and synthetic noise on the acoustic repertoire of the native floodplain toadlet

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Abstract

Vocal communication is critical to a wide range of vertebrates, and its disruption may reduce fitness. Anuran males call to attract females, and the calls of invasive species may interfere with communication by reducing signalling efficacy. To investigate impacts of soundscape invasion on native species, we played invasive cane toad (*Rhinella marina*) calls and control synthetic pure tones to floodplain toadlets (*Uperoleia inundata*) on Groote Eylandt, Australia. We aimed to examine the following questions on floodplain toadlet vocal behaviour: 1) did cane toad calls and synthetic noise elicit a similar response, 2) did loud noises elicit a stronger response than soft noises, and, 3) what was the influence of noise frequencies outside those of their calls. Toadlets increased call effort in response to most noise treatments. Toadlets significantly lowered dominant frequency in response to the loud pure tones, indicating that noise amplitude had a strong influence on call spectral properties. Toadlets adjusted calls similarly in response to cane toad calls and pure tones, although cane toad calls elicited less of a response than some tones. These results revealed a general response, an increase in call effort, to noises. If invasive species calls become dominant in acoustic communities, native anurans could incur fitness costs associated with increased call effort.

Introduction

Across the globe, invasive species are spreading and becoming prevalent, and are among the top threats to biodiversity (Secretariat of the Convention on Biological Diversity 2014).

Invasive species threaten native species in a variety of ways: including consuming them, competing for resources, hybridizing, causing disease or parasite spread, or modifying habitat (Goodenough 2010). Acoustic interference is a potential threat from invasive species that has been largely overlooked (see Chapter 2). Acoustic interference could have important consequences for native species. Co-occurring species that form part of long-established acoustic communities may have evolved to partition the acoustic space, so that each species occupies its own acoustic niche (Wilkins et al. 2013). Invasive species that vocalise can introduce novel noises and could exert pressure on some native species if they interfere with communication. For example, the call of an invasive species may sound like a competitor, or mask the calls of native species, rendering them inaudible.

The presence of calling invasive species may cause native species to compensate for the effects of masking or increased competition *via* vocal plasticity. Vocal plasticity is the short-term adjustment of at least one call property in response to a noise. For example, as masking is primarily influenced by noise amplitude relative to a target signal (i.e., signal-to-noise ratio), individuals may increase call amplitude in response to increased noise (Manabe et al. 1998; Cynx et al. 1998; Lowry et al. 2012). They may also advance (Gil et al. 2015) or delay (Stanley et al. 2016) the onset of daily calling, or vary call rates (Littlejohn and Martin 1969; Schwartz and Wells 1983; Sun and Narins 2005) to avoid temporal overlap with noise. Some species also adjust call spectral properties to avoid calling in the same frequency (in Hz) range as heterospecifics (Both and Grant 2012; Medeiros et al. 2017) or other types of noise (Halfwerk and Slabbekoorn 2009; Cunnington and Fahrig 2010), because the masking potential of noise is greater if it overlaps the frequency of a target signal (Klump 1996; Lohr et al. 2003). Individuals may also increase call effort, for example, by increasing call rate or duration (Penna et al. 2005; Penna and Hamilton-West 2007; Medeiros et al. 2017), to increase call attractiveness, audibility, or both (Gerhardt and Huber 2002; Schwartz and Bee 2013). Calling is energetically costly, and ectotherms expend approximately eight times more energy during calling than when resting (Ophir et al. 2010). Thus, high call rates and long durations probably convey high stamina, and females prefer these traits (Gerhardt 1991; Gerhardt and Huber 2002; Forsman and Hagman 2006). These traits may also increase the chance that at least some calls or parts of calls are heard by females (Schwartz and Bee 2013).

Recently, a small number of studies have examined the influence of invasive species' calls on vocal plasticity in native species. All of these studies have examined anuran acoustic communities, in which calling plays an important role (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). A male anuran's success in mate attraction generally depends on a conspecific female hearing, recognising, and being attracted to his call in a noisy chorus (Gerhardt 1989). The calls of invasive anurans could, in theory, mask the calls of native anurans, or increase the level of chorus competition. In response to the calls of invasive anurans, some native anurans display vocal plasticity by adjusting spectral (Both and Grant 2012; Medeiros et al. 2017) or temporal (Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017) call properties, while others produce unmodified, stereotypical calls (Bleach et al. 2015; Tennessen et al. 2016). From these few studies, is unclear why particular anurans alter call properties while others do not. Some authors have suggested that the similarity in frequency between a native and invasive species' call (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016), or anthropogenic noise (Parris et al. 2009; Cunnington and Fahrig 2010), influences whether the native species will display vocal plasticity. However, a different study that used playback of several noises, including an invader's call, found that the degree of spectral overlap between native species' calls and playback noises was unimportant in predicting whether a signaller will display vocal plasticity, and that signallers produced stronger responses to other anuran calls than to synthetic noise (Medeiros et al. 2017). It is likely, therefore, that characteristics other than similarity in spectral properties may elicit vocal plasticity in anurans, and more work is needed to identify these characteristics. Examining the call responses of native species to invasive species and other noises that do not overlap in frequency with them, would be useful to clarify the role of noise properties apart from frequency overlap, in eliciting vocal plasticity.

In tropical Australia, the invasive cane toad (*Rhinella marina*) continues to expand its range (Markula et al. 2016), causing negative effects on ecosystems where it is introduced (reviewed by Shine 2010). Cane toads produce a low frequency, trilled, call year round (Brodie et al. 2020). Native marbled frogs (*Limnodynastes convexiusculus*) called more during periods of silence than during playback of cane toad calls (Bleach et al. 2015), suggesting that cane toad calls can affect the vocal behaviour of native anurans. In our study, we were interested in determining the influence of cane toad calls and certain noise properties on the calling behaviour of a native anuran. To do this, we exposed floodplain toadlets (*Uperoleia inundata*), to cane toad calls, and to synthetic pure tones of varying properties (high and low frequency and high and low amplitude), to determine whether particular noise properties elicit vocal plasticity in signallers. The population of floodplain toadlets we examined was naïve to cane toads and their calls. Naïve populations are useful for examining the effects of invaders, because the invader's calls are novel, so behavioural adjustments do not already exist. As cane toad calls are a common sound where they have invaded (Taylor et al. 2017; Brodie et al. 2020) it is important to understand their effect on native Australian anurans. We aimed to answer three main questions relating to floodplain toadlets' responses to noise.

Firstly, we examined if there was more of a response to novel biotic noise versus pure tones in similar frequencies. If so, we expected toadlets to make greater call adjustments in response to a cane toad call than to a tone of the same frequency and amplitude. Secondly, we aimed to assess the importance of noise amplitude. As masking is a function of signal-to-noise ratio (Klump 1996; Lohr et al. 2003), louder noises may elicit greater vocal plasticity than softer noises. Additionally, if anurans use noise levels as an indicator of the amount of competition, loud noise may elicit an increase in call attractiveness (Schwartz and Bee 2013). If noise amplitude is important, we expected floodplain toadlets to make more or larger call adjustments in response to loud than soft noises. Finally, we wanted to determine the influence of noise frequency (in Hz) on the vocal responses of toadlets. Anurans may alter calls when there is no overlap in frequency (Medeiros et al. 2017), but noise frequency may cause masking *via* mechanisms other than overlap. For example, low frequency sounds generally propagate further than high frequency sounds (Gerhardt and Huber 2002; Ey and Fischer 2009), so a signaller may perceive a lower frequency noise as more competitive than higher frequency noise. Also, low frequency calls are often attractive to females, potentially causing surrounding males to lower their own calls (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002). We exposed toadlets to noises that did not overlap in frequency with their calls, to determine whether a noise outside that of the signal may cause the signaller to alter their calls.

Methods

Study area and species

This study occurred on Groote Eylandt in the Gulf of Carpentaria, Northern Territory (13°50'50" S 136°24'56" E) in January 2018, during the wet season. Although cane toads occur on the nearby mainland, Groote Eylandt is presently toad-free (Brereton et al. 2012;

Anindilyakwa Land Council 2019). Native frogs on Groote Eylandt are, therefore, naïve to the vocalisations of cane toads.

Floodplain toadlets are native anurans that occur in the far north-eastern Northern Territory, including Groote Eylandt. They often occupy ephemeral water bodies of grasslands and woodlands during monsoonal rain (Tyler et al. 1981, 1986; Cogger 2018). Male floodplain toadlets call during the wet season (approximately mid-December to late-February) to attract females (Dostine et al. 2013). Their call consists of a raspy, single chirp with a dominant frequency of approximately 2200-2800 Hz (Tyler et al. 1981). Groote Eylandt populations of floodplain toadlets produce two variations of their call, a long call with slow pulses, and a short one with faster pulses (Tyler et al. 1986). Cane toad calls consist of a series of rapid pulses (approximately 16 pulses per second) and a dominant frequency of approximately 517 Hz (Bleach et al. 2015). In tropical Australia, cane toads call year-round, but most intensely during the wet season (Brodie et al. 2020), so that where floodplain toadlets and cane toads co-occur, there is likely temporal overlap in calling.

Stimulus noises

We were interested in the potential changes in floodplain toadlet call properties when toadlets were exposed to playback of cane toad calls and synthetic pure tones. The cane toad call stimulus consisted of a one-minute, looped recording of a natural toad call modified to reflect the median call parameters of several cane toad populations (Yasumiba et al. 2015; Muller et al. 2016). During experiments, we played cane toad calls at 68 dB SPL(C), measured with a Lutron sound level meter (model: SL-4013, C weighted), 50 cm from the speaker. The pure tones were generated in Adobe Audition 1.5 (San Jose, CA, U.S.A.) as one-minute files with a sampling rate of 44100 Hz. In experiments we called these a high-frequency, soft tone, a high-frequency, loud tone, a low-frequency, soft tone and a low-frequency, loud tone (Table 4.1). The low-frequency, soft tone included the same frequency and amplitude as the cane toad call. The frequency range of floodplain toadlets calls measured during this experiment fell approximately between the low-frequency and high-frequency noises, with little frequency overlap (Fig. 4.1).

Table 4.1 Sound properties of toad calls and pure tones used as noise stimuli. (*) indicates similar frequency and amplitude to cane toad call.

	500 Hz	**3500 Hz**
68 dB SPL(C)	Low-frequency, soft tone *	High-frequency, soft tone
90 dB SPL(C)	Low-frequency, loud tone	High-frequency, loud tone

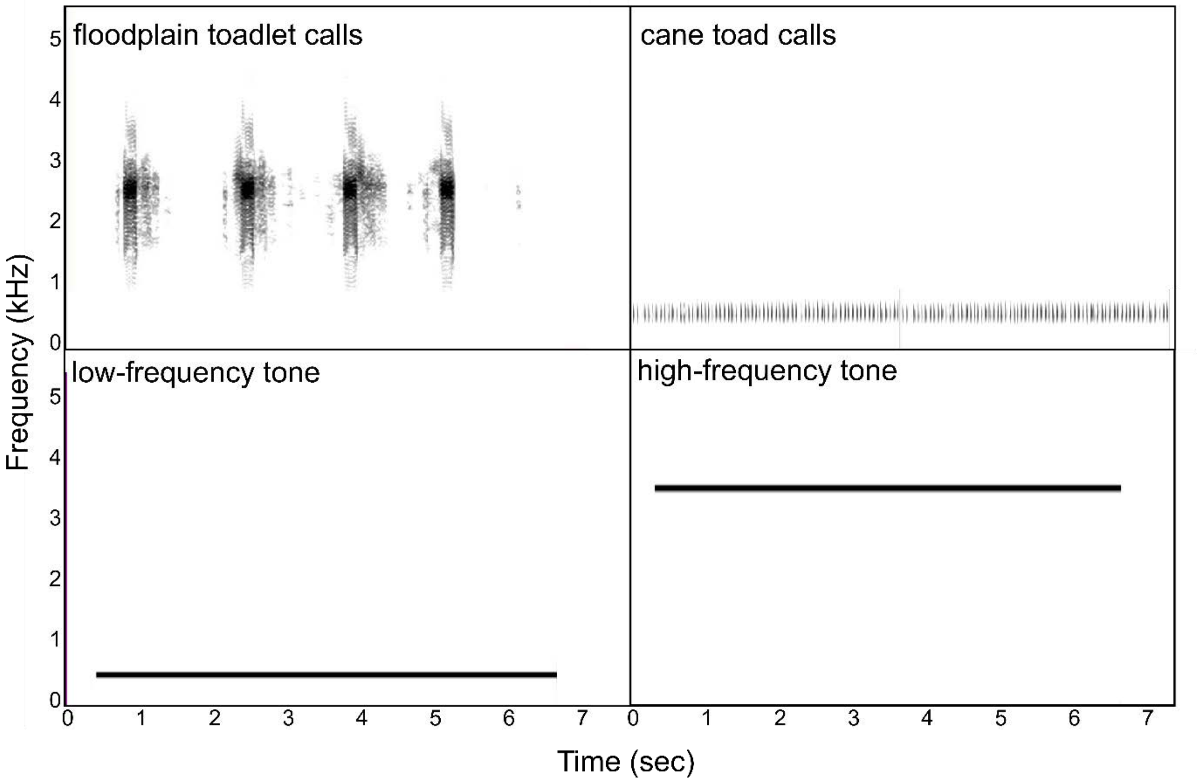

Fig. 4.1 Examples from spectrograms (2048 FFT) of floodplain toadlet calls (measured before playback during an experimental trial), cane toad calls, and pure tones.

Experiment

Experimental trials were carried out between the hours of 2000 and 0030, on 14 male floodplain toadlets. Once a calling male toadlet was located, a Sony Bluetooth speaker (SRS-X11) was placed 50 cm from the individual. Stimulus noises were played through an iPhone 6S connected to the speaker. Toadlet calls were recorded with a Rode NTF3 shotgun microphone connected to a Marantz solid state recorder (PMD661 MKII). Each individual trial lasted approximately eleven minutes. First, toadlet calls were recorded for one minute before playback of any noise. Then, each of the five noise stimuli were played in random order to individuals with at least one minute of silence between each stimulus. Recordings of calls made by male subjects were generated as WAV files with a sampling rate of 96000 Hz/24 bits. Each individual's location was recorded to ensure they were not resampled in the same night. This study was approved by James Cook University's Animal Ethics Committee (A2507).

Acoustic analysis

Measurements of floodplain toadlet call properties were taken in Raven Pro 1.5. WAV files were imported, and spectrograms generated with a fast Fourier transform. The selection spectrum was configured to a window size of 4096 samples. For each individual, we recorded; (i) the number of calls that occurred before any of the noises were played, (ii) the number of calls that occurred during each of the stimulus noises, and (iii) the number of calls that occurred in the one-minute periods after each of the noises. As five stimulus noises were played per trial, a total of eleven call counts were recorded per individual (Fig. 4.2). For each of the calls, we measured: (i) the intercall interval (the time from the end of the previous call to the beginning of the focal call), (ii) call duration, (iii) pulse rate (number of pulses per second), and (iv) dominant frequency (frequency with highest amplitude) (Fig. 4.2).

Statistical analysis

Data exploration and analyses were carried out in R (R Core Team 2019). Exploratory data analysis revealed several patterns that needed consideration. Firstly, toadlets produced both "long" and "short" call types described by Tyler et al. (1986). We considered calls longer than 0.1 sec with pulse rates slower than 87 pulses sec^{-1} 'long' calls and calls 0.1 sec or shorter and with pulse rates of 87 pulses sec^{-1} or faster 'short' calls. The remaining few calls (i.e., calls that met only one of the two conditions for classification) were considered 'intermediate' calls (Fig. S3.1). As long and short calls had distinctly different properties, we analysed them separately. Secondly, among-individual variation in call properties was extremely high, so an individual identifier needed to be included in analyses. Thirdly, we found that toadlets call in bouts, so the analysis of intercall interval was restricted to periods of silence within bouts, and excluded long periods of silence between bouts (> 4 sec). A histogram of intercall intervals demonstrated that intervals greater than four seconds were uncommon, so we considered sequential calls with intercall intervals of less than four

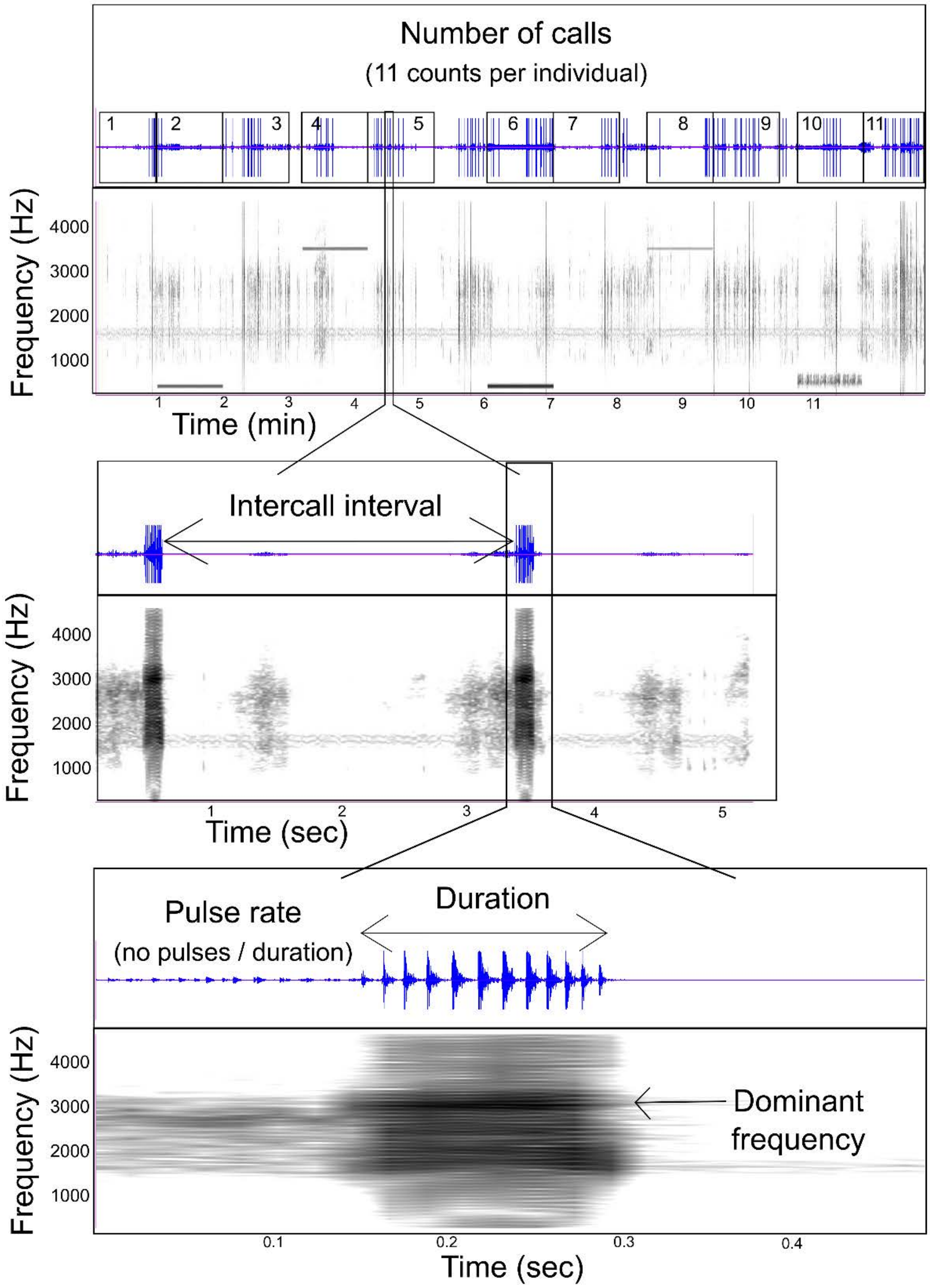

Fig. 4.2 Paired waveform and spectrogram windows from Raven Pro, showing call counts and measurements taken of floodplain toadlet (*Uperoleia inundata*) calls. Each panel is an explosion of the panel above to demonstrate the hierarchical nature of measurements. Waveforms are depicted in the upper half of each panel, and spectrograms (4096 FFT) in the lower.

seconds part of a single bout. All intercall intervals greater than four seconds were excluded from the analysis. Additionally, we only included intercall intervals that occurred either between consecutive long calls or consecutive short calls.

To determine whether treatment influenced the relative number of each call type (i.e., long or short calls), we performed a zero-inflated negative binomial generalised linear model (link = "log", parameterisation = quadratic) using the *glmmTMB* package (Brooks et al. 2017). The response variable in the model was the number of calls, and call type (i.e., long or short call), treatment, individual and the interaction between call type and treatment were included as fixed effects. Treatment was an eleven-level variable that consisted of 'before playback' (i.e., the one-minute period at the start of each individual recording, before that individual had been exposed to any noises), and 'during' and 'after' playback of each of the five stimulus sounds. Individual was a 14-level variable that consisted of individual toadlet identifiers. To examine the effect of treatment on the number of calls, we again performed zero-inflated negative binomial generalised linear models. In three separate models, we examined the total number of calls (i.e., long + short + intermediate calls), the number of long calls and the number of short calls produced by toadlets as response variables. In each model, treatment and individual were included as fixed effects. Residual plots were examined to ensure the models fitted the data.

For each of the call measurements (intercall interval, duration, pulse rate and dominant frequency), long and short calls were analysed in separate models, and treatment and individual were entered as fixed effects. The data were distributed differently at each level of the fixed effects, which violated the assumption of homogeneity in linear models. Therefore, we had to allow different variances for each level of fixed effect in our models (Zuur et al. 2009). For each call measurement, we used the *nlme* package (Pinheiro et al. 2020) to create generalised least squares models on the call properties, that either had no weighting structure, or were weighted either by treatment, individual or both. We selected the model with the best weighting structure for each call measurement, on which we then performed our hypothesis tests using Akaike's information criterion.

The final models used for hypothesis testing are summarised in Table 4.2. The significance of treatment effects on call counts was examined with a chi squared test and on measurements with a likelihood ratio test. In models in which treatment was statistically significant, we

Table 4.2 Statistical models used for hypothesis testing on each call parameter, and obtaining fitted values for PCA.

Call parameter	Model type	Fixed effects	Weighted by
Total number of calls	zero-inflated negative binomial GLM	treatment + individual	-
<u>Long calls</u>			
Number of calls	zero-inflated negative binomial GLM	treatment + individual	-
Intercall interval	generalised least squares	treatment + individual	individual
Duration	generalised least squares	treatment + individual	individual
Pulse rate	generalised least squares	treatment + individual	treatment & individual
Dominant frequency	generalised least squares	treatment + individual	treatment & individual
<u>Short calls</u>			
Number of calls	zero-inflated negative binomial GLM	treatment + individual	-
Intercall interval	generalised least squares	treatment + individual	treatment
Duration	generalised least squares	treatment + individual	treatment & individual
Pulse rate	generalised least squares	treatment + individual	individual
Dominant frequency	generalised least squares	treatment + individual	treatment & individual

assessed the differences in call counts or measurements between before and during, and during and after playback of each of the five noises with pairwise comparisons.

Once statistical models were fitted on call counts and properties individually, we assessed the overall changes to long and short calls in response to the interfering noises using a multivariate analysis. For long and short calls separately, we performed a principal component analysis on fitted values derived from the models examining the number of calls, intercall interval, duration, pulse rate and dominant frequency. Again, because there was a high degree of variability associated with among-individual differences, we firstly centred and scaled all fitted values by calculating standardised z-scores for each response for each individual to reduce among-individual variation. PCA of long and short calls were undertaken using the *vegan* package (Oksanen et al. 2019) and plotted using the *ggplot2* package (Wickham 2016).

Results

Generalised linear and generalised least squares models

Floodplain toadlets called throughout all treatments, and we recorded a total of 1509 calls from 14 individuals. The total number of calls (i.e., long + short + intermediate calls) produced by toadlets was influenced significantly by treatment ($\chi^2_{10} = 21.01$, $p = 0.02$), however, there were no significant differences in the total number of calls produced between before and during, and during and after playback of each of the noises (Table S3.1). We recorded 1077 long calls, 421 short calls, and 11 intermediate calls. All 14 individuals produced long calls, but only nine produced short calls. Toadlets produced marginally more long than short calls overall ($\chi^2_{10} = 3.62$, $p = 0.06$). The interaction between call type and treatment was not significant ($\chi^2_{10} = 12.76$, $p = 0.24$), meaning that toadlets generally produced more long than short calls during the different treatments. Because toadlets produced more long than short calls, only the analysis of long calls is reported and discussed in the main text; the analysis of short calls is appended to this thesis (Table S3.1; Table S3.2; Table S3.3; Fig. S3.2).

There was no significant effect of treatment on the number of long calls produced ($\chi^2_{10} = 13.00$, $p = 0.22$). Treatment marginally influenced intercall interval ($LRT_{10} = 17.06$, $p = 0.07$), with toadlets sometimes producing shorter intercall intervals during noise playback than before or after. Call duration was influenced significantly by treatment ($LRT_{10} = 45.10$, $p < 0.01$). Toadlets, in general, produced longer calls during noise playback than before, then shorter calls after noise playback than during. Treatment was also significant in predicting the pulse rate of calls ($LRT_{10} = 38.52$, $p < 0.01$), with toadlets generally producing slower-pulsed calls during noise playback than before, and faster-pulsed calls after noise playback than during. Finally, dominant frequency was significantly influenced by treatment ($LRT_{10} = 98.42$, $p < 0.01$). Toadlets mostly lowered dominant frequency during noise playback compared to before, but changes to dominant frequency after noise playback were not uniform across noise stimulus types. Toadlets produced much lower-frequency calls during playback of the loud tones than before, then higher-frequency calls after. Additionally, toadlets produced lower-frequency calls after playback of the toad call and soft tones than during playback. For models with significant treatment effects, pairwise comparisons of call parameters from the before and during periods, and during and after periods for each noise are shown in Table S3.1.

Principal component analysis

The first two principal components accounted for 73% of the variation in call property measurements (Table 4.3). The temporal properties were weighted heavily on PC1, and the spectral measurement, dominant frequency, was weighted heavily on PC2 (Table 4.3). The number of calls and the duration of calls were highly positively correlated, and intercall interval and pulse rate of calls were also positively correlated. Those pairs were also negatively correlated with one another, so that the lower end of PC1 included calls with long intercall intervals and high pulse rates, and the higher end of PC1 included many calls and long duration calls (Fig. 4.3). Dominant frequency was not strongly correlated with any of the other call property measurements.

The principal components demonstrated a clear shift in call properties in response to noise (Fig. 4.3). In general, toadlets called more often in response to noise treatments, and produced calls with shorter intercall intervals, longer durations, slower pulse rates and lower dominant frequencies, as demonstrated by the positive shifts along PC1 and negative shifts along PC2 from before to during noise playback. The low-frequency, loud tone caused the greatest changes in toadlet call properties, with toadlets producing the most calls, the shortest intercall intervals, and the longest, slowest-pulsed, lowest-frequency calls during playback of that tone. Toadlets responded fairly similarly to the toad call and the low-frequency, soft tone (which resembled the toad call), and these noises caused the smallest overall change to toadlet calls. Dominant frequency was highest before noise playback and lowest during playback of both the low-frequency and high-frequency loud tones. It increased slightly after playback of the loud tones, but the calls were still lower frequency than before-playback calls. Dominant frequency also decreased slightly during playback of the toad call and the low-frequency soft tone, but unlike post-playback responses to the loud tones, toadlets' dominant frequency remained unchanged after noise playback. In general, the number of calls, intercall intervals, call duration, and pulse rate reverted back towards their before-playback states after noise playback, but did not return completely.

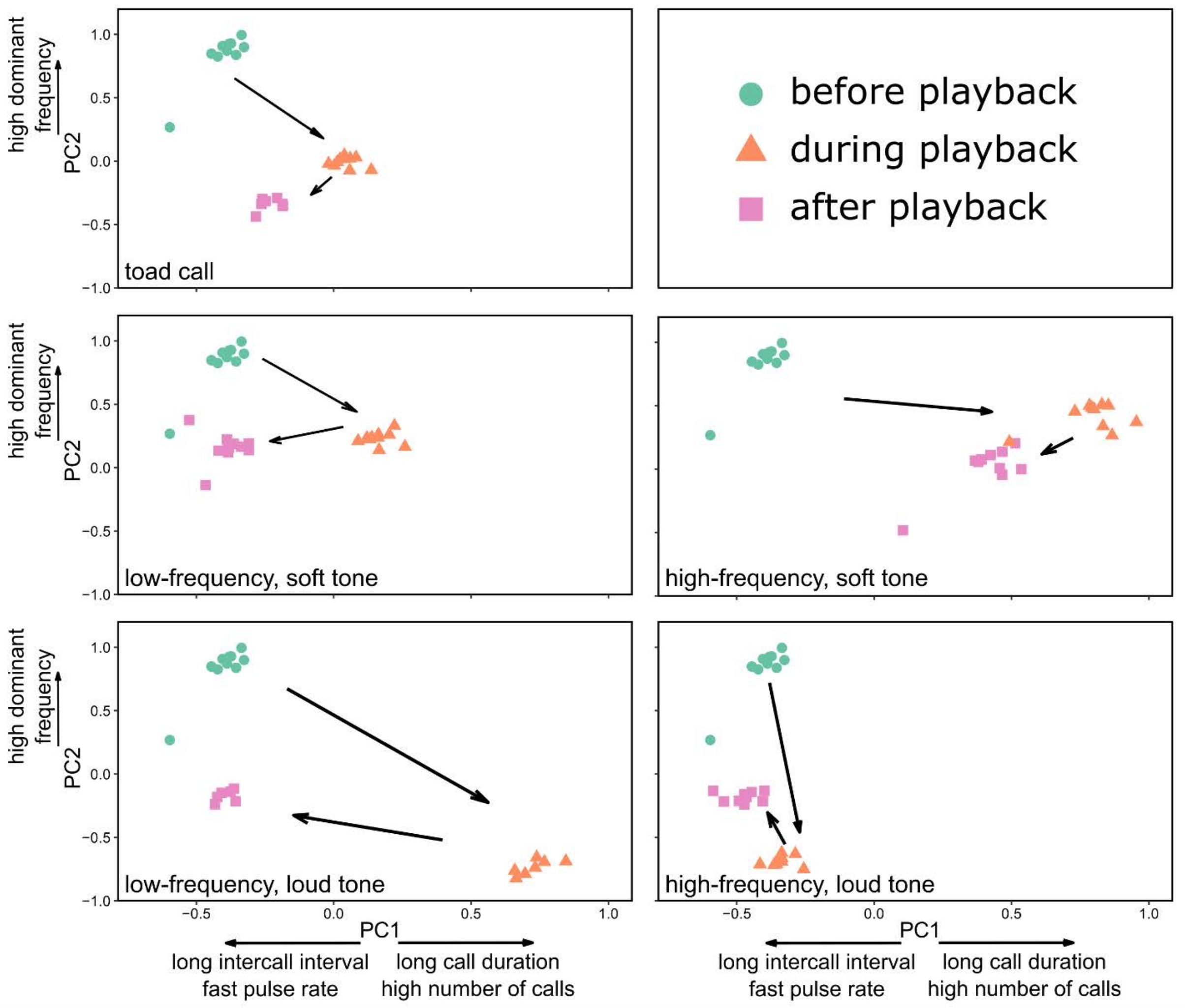

Fig. 4.3 PC1 and PC2 scores for floodplain toadlet long call property measurements. Points are the fitted values derived from the models, standardised (i.e., z-scores) by individual. Arrows indicate the shift in calls from "before" to "during" noise playback, and from "during" to "after" noise playback.

Table 4.3 Weightings of long call property measurements along principal components one and two.

	PC1	PC2
% Explained variance	50.4%	22.9%
Number of calls	1.8570	0.2691
Intercall interval	-1.3250	-0.3308
Duration	1.8470	-0.3855
Pulse rate	-1.6740	1.1634
Dominant frequency	0.8770	1.9626

Discussion

We recorded both long and short calls from floodplain toadlets during this study. It is not known whether these calls function differently (Tyler et al. 1986). We found that toadlets produce marginally more long than short calls, suggesting that long calls are the dominant vocalisation used. Short call properties appeared somewhat influenced by the noise treatments (Table S3.2; Fig. S3.2), however, given the small number of short calls recorded in this study, the conclusions able to be drawn on the influence of treatment on short call properties are limited. More work is needed to determine the function of the short and long calls and how plasticity in short calls is influenced by noise.

Floodplain toadlets responded to noise playback by adjusting properties of their long calls. When presented with any of the noise treatments (synthetic tones or the toad call), floodplain toadlets exhibited vocal plasticity by increasing the amount of time spent calling and changing the parameters of those calls. That is, they produced more calls, and calls with shorter intercall intervals and longer-duration, coupled with slower pulse rates. After call playback, toadlets generally reverted towards pre-playback temporal properties. However, call properties did not completely return to levels of the pre-playback states. This indicates that the effects of novel noise might not only be important while novel noise is present, instead calling behaviour may be affected for some time after exposure to novel noise. Overall, the observed call adjustments indicate that toadlets increase call effort in response to noise. Because calling is energetically costly (Ophir et al. 2010) and correlated with female preference in anurans (Schwartz 1986; Gerhardt 1991; Forsman and Hagman 2006; Martínez-Rivera and Gerhardt 2008), these adjustments could potentially affect toadlet fitness and reproductive success.

Rather than demonstrating specific responses to biotic versus synthetic noise, floodplain toadlets generally increased their call rate in response to all noises played during experimental trials. This was evident from the increase in the number of calls and the decrease in intercall intervals. Increased call rates occur in several anuran species exposed to interfering noise (Penna et al. 2005; Penna and Hamilton-West 2007; Kaiser and Hammers 2009; Medeiros et al. 2017), but some anuran species have reduced call rates in response to noise playback (Schwartz and Wells 1983; Sun and Narins 2005; Cunnington and Fahrig 2010; Bleach et al. 2015; Medeiros et al. 2017), while others do not alter call rates (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016). This evidence suggests that different species use different strategies to compete with noise (e.g., calling more intensely during lulls in chorus noise), even if those species are taxonomically similar, such as congeners (Penna and Hamilton-West 2007). Male anurans probably increase call rates in response to increased noise levels to enhance the attractiveness, or audibility of calls, or both (Gerhardt and Huber 2002; Schwartz and Bee 2013). High call rates likely convey high stamina, and females prefer this trait (Schwartz 1986; Gerhardt 1991; Gerhardt and Huber 2002; Forsman and Hagman 2006). Therefore, when males call in noisy choruses, they often increase call rate as a competitive mechanism (Gerhardt and Huber 2002). Anurans may interpret high noise levels as high competition, and increase call rates regardless of the noise type (Schwartz and Bee 2013). By increasing call rates to improve attractiveness, male anurans likely also increase the chance that at least some calls might be heard by females (Schwartz 1986; Gerhardt and Huber 2002). We suggest that floodplain toadlets increased call rates to improve signal attractiveness against ‘competing’ noises, and perhaps also to increase call audibility.

Floodplain toadlets generally increased duration of long calls during playback of noises, a response which has also been observed in several other anuran species (Penna et al. 2005; Penna and Hamilton-West 2007; Martínez-Rivera and Gerhardt 2008; Medeiros et al. 2017). Like call rates, increased call duration is thought to convey male anuran stamina and quality to females (Gerhardt 1991; Gerhardt and Huber 2002; Forsman and Hagman 2006; Martínez-Rivera and Gerhardt 2008) and may also increase the chance that at least some call pulses are heard by females (Schwartz and Bee 2013). However, based on several studies of different species, female anurans typically prefer longer calls when that species’ calls exceed approximately 0.5 seconds (Gerhardt and Huber 2002). Floodplain toadlet calls are much shorter than that (approximately 0.05-0.23 seconds in duration), so it is unlikely that the increase in duration during noise playback we observed was an attempt to increase call attractiveness. Furthermore, toadlets reduced pulse rates of calls during noise playback, an adjustment that typically reduces call attractiveness. High pulse rate indicates high stamina and quality in male anurans, and females tend to prefer it (Gerhardt and Huber 2002). Therefore, lowering pulse rates does not seem to be an attempt to enhance call attractiveness. Rather, reduced pulse rates may have been traded-off with increased call duration, as high pulse rate and long duration are both energetically costly strategies (Gerhardt and Huber 2002). The observed increase in call duration could be driven by a need for increased audibility. Controlled experiments have demonstrated that, when target signals are less than

0.2 seconds in duration, longer-duration sounds are easier to hear and discriminate amidst noise than are shorter sounds (Okanoya and Dooling 1990; Brumm and Slabbekoorn 2005). Therefore, when anurans, such as the floodplain toadlet, produce short calls, increasing call duration is likely a tactic that increases call audibility, rather than call attractiveness, in noisy landscapes.

When considering the overall function of temporal properties of calls operating together, it is possible that longer duration (and slower-pulsed) calls increase call audibility, and faster call rates (i.e., more calls and shorter intercall intervals) increase calls attractiveness, and further improve call audibility. Controlled studies, using female orientation and phonotaxis as a response, would be useful in determining whether increased call rates or durations, or both, improve signal audibility, attractiveness, or both, under noisy conditions. For example, manipulating call rate and duration in the absence of noise would provide baseline indications of female preference, which could then be further manipulated under noisy conditions to test audibility of different call rates and durations.

The amplitude of the noise playback affected some call parameters of floodplain toadlets. Toadlets lowered the dominant frequency of long calls in response to the two loud tones, but not soft tones or the toad call, indicating that loud noise can cause a shift in the spectral components of some species' calls. Noise that overlaps the frequency range of a signal has a higher chance of masking that signal than does a non-overlapping noise, but signal-to-noise ratio is more important than spectral overlap in influencing the severity of masking (Klump 1996; Dooling et al. 2000; Dooling 2004). Therefore, despite the lack of spectral overlap between the noises used in our study and the floodplain toadlet calls, it is possible that the adjustment to dominant frequency was an attempt by floodplain toadlets to improve call audibility. Native frogs exposed to invasive American bullfrog (*Lithobates catesbeianus*) calls and other noises, often lowered call dominant frequencies in response to noise playback, despite the adjustment increasing spectral overlap between signal and noise (Medeiros et al. 2017). Rather than lowering call dominant frequency to reduce spectral overlap, Medeiros et al. (2017) suggested frogs were instead producing calls that transmitted further and reached more females (Medeiros et al. 2017). High frequency signals attenuate and degrade more than low frequency signals (Gerhardt and Huber 2002; Ey and Fischer 2009) in terrestrial (Boonman and Kurniati 2011; Llusia et al. 2013; Ladich and Winkler 2017) and aquatic habitats (Llusia et al. 2013), so it is plausible that switching to lower-frequency calls increases the active space of anuran calls.

While we agree it is possible that these frogs may switch to a lower call frequency to increase call propagation (Medeiros et al. 2017), we also propose an alternative explanation for the observed reduction in dominant frequency. If anurans generally interpret high noise levels as high competition, they may adjust calls as if they were responding to competitors (Schwartz and Bee 2013). As low-frequency calls generally convey larger body sizes and are often preferred by females, male anurans may lower their call frequency as a competitive mechanism (Ryan and Keddy-Hector 1992; Gerhardt and Huber 2002). We suggest that during the playback of the loud tones, floodplain toadlets may have lowered the dominant frequency of long calls primarily to increase call attractiveness to females, and the active space of those calls may have consequently increased because lower frequencies attenuate less across landscapes than higher frequencies (Gerhardt and Huber 2002; Ey and Fischer 2009). To better understand the mechanism behind reduced dominant frequencies in anurans signalling amidst noise, we suggest future studies utilise female orientation and phonotaxis experiments to examine the relationships among dominant frequency, female preference and signal active space in quiet and noisy conditions.

Amplitude also affected call duration and pulse rate of the floodplain toadlet's long call, but this relationship was an interaction, also dependent upon the frequency of the noise playback. Toadlet calls had significantly longer durations and slower pulse rates during playback of the low-frequency, loud tone than before it. Although trends were similar during playback of the low-frequency, soft tone and the high-frequency, loud tone, call properties were not significantly different from those of pre-playback calls. Therefore, neither low-frequency nor loud noise were strong enough on their own alone to cause statistically significant changes to toadlet call durations or call pulse rates. Only when a noise had both characteristics did the alterations to temporal properties of calls reach statistical significance.

Although cane toad calls did not have specific effects on floodplain toadlet calls, floodplain toadlets did adjust calls when presented with cane toad calls and other noises, suggesting that toadlets make a stereotypic response to noise, including when they hear cane toad calls. By doing so, toadlets that co-occur with cane toads may be disadvantaged. Cane toads call year-round (Brodie et al. 2020) and can produce loud calls (85 dB) (Bleach et al. 2015), making them a dominant presence in acoustic communities. If cane toads reach high abundances in areas occupied by floodplain toadlets, or other anurans that respond similarly to exogeneous noise, the level of noise to which the native frogs are exposed will be increased, thus encouraging toadlets to increase call rate and duration. Although an increase in call rate or duration may increase reproductive success (Schwartz 1986; Gerhardt 1991; Forsman and Hagman 2006; Martínez-Rivera and Gerhardt 2008), it can be energetically expensive (Ophir et al. 2010), which may incur fitness costs. Future research should examine the physiological and fitness effects of cane toad calls on anurans to better understand the scope and severity of this issue.

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Chapter 5

Signaller and receiver behaviour of native Australian Peaceful Doves exposed to invasive Spotted Dove coos

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Abstract

In several vertebrate species, males vocalise to attract females and defend territories from rivals. Novel noise can mask these signals, preventing individuals from hearing and responding to them. Typically, adjustments to call properties, or vocal plasticity responses from signallers, are interpreted as mechanisms that reduce masking. For example, signallers may adjust calls in ways that increase audibility, or reduce call effort during times when calls are likely to be masked. The best way, however, to identify masking is by examining receiver behaviour because masking is, by definition, an impediment to calls reaching a receiver. Signallers may use vocal plasticity even if masking is not occurring, and adjustments by vocal plasticity do not guarantee signals will reach receivers. In this study, we examined both signaller and receiver behaviour of Australian Peaceful Doves (*Geopelia placida*), to determine if masking occurred, and if vocal plasticity mediated it. To assess this, wild individual Peaceful Doves were exposed to conspecific calls alone, and conspecific calls in concert with either the calls of the invasive Spotted Dove (*Streptopelia chinensis*), or a pure tone. At three signal-to-noise ratios, we played Peaceful Dove calls before and during playback of one of the noises, while recording the calls of the target individual. The signaller's calls were subsequently analysed. Peaceful Doves called equally frequently before and during noise playback, regardless of the noise type or signal-to-noise ratio, indicating that conspecific calls were not masked by either of the noises. Peaceful Dove signallers displayed vocal plasticity in response to the noises, by producing longer intercall intervals and shorter calls during noise playback than before, suggesting that doves adjust call temporal properties when faced with noise. There were no differences in Peaceful Dove responses to the two

noises, meaning that the calls of invasive species had no greater effect on Peaceful Dove calling behaviour than a biologically irrelevant noise. These results indicate that vocal plasticity in a signaller is an unreliable indicator of masking in a communication system.

Introduction

Vocalisation is a key form of communication in many animals. Notably, males of several animal groups (e.g., birds, anurans, orthopterans) call to attract females and ward off rival males (Kroodsma and Miller 1996; Gerhardt and Huber 2002). Effective call transmission is important for reproduction and territory maintenance, and noise can interfere with these processes (reviewed by Shannon et al., 2016). Masking is a problem caused by noise (Brumm and Slabbekoorn 2005) and occurs when a receiver cannot hear a signal properly (Klump 1996). Masking is, first and foremost, a function of signal-to-noise ratio (i.e., how loud the signal is relative to the noise) (Klump 1996; Dooling et al. 2000; Dooling 2004). Spectral overlap, in frequency or pitch, between a signal and noise also influences the severity of masking, particularly when the noise has high energy at the frequency in which the target signal occurs (Klump 1996; Lohr et al. 2003; Francis and Barber 2013; Francis 2015).

Field studies examining the effects of masking have generally focussed on the vocal behaviour of signallers (e.g., Slabbekoorn and Peet 2003; Brumm 2004; Sun and Narins 2005; Slabbekoorn and den Boer-Visser 2006; Halfwerk and Slabbekoorn 2009; Cunnington and Fahrig 2010). Vocal plasticity is the short-term adjustment of one or several call properties, and some adjustments are thought to increase call audibility and decrease masking in noisy environments (Brumm and Slabbekoorn 2005). For example, when exposed to noise, some signallers increase call amplitude, and, therefore, the signal-to-noise ratio (Brumm 2004; Lowry et al. 2012) or adjust calls to reduce spectral (Slabbekoorn and Peet 2003; Wood and Yezerinac 2006; Slabbekoorn and den Boer-Visser 2006; Parris et al. 2009; Halfwerk and Slabbekoorn 2009; Gross et al. 2010) or temporal overlap (Sun and Narins 2005; Slabbekoorn and den Boer-Visser 2006; Vargas-Salinas et al. 2014; Gil et al. 2015; Orci et al. 2016). Other vocal adjustments may conserve energy, rather than expending it on signals unlikely to reach receivers. For example, some signallers reduce call rate (Gross et al. 2010; Potvin et al. 2011) or duration (Slabbekoorn and den Boer-Visser 2006; Mockford and Marshall 2009; Nemeth and Brumm 2009) when exposed to noise.

Signallers may use vocal plasticity to avoid masking, for example if they can identify masking potential using noise properties. Loud and spectrally overlapping sounds are more likely to mask others. It is plausible that signallers may respond to these properties by altering calls to increase call audibility (so that calls reach receivers) or save energy (so that energy is not wasted on calls unlikely to reach receivers). However, changes in signaller behaviour may not be designed to avoid masking. For example, in loud choruses, when there is high competition from other males, anurans lower the frequency of their calls to sound more attractive to potential mates (Schwartz and Bee 2013). Also, vocal plasticity may not actually succeed in improving signal transmission amidst noise (Nemeth and Brumm 2010; Templeton et al. 2016). Therefore, conclusions drawn about masking from studies examining signallers are limited.

To determine if masking is occurring, we must examine receiver responses to target signals amidst noise. To date, only a small number of studies have examined receiver responses in field experiments. Such studies indicate that novel noise (i.e., new to the landscape on an evolutionary timescale) has the potential to prevent receivers from hearing conspecific signals in the wild (Halfwerk et al. 2011, 2012; Kleist et al. 2016; Templeton et al. 2016). Further work is needed to determine whether novel noise both elicits vocal plasticity (indicated by signaller behaviour) and masks stereotypical target signals (indicated by receiver behaviour) in a single system. Such work would be useful in determining whether vocal plasticity reliably indicates that the stereotypical calls of a species would be masked by a novel noise.

Columbids (i.e., family Columbidae: doves and pigeons) produce a variety of vocalisations with different functions, the most common being the perch coo (ten Cate et al. 2002). Perch coos can be produced by both sexes (Davies 1974; Ballintijn and ten Cate 1997), however, they are mostly emitted by males (ten Cate et al. 2002), and are used for territory defence and mate attraction (Craig 1908; Baptista 1996; ten Cate et al. 2002). They are typically produced in bouts (i.e., in a series of repeated perch coos) (Slabbekoorn and ten Cate 1996; Ballintijn and ten Cate 1999) from elevated positions (Slabbekoorn and ten Cate 1996), Columbids are good model systems in which to study masking, because they generally respond to conspecific perch coos (Slabbekoorn and ten Cate 1996), making it clear that receivers have heard a signal.

Peaceful Doves (*Geopelia placida*) are native Australian columbids that co-occur with an invasive columbid (Spotted Doves, *Streptopelia chinensis*) in some Australian urban areas. In this study, we quantified the responses of Peaceful Doves to conspecific perch coos in the presence of two noises, Spotted Dove perch coos and a pure tone of the same frequency. Although Spotted Doves were introduced to Australia over 100 years ago (Morcombe 2000), Peaceful Dove perch coos evolved in the absence of Spotted Doves, so the two species may experience some acoustic niche overlap, as in other avian communities including invasive species (Azar and Bell 2016). We aimed to determine whether: 1) Peaceful Dove receivers listening for conspecific calls were unable to respond when Spotted Dove perch coos or a pure tone were played, and if so, how this masking was influenced by signal-to-noise ratio; 2) Peaceful Dove signallers displayed vocal plasticity when presented with noise; and 3) the type of noise (i.e., an invasive species' call or a similar noise) affected Peaceful Doves' responses. Our study contributes to the few field-based studies that have examined the responses of both signallers and receivers to potentially masking noise, in this case the calls of an invasive species.

Methods

Study site and species

The study was conducted in suburban areas of Townsville, Australia (-19.258965ºS, 146.816956ºE) between June and August 2019. In these areas, both native Peaceful Doves and invasive Spotted Doves are common. Peaceful Dove perch coos typically consist of three elements, lasting for 0.25-0.6 seconds, with a frequency range of ~900-1200 Hz. Spotted Dove perch coos have three to four elements, last ~1.5-1.7 seconds, and typically range in frequency from 600-800 Hz (Fig. 5.1). We exposed wild Peaceful Doves to recordings of conspecific perch coos (hereby referred to as calls) alongside Spotted Dove calls or a pure tone at the same frequency. The pure tone acted as a control to determine if observed effects were specific to Spotted Dove calls.

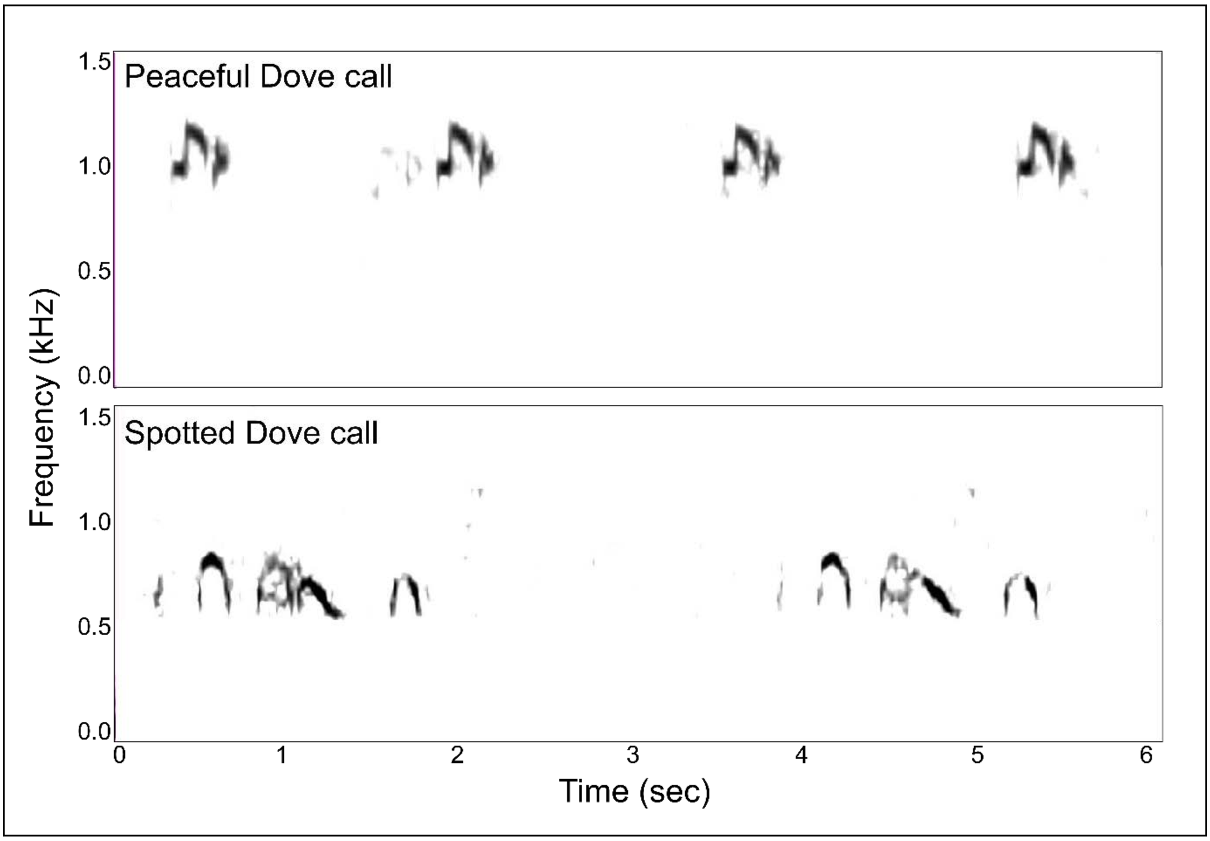

Fig. 5.1 Spectrographic examples of Peaceful Dove and Spotted Dove perch coos.

Preparation of playback sounds

Three different sounds were generated for playback: (i) Peaceful Dove calls (used to stimulate calling in target individuals), (ii) Spotted Dove calls (used to test the effects of an invader's call on target individuals), and (iii) a pure tone (control). The calls of four Peaceful Doves and two Spotted Doves that were used in playback were recorded from local, wild individuals. Recordings were made using a Rode NTF3 shotgun microphone connected to a Marantz solid state recorder (PMD661 MKII) with a sampling rate of 96000 Hz, and encoded as 16-bit wav files. Recorded calls were combined to create a single audio file per dove species, and amplitude of calls was standardised within files to ensure all calls played at the same volume. For Peaceful Doves, the audio file consisted of a 60-second bout of calling. For Spotted Doves, the audio file consisted of a 30-second bout of calling. The pure tone was generated using Audacity software (Audacity Team 2019), with a frequency of 600-800 Hz (approximately the same as Spotted Dove calls) and a sampling rate of 44100 Hz, encoded as a 30 second 16-bit wav file.

Playback trials

Peaceful Doves were located in perched positions on trees, powerlines, or fences around suburban Townsville, and were subjected to one trial each, during which their calls were recorded. All records of responses from targets were recorded with the same equipment, with the same specifications, as the playback sounds. If the target individual flew to a nearby location during the trial period, it was followed and recording continued. If it flew out of sight, the trial was abandoned. Similarly, trials in which the target individual was clearly calling to another conspecific in the vicinity were abandoned. In total, we completed trials with 60 individuals.

The influence of noise on signaller and receiver responses

The experiment was designed to obtain Peaceful Dove calls in the absence of potentially masking noise and also while such noise was occurring, to allow us to compare their responses to conspecific calls in both the presence and absence of noise (Fig. 5.2). Stimulus sounds were played from two speakers (JBL Portable 'Go') attached to a pole. Typically, individuals have greater success identifying target signals amidst noise when the signal and

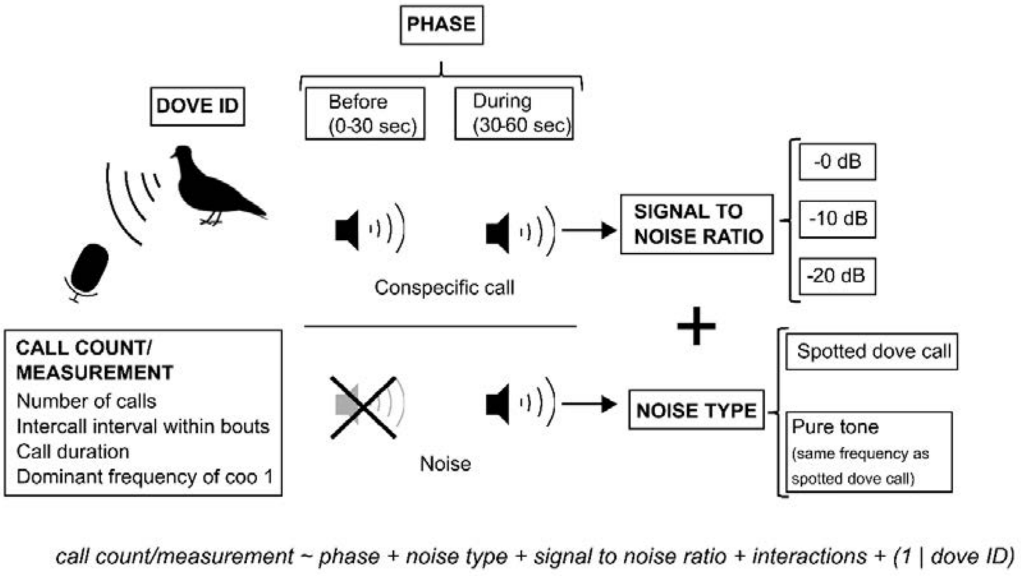

Fig. 5.2 Experimental design and statistical model formula used for analysing Peaceful Dove (*Geopelia placida*) call characteristics in the presence and absence of noise. Each Peaceful Dove was played a conspecific call and a noise, which was either an invasive Spotted Dove (*Streptopelia chinensis*) call or a pure tone of the same frequency.

noise are spatially separated (Dooling 2004; Bee 2008), so the speakers were positioned together on the pole to maximise the probability of masking occurring. The pole was extended before each trial so that the speakers were less than seven metres from the target individual at the same elevation. Sounds were played using two iPhones, connected to the speakers *via* Bluetooth. Trials ran for 60 seconds and consisted of two 30-sec phases: before- and during playback. As Peaceful Doves usually respond to the calls of conspecifics, we presented all target individuals with the pre-recorded 60-sec bout of conspecific calls for the duration of trials through the first speaker, to stimulate calling. For the first 30-sec period, no noise was played from the second speaker (labelled "before" in Fig. 5.2). For the final 30-sec period, noise (Spotted Dove calls or the pure tone) was presented through the second speaker (labelled "during" in Fig. 5.2). Half (n=30) of the trials used Spotted Dove call recordings as potentially masking noise, and the other half used pure tones.

The influence of signal-to-noise ratio on signaller and receiver responses

Spotted Dove calls and the pure tone were always played at a volume of 73-78 dB, measured at a distance of 1 m from the source, selected based on sound pressure measurements from Spotted Doves (Lutron Sound Level Meter SL-4013). We assessed the influence of volume of potentially masking noise by altering the signal-to-noise ratio of conspecific calls relative to the noises. Three levels of signal-to-noise ratio were imposed, replicated equally across trials (n=10 per signal-to-noise ratio x masking noise treatment combinations). First, conspecific calls were played at the same volume as the noise (-0 dB), then -10 dB, and then -20 dB. This design provided a decreasing volume of conspecific signal relative to noise (see Fig. 5.2). To provide different signal-to-noise ratios, we altered the signal (i.e., the conspecific call) rather than the noise (i.e., the invasive Spotted Dove call or the tone) because it was more representative of what we observed in the wild. Spotted Dove calls were played at a realistic volume, based on real measurements of their calls. Increasing the volume of Spotted Dove calls to manipulate signal-to-noise ratio would have created unrealistically loud noise. By manipulating the conspecific signal, we ensured calls never exceeded the volume of natural signals. In the wild, Peaceful Doves are exposed to conspecific calls of varying volumes, usually in relation to signaller proximity. To manipulate signal-to-noise ratio therefore, it was more realistic to decrease the volume of conspecific calls, rather than increase the volume of noise.

Acoustic analyses

Peaceful Dove calls were analysed using RavenPro 1.5 (Bioacoustics Research Program 2014). To visualise, count and measure calls, spectrograms with a window size of 8192 samples were generated from wav files. The number of calls per 30-sec phase, i.e., before or during playback, were counted. Additionally, we classed consecutive calls that occurred within three seconds of preceding calls as 'bouts' (Fig. 5.3). We measured the intercall interval between each Peaceful Dove call within each bout, excluding intercall intervals that ran over both phases. The duration of each call was also measured, as was the dominant frequency and minimum frequency of the first coo of each call (Fig. 5.3). Dominant frequency and minimum frequency were highly correlated, so minimum frequency was removed from the analysis.

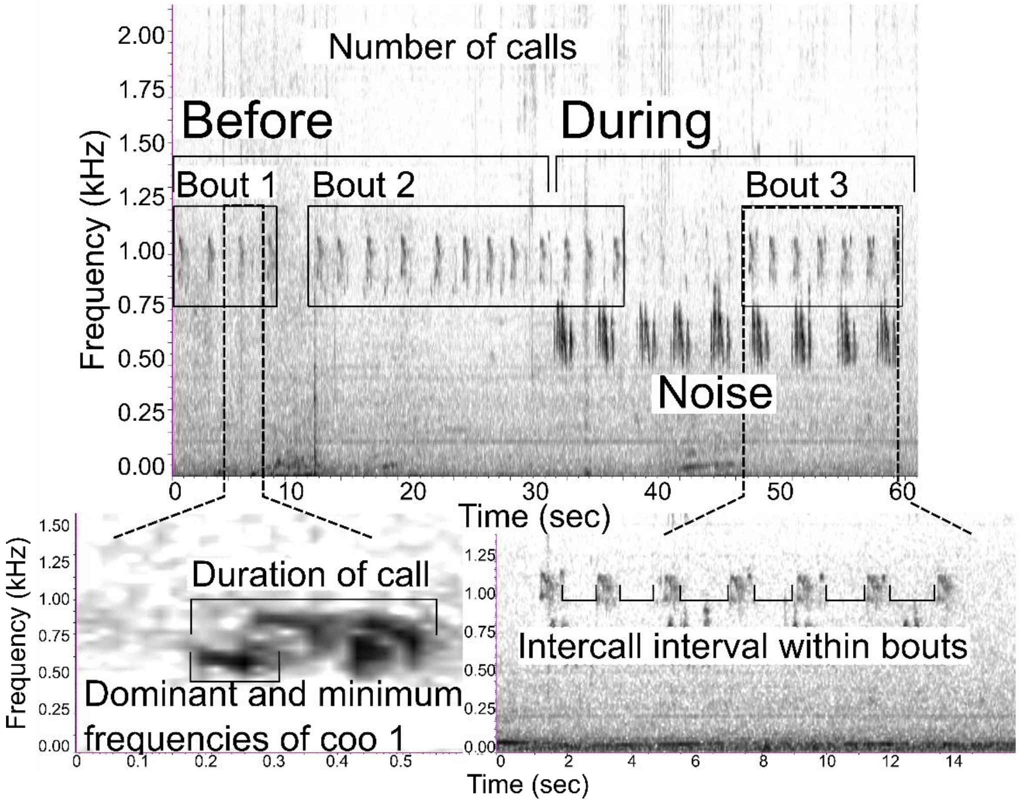

Fig. 5.3 Spectrogram window (8192 FFT) from Raven Pro, showing call counts and measurements taken of Peaceful Dove (*Geopelia placida*) coos. Each panel is an explosion of the panel above to demonstrate the hierarchical nature of measurements. Call counts and measurements were taken before and during noise playback.

Statistical analyses

All statistical analyses were performed in R (R Core Team 2019). During our experiments, we counted calls produced by focal Peaceful Doves. Because Peaceful Doves respond to calls of conspecifics, number of calls was an appropriate response variable to quantify masking by external noise sources. If there was no effect of noise on number of calls, it suggests that conspecific signals were not masked by the noise. If calling rates decreased during noise playback, it could be that conspecific calls were masked, among other things. Because Peaceful Dove calls and responses are territorial signals, we expected stronger call back responses to louder conspecific calls, i.e., calls played at a higher signal-to-noise ratio. If conspecific calls were masked as they got softer, we expected that birds would be less likely to respond to softer signals, which would give rise to a significant interaction of phase (before/during) and signal-to-noise ratio, with a significant reduction in call number when conspecific calls were the relatively quietest.

To determine whether the noises reduced the number of focal dove calls; because the counts of focal dove calls were zero-inflated, we used a zero-altered Poisson model (i.e., Poisson hurdle model) using the *lme4* package (Bates et al. 2015). The zero-altered Poisson model has two parts, first modelling zeros and non-zeros, i.e., whether or not the focal dove called, with a binomial model, and then counting values > zero using a truncated Poisson model (Zuur et al. 2009). The global model included the following fixed effects: phase (before or during), noise type (spotted dove or pure tone), signal-to-noise ratio (-0 dB, -10 dB, -20 dB), the three-way interaction, and the interactions between phase and noise type, and between phase and signal-to-noise ratio. The model also included focal dove identifier as a random effect, because we used the same focal dove before and during (Fig. 5.2). Backwards stepwise selection, based on Akaike's information criterion and likelihood ratio tests, was used to remove non-significant interactions from the model. The significance of fixed effects in the final model was assessed using likelihood ratio tests and pairwise comparisons were generated where necessary by comparing estimated marginal means using the *emmeans* package (Lenth 2019).

To determine whether focal Peaceful Doves displayed vocal plasticity in response to the noises, i.e., to quantify the signaller response, we examined call properties. As multiple call measurements were taken per dove per phase, means of each measurement were generated for each dove for each phase. Only doves for which we obtained measurements in both phases were included in analyses. To assess the influence of noise treatments on mean call characteristics, we used linear mixed effect models for each mean measurement, separately, using the *lmer* package (Bates et al. 2015). Model formulation, model selection, and significance of fixed effects were determined as described above for the number of calls.

Results

The interaction between phase, noise type and signal-to-noise ratio was not significant in any models examining call counts and properties. Similarly, the interactions between phase and noise type, and between phase and signal-to-noise ratio were not significant (Table 5.1). If conspecific calls, i.e., target signals, were masked by noise, the interaction between phase and signal-to-noise ratio would have been significant. Focal doves would have been less responsive to conspecific calls during playback of noise at the lowest signal-to-noise ratio, or

Table 5.1 Interactions removed from the final models, based on backwards stepwise selection. AIC and likelihood ratio tests were used to assess whether interactions should be included in final models.

	Interaction	DF effect	Δ AIC	LRT	P value
Number of calls (binomial)	phase * noise type * signal-to-noise ratio	2	3.01	1.00	0.61
	phase * noise type	1	5.33	0.01	0.92
	phase * signal-to-noise ratio	2	0.05	3.96	0.14
Number of calls (truncated Poisson)	phase * noise type * signal-to-noise ratio	2	3.99	0.01	1.00
	phase * signal-to-noise ratio	2	6.74	0.94	0.63
	phase * noise type	1	1.45	0.55	0.46
Intercall interval within bouts	phase * noise type * signal-to-noise ratio	2	1.86	2.14	0.34
	phase * signal-to-noise ratio	2	3.80	0.20	0.90
	phase * noise type	1	1.56	0.44	0.51
Call duration	phase * noise type * signal-to-noise ratio	2	0.18	3.82	0.15
	phase * signal-to-noise ratio	2	3.04	0.96	0.62
	phase * noise type	1	1.88	0.12	0.73
Dominant frequency	phase * noise type * signal-to-noise ratio	2	3.81	0.19	0.91
	phase * noise type	1	1.95	0.05	0.83
	phase * signal-to-noise ratio	2	1.87	2.13	0.35

when conspecific calls were at their quietest. The non-significant interaction indicated that Peaceful Dove calls were not masked by either of the noises. Interactions were not included in final models examining call counts and properties.

The number of calls produced by focal Peaceful Doves was not influenced by phase or noise type. The binomial part of the zero-altered Poisson model, quantifying whether or not doves called, demonstrated that signal-to-noise ratio was marginally significant in predicting whether Peaceful Doves called ($LRT_2 = 4.98$, $p = 0.08$; Table 5.2). Pairwise comparisons showed that doves were marginally less likely to call when the signal-to-noise ratio was -20 dB than -0 dB ($z = 1.95$, $p = 0.05$), but there were no significant differences in the probability of calling when the signal-to-noise ratio was -10 dB compared to -0 dB ($z = 1.34$, $p = 0.17$) or -20 dB ($z = 0.62$, $p = 0.53$). We expected this, because Peaceful Dove perch coos are territorial vocalisations, so louder conspecific calls should have elicited a stronger territorial response in receivers. The result was not, however, due to masking, as a significant

Table 5.2 Significance of fixed effects in models on call counts and measurements, based on likelihood ratio tests. (*) indicates statistically significant results ($P < 0.05$) and (.) indicates marginally significant results ($P > 0.05$ & < 0.10).

	Fixed effect	DF	LRT	P value
Number of calls (binomial)	phase	1	2.64	0.10
	noise type	1	1.94	0.16
	signal-to-noise ratio	2	4.98	0.08 .
Number of calls (truncated Poisson)	phase	1	0.07	0.79
	noise type	1	0.65	0.42
	signal-to-noise ratio	2	0.42	0.81
Intercall interval within bouts	phase	1	5.71	0.02 *
	noise type	1	0.00	0.98
	signal-to-noise ratio	2	3.39	0.18
Call duration	phase	1	3.20	0.07 .
	noise type	1	0.36	0.55
	signal-to-noise ratio	2	0.31	0.86
Dominant frequency	phase	1	0.01	0.92
	noise type	1	0.27	0.61
	signal-to-noise ratio	2	1.96	0.38

interaction between phase and signal-to-noise ratio would indicate masking, not significant responses to signal-to-noise ratio alone. The truncated Poisson part of the model, which only included counts greater than zero, revealed that none of the fixed effects were significant in predicting the number of calls produced by focal doves (Table 5.2).

In all models examining mean focal Peaceful Dove call properties, noise type and signal-to-noise ratio did not influence calls (Table 5.2). Phase, however, did influence temporal properties. Intercall intervals were significantly longer during noise playback than before it (Fig. 5.4A). Doves also produced marginally shorter calls during noise playback than before it (Fig. 5.4B). The dominant frequency of calls was not influenced by phase.

Discussion

In this study, we found that the calling behaviour of signalling focal Peaceful Doves was influenced by noise, in the form of invasive Spotted Dove calls and pure tones in the same frequency. Focal signallers reduced the duration of calls and increased intercall interval when the noises were played. In contrast, we found no difference before and during noise playback in the number of calls produced by focal doves, including at low signal-to-noise ratios, demonstrating that they continued to receive and respond to conspecific calls, even when noises were much louder. Thus, although Peaceful Doves could hear and respond to signals in the presence of noise, i.e., masking did not occur, they still displayed vocal plasticity in temporal call properties when presented with noise. Many studies have interpreted vocal plasticity as evidence of masking, but our results demonstrate that, at least for these birds, vocal plasticity occurred even in the absence of masking. Finally, we found that the response of the doves was not dependent on the type of noise. Thus, it made no significant difference to Peaceful Doves’ responses whether the noise was made by an invasive species or was a pure tone in the same frequency.

Receiver responses to conspecific calls amidst noise

We found no evidence that Peaceful Dove calls were masked by either of the noises. Doves are highly responsive to conspecific calls, often calling back and forth with them (Slabbekoorn and ten Cate 1996). In our experiment, target individuals called as frequently, and were as likely to call during noise playback as they were before it. When a target signal and a noise are spatially close, the masking effect of the noise is greater (Dooling 2004; Bee

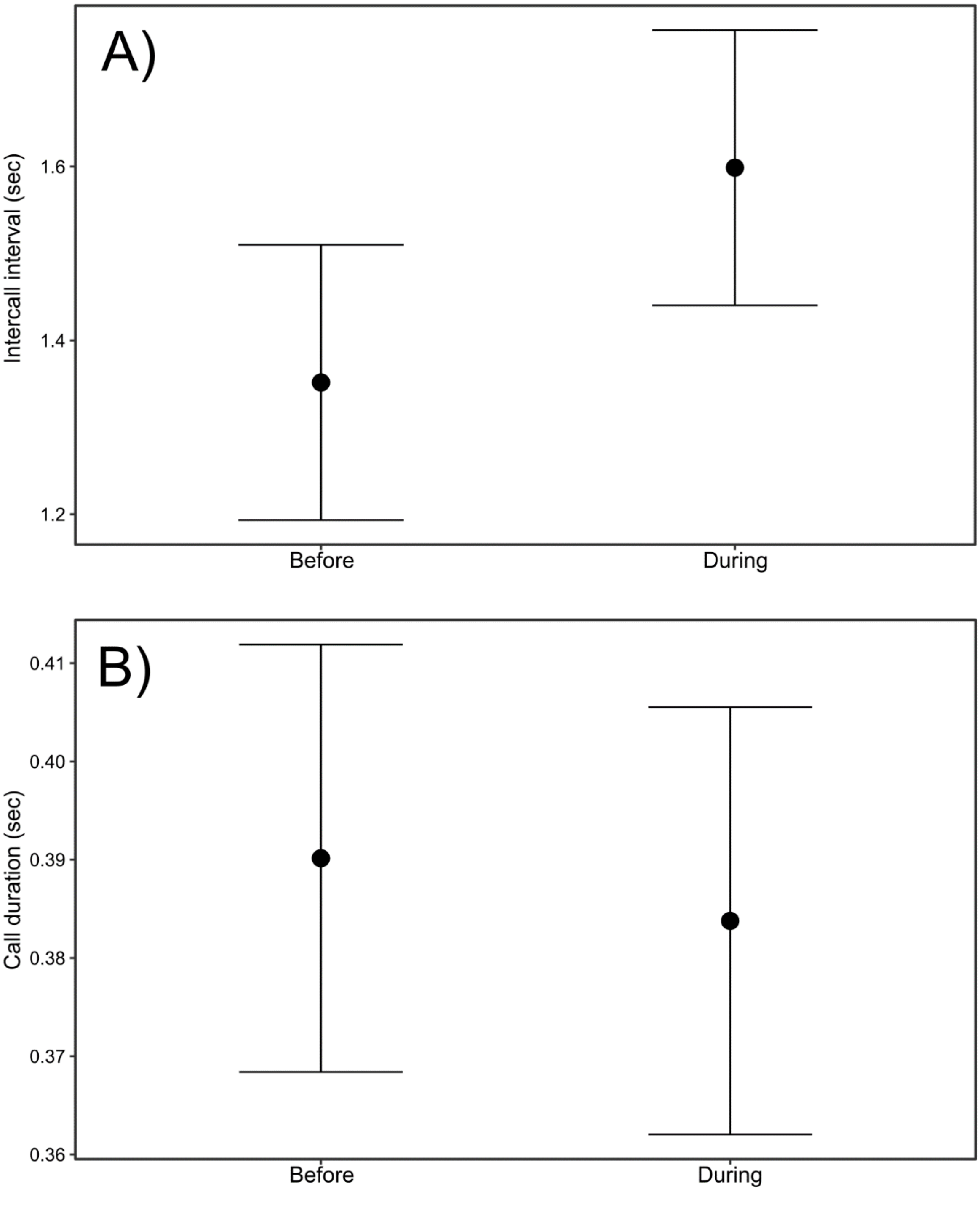

Fig. 5.4 Fitted values (points) and confidence intervals (error bars) of mean Peaceful Dove call property measurements, derived from linear mixed-effect models. Intercall interval (A) is longer during noise playback than before, and call duration (B) is shorter during noise playback than before, indicating reduced call effort during noise playback.

2008), and we projected the target signal and noise from speakers positioned together to maximise the masking potential of the noise, yet doves continued to respond to conspecific calls. Thus, conspecific calls were both received, and responded to, while an invasive species call, or similar sound, was playing. Furthermore, when conspecific calls that were harder to hear, i.e., they were projected 20 dB lower than the noises, they still elicited responses. Unlike ours, other studies have found evidence of signal masking using receivers. For example, some experiments using phonotaxis by receptive female frogs indicated that male advertisement calls could be masked by noise (Bee and Swanson 2007; Bee 2008). Similarly, operant conditioning experiments have shown that noise interferes with signal detection and discrimination in (receiving) birds (Lohr et al. 2003; Pohl et al. 2009). Also, under field conditions, birds have: failed to respond to conspecific alarm calls in the presence of road noise (Templeton et al. 2016), demonstrated delayed responses to territorial conspecific intruder songs amidst industrial noise (Kleist et al. 2016), and shown lower rates of emergence and singing in female songbirds exposed to male advertisement songs alongside noise (Halfwerk et al. 2011, 2012). So, there is evidence from receivers that noise can mask signals.

We suggest that spectral differences between Spotted and Peaceful Dove calls explains the apparent lack of masking observed here. Spotted Doves call at approximately 600-800 Hz, at a slightly lower frequency than Peaceful Dove calls (900-1200 Hz). In mammals, lower frequency signals mask higher frequency signals, a process known as ‘upward spread of masking’ (Dooling et al. 2001; Gelfand 2009). However, studies examining the responses of several other species to target signals amidst pure tone (i.e., narrowband) noise have found no evidence of upward spread of masking in birds. Instead, pure tones of the same frequency as a target signal have the greatest masking effect in birds, and the masking effect decreases as tones become more spectrally separated from the signal, regardless of whether the tone is higher or lower in frequency than the signal (Dooling and Searcy 1985; Dooling et al. 2001; Brown et al. 2002; Lauer et al. 2009). Similarly, broadband noise overlapping the frequency range of a signal has the maximum masking potential when the noise has high energy in a similar frequency (Lohr et al. 2003). Because Spotted Dove calls did not overlap the frequency range of Peaceful Dove calls, it is likely that Peaceful Doves could still hear and identify conspecific calls amidst the invader’s calls, despite the invader’s calls being up to 20 dB louder. We suggest that, in birds, spectral separation between an invasive and native call can release the native call from masking, even when the invader's call is louder than the native's call.

Signaller responses to noise

The Peaceful Doves examined in this study did display some plasticity in signal production, indicating they can adjust behaviourally to noise. Intercall intervals were longer and call durations marginally shorter during noise playback than before, so that doves spent less time calling and more time resting when the experimental stimuli were played. Male songbirds exposed to anthropogenic noise show similar responses. For example, silvereyes (*Zosterops lateralis*) reduce syllable rates in noisy urban environments (Potvin et al. 2011), and reed buntings (*Emberiza schoeniclus*) lower song rates during noise playback (Gross et al. 2010). Similarly, shorter songs or song elements have been observed in great tits (*Parus major*) (Slabbekoorn and den Boer-Visser 2006; Mockford and Marshall 2009) and blackbirds (*Turdus merula*) (Nemeth and Brumm 2009) living in noisier areas.

Bird calling is approximately twice as energetically expensive as resting (Ophir et al. 2010). Thus, energy conservation, because they might not be heard, could have been a reason Peaceful Doves reduced calling effort during playback. However, as demonstrated above, Peaceful Dove calls were not masked by Spotted Dove calls. Similarly, reductions in call rate occurred in *Oecanthus* tree crickets exposed to road noise; male signallers were less likely to call but female receivers continued to approach male calls during noise playback (Costello and Symes 2014). Peaceful Doves may always respond to noise by reducing call effort, regardless of the noises' masking ability, perhaps because they are wary or distracted by the noises, and spending more time listening or looking for the noise source and less time calling. Vocal plasticity in signallers coupled with a lack of masking indicates that signaller responses to noise are not a reliable indicator of masking, in a system. Signallers may display vocal plasticity even when there is no evidence of masking. Future studies aiming to identify masking should focus on receiver responses, or both signaller and receiver responses.

Although we observed plasticity in intercall interval and duration, the dominant frequency of Peaceful Dove calls did not change in response to noise playback. Some birds (Halfwerk and Slabbekoorn 2009; Gross et al. 2010; Bermúdez-Cuamatzin et al. 2011; Francis et al. 2011; Davidson et al. 2017) and anurans (Cunnington and Fahrig 2010; Both and Grant 2012) immediately adjust frequency components of calls to avoid frequency overlap during experimental playbacks. The lack of adjustment of dominant frequency observed in our experiment was probably due to the spectral separation between Peaceful Dove and Spotted Dove calls. Conspecific calls were audible to Peaceful Doves during playback of Spotted Dove calls (as indicated by the positive receiver responses), probably due to spectral separation, so Peaceful Dove signallers should have, in theory, no reason to adjust the dominant frequency of their calls.

The effect of invasive species' calls

This study used two different noises to examine the effects of the calls of an invasive species on a native species. We found that Peaceful Doves responded in the same way to invasive Spotted Dove calls as they did to a pure tone in the same frequency, demonstrating that Peaceful Doves showed no specialised response to the calls of Spotted Doves, *per se*. Only a few other studies have examined the effects of the calls of invasive species on native species. A native Australian frog (*Limnodynastes convexiusculus*) had longer intercall intervals during playback of invasive cane toad (*Rhinella marina*) calls, native frog (*Litoria pallida*) calls, and lawn mower noise (Bleach et al. 2015). Similarly, a native American frog (*Hyla cinerea*) shortened calls in response to both invasive Cuban treefrog (*Osteopilus septentrionalis*) calls and white noise (Tennessen et al. 2016). We suggest that in some species, individuals display vocal plasticity as a generalised response to noise, rather than to the calls of invasive species specifically.

In this study, we found that the calls of an invasive species had similar effects on the vocal behaviour of native species to a tone in the same frequency. However, this result does not mean that the calls of invasive species are of no concern. Biological invasions are increasing globally, simultaneously introducing extra noise for native species (Both and Grant 2012; Farina et al. 2013; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). The calls of invasive species can elicit vocal plasticity in native species, as demonstrated in our short-term study and others (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). In the long term, if the vocal adjustment used by native species reduces call reception or signal quality, the signal will not function as effectively, potentially impacting fitness. For example, if fewer calls are received by conspecifics or calls signal lower individual quality, individuals may lose their territory or fail to attract mates. Future studies should aim to examine the costs associated with call modification to determine whether this behavioural adjustment reduces fitness.

Conclusion

Our study addressed several knowledge gaps in the field of acoustic masking. We examined the effect of masking by assessing receiver responses in a field-based experiment, and demonstrated that a noise that does not overlap spectrally with a target signal does not mask the signal, even if it is up to 20 dB louder. We have also highlighted the importance of studying receiver, rather than signaller, behaviour when aiming to identify masking in a system. Finally, we have examined a real-world example of soundscape invasion, a first for birds. Very few studies have assessed the masking potential of the calls of invasive species on the calls of native species, and those that have, have examined anurans (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). We demonstrate that the calls of an invasive species have no greater masking effect than a different noise of the same frequency, but novel noises that affect spectral and temporal characteristics of native species' signals may be of conservation concern, because in the long run such effects could reduce fitness. Future research should examine the direct fitness effects associated with soundscape invasion.

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Chapter 6

Discussion and future directions

Discussion

In this thesis, I assessed the changes to a functionally important behaviour, acoustic signalling, in native species exposed to the calls of invasive species. I found that native species display vocal plasticity when faced with these noises. It is generally thought that signallers may use vocal plasticity to prevent their calls from being masked by noise (Luo et al. 2016). However, my results indicate that signallers sometimes use vocal plasticity for reasons unrelated to masking. The overall aim of this thesis was to determine the effects of soundscape invasion on native species. To address that aim, I asked four main questions, which I will now answer:

Do native species alter behaviour in response to the calls of invasive species?

Vocalisation is an important form of communication in several species, so changes to the amount of calling or call properties could influence how well vocalisation functions. In all three native species examined, signallers altered their vocal behaviour when exposed to the calls of invasive species. In Chapter 3, Black-throated Finches called significantly less when exposed to invasive Common Myna (*Acridotheres tristis*) calls compared to silent controls, but not invasive Nutmeg Mannikin (*Lonchura punctulata*) calls. This may have occurred because Black-throated Finches left areas exposed to noise, or remained in the areas, but called less. The different responses to the two invasive species' calls indicate that not all invasive species' vocalisations elicit behavioural changes and that the responses must have been influenced by some property of the noise itself rather than pure novelty. I suggested that overlap in frequency between the invasive bird calls and Black-throated Finch calls may be the primary cause (discussed further below), but more work is required to conclusively determine this.

The call properties of signallers were also affected. In Chapter 4, a multivariate analysis floodplain toadlet (*Uperoleia inundata*) calls clearly showed consistent shifts in call properties in response to invasive cane toad (*Rhinella marina*) calls. Consistent shifts included calling more often and producing longer calls with shorter intercall intervals. After noise playback, call properties generally reverted back towards pre-playback states, but did not do so entirely, suggesting that call properties can be affected for some time after noise exposure. Increased call effort has also been shown in other native anurans exposed to invasive anuran calls (Medeiros et al. 2017). This may not be universal, however, as some native anurans decrease call effort (Bleach et al. 2015) or continue to produce stereotypical calls (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016) when exposed to invasive anuran calls.

In Chapter 5, signalling native Peaceful Doves (*Geopelia placida*) exposed to invasive Spotted Dove (*Streptopelia chinensis*) calls similarly altered call behaviour. Signalling Peaceful Doves produced longer intercall intervals and marginally shorter calls, indicative of reduced call effort, when faced with the invader's call. In contrast to signaller responses, receivers continued to respond normally to the conspecific call during playback of the invader's call, indicating the signal was not masked. Thus, in this system, the signaller's behaviour changed in response to the invader's call; the receiver's behaviour did not.

These studies involved three distinct study systems, which encompassed unrelated species, that used acoustic signalling for different functions. All three species changed vocal behaviour in response to the calls of invasive species. This finding indicates that invasive species' calls have the potential to affect at least one important functional behaviour of native species, which could affect fitness.

Do native species respond similarly to the calls of invasive species and other noise of similar properties?

In each of the three studies, synthetic noises elicited similar responses in native species as did invasive species' calls, indicating that native species display vocal plasticity when exposed to noise, regardless of whether it belongs to an invasive animal or whether it is non-biological in origin.

In Chapter 3, Black-throated Finches reduced calling activity when exposed to pink noise, just as they did when exposed to Common Myna calls. However, Black-throated Finches did not reduce calling activity when exposed to Nutmeg Mannikin calls (see above). Thus, whether a noise came from an invasive species was unimportant in eliciting vocal plasticity in Black-throated Finches. Rather, the results suggest that pink noise and Common Myna calls share some property that inhibits Black-throated finches, but this is absent in Nutmeg

Mannikin calls (or *vice versa*). One possibility is that the response may be related to overlap in frequency between the noises and the Black-throated Finch call (discussed further in the next section), but more work is needed to fully resolve this.

In Chapter 4, Floodplain toadlets responded similarly to cane toad calls and the tone of the same frequency (in Hz) and amplitude by increasing call effort. Cane toad calls are trilled, whereas the tone was a sinusoidal wave. Fine-scale temporal properties are important for species identification in anurans, so they probably pay attention to such traits (Gerhardt and Huber 2002). Therefore, it was possible that floodplain toadlets could identify the cane toad call as biotic, based on its trilled nature. However, they produced similar types and levels of plasticity when exposed to both cane toad calls and the pure tone, suggesting that the trill of the cane toad call (and lack thereof in the tone) was unimportant in influencing vocal plasticity in floodplain toadlets. In other words, it did not matter whether the noise belonged to an invasive anuran.

In Chapter 5, Peaceful Doves were exposed to a pure tone of the same frequency and amplitude as the invasive Spotted Dove call. Peaceful Dove signallers responded to Spotted Dove calls and the pure tone by reducing call effort. These results indicate that the response is not unique to the calls of invasive species, but, rather, extended to other noise types of similar properties. Further, receivers continued to respond to conspecific calls presented alongside both Spotted Dove calls and the pure tone, suggesting that neither noise masked Peaceful Dove calls.

The findings from these aspects of my thesis demonstrate that invasive species' calls cause no greater effects on the vocal behaviour of native species than does noise in general. However, it does not, therefore, follow that the calls of invasive species are irrelevant to native species, because both sounds and calls elicited responses in signallers. Acoustic signalling is highly important in animal communication, serving several crucial functions, including maintaining contact among group members, attracting mates and defending territories (Gerhardt and Huber 2002; Marler 2004; Collins 2004). Therefore, a reduction in call effort will likely affect those processes. Conversely, increasing call effort is energetically costly (Ophir et al. 2010), so, although higher call rates or longer calls may increase signal reception, it is physically demanding on the signaller. If an invasive species calls incessantly or at the same time as a native species, that native species' calling behaviour is likely to be impacted. Therefore, even though the calls of invasive species may cause no greater effects than other noise of similar properties, they still have high potential to negatively impact native species. More work is needed to determine the fitness costs in native species associated with calling alongside invasive species.

Which noise properties are important in eliciting behavioural changes in native species?

I found varying types of vocal plasticity in the three focal study species in response to noise of varying properties. Masking is greatest when noise substantially overlaps in frequency with a signal (Klump 1996; Dooling et al. 2000; Dooling 2004). In a recipient ecosystem, the call of an invasive species will likely overlap in frequency with some native species' calls, but not others (Azar and Bell 2016). It is important to understand how native species will respond under both scenarios. The Black-throated Finch (Chapter 3) was the only focal native species in this thesis exposed to noise that overlapped the frequency range of its own call. Native floodplain toadlets (Chapter 4) and Peaceful Doves (Chapter 5) were exposed to noise that did not overlap in frequency with their own calls. I found that native species use vocal plasticity when exposed to noise, regardless of whether that noise overlaps in frequency with their acoustic signals.

It is possible that, in Chapter 3, spectral overlap between noise and conspecific signals affected calling activity in Black-throated Finches, although I did not explicitly test for it. Black-throated Finches reduced calling when exposed to noises that significantly overlapped the frequency range of their calls (pink noise and Common Myna calls) but continued to call normally when exposed to the noise with the least degree of overlap (Nutmeg Mannikin calls). Black-throated Finch calls may have been masked by pink noise and Common Myna calls, causing a reduction in conspecific call-back responses. In contrast, floodplain toadlets (Chapter 4) increased call effort in response to noise, a response thought to be used to increase the chance that calls reach receivers (Brumm and Slabbekoorn 2005) even though their calls should not have been masked (due to lack of spectral overlap). An alternative reason frogs may increase call effort in response to noisy environments is that noise increases the perception of competition. Anurans often use noise amplitude as a measurement of chorus competition intensity (Schwartz and Bee 2013), and high call rates are preferred by females because they indicate high stamina (Gerhardt and Huber 2002). Therefore, increased call effort in response to increased noise levels in floodplain toadlets could increase individual attractiveness amidst noise, rather than call audibility. Similarly, in Chapter 5, Peaceful Doves displayed vocal plasticity when exposed to noise that did not overlap their calling frequency. They increased intercall intervals and shortened calls, responses considered to save energy amidst masking noise (Costello and Symes 2014). However, Peaceful Doves continued to call back to conspecific calls amidst noise, indicating that signals were not masked (discussed further below). Therefore, reduced call effort in Peaceful Doves may have arisen for reasons unrelated to masking. One alternative possibility is increased vigilance as a fear response, where individuals spent more time looking or listening for potential intruders or threats and, consequently, less time calling.

The responses of both floodplain toadlets (Chapter 4) and Peaceful Doves (Chapter 5) show that signallers may display vocal plasticity in response to noise that does not overlap in frequency with their own vocalisations. Therefore, invasive species that call at a different frequency to native species still have the potential to affect the vocal behaviour of native species, probably due to factors other than masking. More research is needed to understand why signallers display vocal plasticity when exposed to noise outside their calling frequency.

One additional property of noise that may be important in vocal plasticity is loudness. To investigate this possible effect, I examined the influence of noise amplitude on native floodplain toadlet signallers (Chapter 4), and the effect of signal-to-noise ratio on native Peaceful Dove signallers and receivers (Chapter 5). Floodplain toadlets increased call effort in response to loud and soft noises. However, they significantly reduced dominant frequency in response to loud noises, specifically. Floodplain toadlets calls did not overlap in frequency with any of the noises, so clearly this adjustment was not being used by signallers to reduce spectral overlap (and masking effects). It is possible floodplain toadlets interpreted loud noise as a highly competitive chorus, like several anurans often do (Schwartz and Bee 2013). They may have lowered dominant frequency to make themselves sound more attractive, as low frequency calls convey large body size and are preferred by females (Gerhardt and Huber 2002). Peaceful Doves, both signallers and receivers, were not affected by signal-to-noise ratio. Signallers produced longer intercall intervals and shorter calls during noise exposure (compared to no noise exposure) at three different signal-to-noise ratios, indicating that these signal adjustments may be general responses to noise. Receivers continued to call back to the conspecific signal regardless of signal-to-noise ratio, suggesting that even soft target signals were not masked by the noises.

To predict which vocal invasive species will affect native species, and which native species will be affected, it is important to identify the noise properties that are likely to cause behavioural changes in native species. Spectral overlap between a noise and a species' own signal appears unimportant in predicting whether a species will display vocal plasticity when exposed to noise. The importance of amplitude may vary among species, but more work is needed to fully resolve this, as I only examined this question in one anuran and one bird.

Is vocal plasticity in signallers a reliable indicator of masking?

Several studies examining the effects of noise, such as anthropogenic noise, synthetic noise and the calls of invasive species, have concluded that vocal plasticity in a signaller exposed to noise indicates the presence of masking; unmodified calls would be masked by the noise which is why signallers alter call properties (Brumm and Slabbekoorn 2005). For example, Great Tits (*Parus major*) raise frequency components of song elements when exposed to urban noise (Halfwerk and Slabbekoorn 2009), which allows receivers to better detect and discriminate songs amidst urban noise (Pohl et al. 2012). Vocal plasticity is clearly used to mitigate masking in some scenarios, but it may not be a reliable indicator of masking in all communication systems. Signallers could alter calls for reasons unrelated to masking, as discussed above. Additionally, modified calls may not always reach receivers (Templeton et al. 2016), so vocal plasticity does not necessarily reduce masking.

In Chapter 5, I examined both signaller and receiver responses in Peaceful Doves to determine whether vocal plasticity reliably indicates whether masking occurs in a communication system. To assess whether Peaceful Doves use vocal plasticity when exposed to noise (invasive Spotted Dove calls and a pure tone), I measured signaller call properties. To establish whether Peaceful Dove calls are masked by noise, I quantified the call-back response of receivers to conspecific calls. Signallers displayed vocal plasticity by increasing intercall interval and reducing call duration when exposed to the noises, a strategy which may be used to conserve energy amidst masking noise (Costello and Symes 2014). However, the observed vocal plasticity was not indicative of masking in this case. Receivers continued to call back to conspecific calls during noise exposure, even at low signal-to-noise ratios, demonstrating that Peaceful Dove calls were not masked by either of the noises. A similar result was found in *Oecanthus* tree crickets exposed to road noise. Male crickets called significantly less than silent controls when exposed to road noise, yet female crickets were as responsive to male calls during road noise exposure as they were during silent controls (Costello and Symes 2014). Therefore, vocal plasticity in signallers is not a reliable indicator of masking in a communication system and it is important to include receiver responses in experiments aimed to assess masking.

Future directions

This thesis provides the first detailed examination of the effects of soundscape invasion on native species. I examined the vocal behaviour of native species in response to the calls of invasive species because vocal behaviour is affected by noise (e.g., anthropogenic noise, synthetic noise, the calls of other animals). This allowed me to compare my results with those of other studies (e.g., studies examining the effects of anthropogenic noise). I also examined vocal behaviour because vocalisation is a vital form of communication in many animal groups (Gerhardt and Huber 2002; Marler 2004; Collins 2004), so changes may impact individual fitness. This thesis provides the groundwork for future studies to explore the effects of soundscape invasion on native species, both in relation to vocal- and other important behaviours, as well as the fitness costs associated with behavioural modification, or lack thereof.

One of the major themes discussed in the thesis is the reason behind the use of vocal plasticity in signallers. Some species undoubtedly use vocal plasticity to mitigate the effects of masking, as demonstrated by improved receiver responses to modified calls (Pohl et al. 2012). I have, however, highlighted that vocal plasticity may not always be used in this sense and suggest some alternative explanations for its occurrence. Future work should explore other reasons why signallers use vocal plasticity in response to the calls of invasive species, rather than just assuming it is avoiding masking. For example, more studies of receiver responses to different conspecific calls, presented both with and without noise exposure, could be useful for assessing shifts in call audibility or attractiveness, providing insight into why signallers adjust calls. Similarly, examination of other behaviours that accompany calling in signallers could help to determine why signallers adjust call rates. For example, if signallers spend more time visually scanning or hiding, and less time calling when exposed to an invasive species' call, a reduced call rate may be better explained by fear of the noise, rather than trying to save energy on calls likely to be masked by the noise.

Although I found little evidence of masking of native species' calls by invasive species' calls and synthetic noise (except in the responses of Black-throated Finches; Chapter 3), masking is one of the most reported effects of noise on animals (Shannon et al. 2016). More detailed studies of the effects of certain noise properties are required to understand which invasive species' calls mask which native species' calls. For example, controlled lab-based studies in which subjects are exposed to conspecific calls amidst different noises would be useful in determining how loud a noise needs to be in order to mask a signal, and how masking is further influenced by the frequency (in Hz) or duration of noise and a target signal.

Changes to dominant frequency are one common way in which signallers alter their calls when exposed to noise (Brumm and Slabbekoorn 2005). In Chapter 4, I found that floodplain toadlets lower call dominant frequency when exposed to loud noise, despite spectral separation between the noises and floodplain toadlet calls, indicating that the response was not used to reduce spectral overlap. Similar findings have been reported in other anuran species exposed to noise that did not overlap in frequency with the anurans' calls. It was suggested that anurans may have lowered dominant frequency to increase call transmission distance and, potentially, the number of conspecific receivers of calls (Medeiros et al. 2017). It is certainly possible that floodplain toadlets lowered call dominant frequency for the same reason, but I have also suggested that floodplain toadlets may lower dominant frequency to increase call attractiveness. More work is required to determine the generality of this response across species, both anuran and non-anuran, and why signallers lower call dominant frequency when exposed to noise that does not overlap in frequency with their own signals.

Native species that co-occur in an acoustic community may partition the acoustic space, so that each species occupies its own acoustic niche (Krause 1987, 1993). The acoustic signals of native species may, in theory, evolve further following the introduction of invasive species that intrude on their acoustic niches. To understand the scope and severity of soundscape invasion, it would be useful to assess any evidence of acoustic niche evolution in invaded habitats. For example, opportunistic, long-term passive acoustic monitoring studies could assess changes to the acoustic niches of native species faced with the calls of an invader. If native species alter the timing of calling (e.g., call at a different time of day or year) to avoid calling alongside the invader, or alter spectral properties to avoid calling in the same frequency as the invader, it could indicate evolution of their acoustic niche. Understanding whether the calls of invasive species cause such an effect will provide insight into scale of the problem of soundscape invasion (i.e., whether effects are immediate or long term).

It has been demonstrated by the results of this thesis, and other similar studies, that animals alter behaviour when exposed to the calls of invasive species (Both and Grant 2012; Bleach et al. 2015; Tennessen et al. 2016; Medeiros et al. 2017). What remains unclear is whether there are fitness costs associated with these behavioural changes, or whether failing to adjust behaviour incurs fitness costs. For example, individuals may suffer reduced body condition if they increase call effort in response to the calls of invasive species, as calling is energetically costly (Ophir et al. 2010). Similarly, increased vigilance behaviour (e.g., more time visually scanning for predators) in response to the calls of invasive species may contribute to reduced body condition, if individuals are sacrificing foraging opportunities to maintain vigilance. The calls of invasive species may also physically affect individuals by increasing stress levels, a response which has been observed in individuals exposed to anthropogenic noise (Rolland et al. 2012; Blickley et al. 2012). Future studies should examine the physiological fitness costs associated with hearing the calls of invasive species. Finally, because some signallers alter calls in response to the calls of some invasive species, it is important to understand how modified calls influence reproductive success. If signallers are changing their calls in a way that reduces call attractiveness or audibility, it is possible they will attract fewer mates. Studies of receiver responses to stereotypical and modified conspecific calls, presented both with and without the calls of invasive species, will provide insight into the effects of call modification on mate attraction. It is important to understand the fitness costs associated with hearing invasive species' calls as it will help in assessing the degree of impact of this recently discovered invasive species' effect.

Conclusion

I started this research in the hope of addressing why native animals display vocal plasticity in response to the calls of invasive species and which noises are likely to cause vocal plasticity. This is an important and understudied potential impact of invasive species that may have unrecognised consequences. This thesis has provided important groundwork for future studies to examine the effects of soundscape invasion on native species. I found that the calls of invasive species can affect the vocal behaviour of native species in a recipient ecosystem. Native species may respond by calling more, calling less, or altering call properties such as duration and dominant frequency. They often produce similar responses to the calls of invasive species and synthetic noise of similar properties, suggesting that responses are not specific to invasive species' calls, but, rather, to noise in general. Vocal plasticity can occur in native species even when their calls do not overlap in frequency with the calls of invasive species, which indicates that vocal plasticity may not be used exclusively to mitigate the effects of masking. Furthermore, signallers may use vocal plasticity when their calls are not masked by noise, as evidenced by positive receiver responses to signals in the same communication system. Acoustic signalling serves several important functions in animal communication, so it is important to understand how these vocal changes (or lack thereof) in response to the calls of invasive species affect fitness in signallers. If invasive species call frequently, for long periods, or at the same time as native species, they have the potential to greatly impact the fitness of native signallers. Additionally, other effects of invasive species' calls on native species must be assessed in order to understand the scope and severity of soundscape invasion. Biological invasions are a major threatening process to native species (Secretariat of the Convention on Biological Diversity 2014) and in order to understand and prioritise management of invasive species, their full range of impacts must be assessed. I have demonstrated in this thesis that soundscape invasion has the potential to affect native species. Although I have suggested some avenues of future research here, there are many different pathways that could be explored, as soundscape invasion is a completely novel field of research.

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Appendix S1

Calling behaviour in the invasive Asian house gecko (*Hemidactylus frenatus*) and implications for early detection

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Abstract

Acoustic communication is common in some animal groups, with an underlying function typically associated with mating or territoriality. Resolving the function of calls is valuable both in terms of understanding the fundamental biology of the species and, potentially, for applied reasons such as detection. Early detection is a key step in exclusion and eradication of invasive species, and calling behaviour can be used in this regard. The Asian house gecko (*Hemidactylus frenatus*) is one of a minority of lizards that uses acoustic communication. However, despite how conspicuous the call is, its function remains poorly resolved. It is also one of the world's most invasive species, with exclusion via early detection being the key form of control. The aim was to resolve calling patterns and underlying function of the loud, multiple-chirp call ('chik, chik, chik…') in *H. frenatus*, in the context of using the results for developing effective methods for detection of new and establishing populations. The calls of wild *H. frenatus* were recorded to assess peaks in calling activity. Also, laboratory experiments were performed to determine which individuals call, what causes them to call and the degree of call variation among individuals. Assessment of calling behaviour in the wild revealed greater calling activity in warmer months, and five- to 10-fold peaks in calling activity at sunset and 30 min before sunrise. Laboratory experiments revealed that calls were uttered exclusively by males and primarily by adults (although juveniles can call). Males called more when they were paired with females as opposed to other males. Calls differed among geckos, including the expected negative correlation between dominant frequency and body size. The results suggest that the multiple-chirp call functions as a territory or sexual broadcast by males, perhaps containing information such as body size. Detection success can be maximised by performing acoustic surveys (by human or machine) during the calling

peaks at 30 min before sunrise and at sunset, particularly during warm nights. However, these surveys will only be effective for detecting adult males. The results also suggest that good quality recordings could potentially be used to identify individual geckos.

Introduction

Acoustic signals are used by a variety of animals, particularly orthopteran insects (e.g. crickets, katydids), frogs, birds and mammals. These groups have been well studied, especially those in which calls are audible to human hearing. Studies in orthopteran insects and frogs have generally found that call function revolves around mating (species recognition and mate choice) and/or territoriality and aggression (Gerhardt and Huber 2002). In birds and mammals, calls function for mating and territoriality in many species but also for a variety of other reasons such as alarm calls, contact calls and begging (Kroodsma and Byers 1991; Kondo and Watanabe 2009; Hollén and Radford 2009). In contrast, relatively few reptiles produce acoustic signals (audible to human hearing), and the function of these remain less well resolved.

Reptiles can produce sound through hissing, integument movement (e.g. scale rubbing, tail rattling) or vocalisations (i.e. calls) (Gans and Maderson 1973). Of the >10 000 species of squamate reptiles, few are known to use calls, and those that do are primarily geckos (Gans and Maderson 1973). Calls may be more common in geckos than other lizards because geckos are nocturnal and therefore less able to use visual signals (Marcellini 1977; Hibbitts et al. 2007). Geckos from numerous families can produce a sound, including Gekkonidae, Carphodactylidae, Diplodactylidae, Eublepharidae, Phyllodactylidae, Sphaerodactylidae and Pygopodidae (Phongkangsananan et al.). However, only some species in one family, Gekkonidae, possess true vocal cords and use regular, loud vocal communication (Russell et al.; Moore et al. 1991; Rittenhouse et al. 1997, 1998). Gekkonid species also detect sounds across a wider frequency range than other reptiles. This is because they possess complex basilar papillae of the inner ear (Manley et al. 2013). The detection distance of conspecific calls by gekkonids varies greatly across species, with estimates from less than 2 m (e.g. *Gekko japonicus* and *Gekko subpalmatus*; (Jono and Inui 2012; Chen et al. 2016) to greater than 200 m (e.g. *Gekko gecko*; (Tang et al. 2001).

The functions of gecko calls have been shown to include species recognition, mate choice and territoriality. In regards to species recognition, different calls have been demonstrated between sympatric and parapatric species of *Ptenopus* in Africa (Haacke 1976), *Ptyodactylus* in northern Africa and south-west Asia (Frankenberg 1974) and *Gekko* in south-east Asia (Yu et al. 2011; Jono 2016). In regards to mate choice, *Hemidactylus mabouia* calls appear to be primarily used for courtship behaviours (Regalado 2003), whereas calls of congeneric *Hemidactylus frenatus* are thought to be associated with aggression (Marcellini 1974). The calls of most of the studied gecko species, however, appear to function in both mate choice and territoriality (e.g. *Hemidactylus turcicus*, Frankenberg 198); *Gekko gecko*, Brillet and Paillette 1991; Tang et al. 2001; *Ptenopus garrulus*, Hibbitts et al. 2007). Although poorly studied in geckos, characteristics of calls may convey information about the signaller (e.g. body size) (Hibbitts et al. 2007), as has been shown in frogs and other groups that use acoustic communication (Gerhardt and Huber 2002).

Sexual dimorphism in calling behaviour varies across gecko species. In geckos with loud advertisement calls, these are always produced at least by males. In many gecko species, females do not call at all (Frankenberg 1982; Regalado 2003; Hibbitts et al. 2005; Dame and Petren 2006). In species where females do call, different species do one of three things: (1) females may utter the same call (e.g. the 'tokay' call of *Gekko gekko*; Tang et al. 2001); (2) females may utter subtly different versions of the call (e.g. similar calls but of different pitch between sexes in *Gekko japonicus*; Jono and Inui 2012); or (3) the female call may be a call that is completely different to the male call (e.g. calls of *Gehyra dubia*; Phongkangsananan et al.). Rittenhouse et al. (1998) suggests sex-related differences in vocal behaviour are unlikely due to laryngotracheal morphological differences (as in most anurans; Gerhardt and Huber 2002), but rather differing social behaviours or motor control of central pattern generators (as seen in the anuran *Xenopus laevis*; Tobias and Kelley 1988).

Several *Hemidactylus* gecko species are spreading rapidly, globally. Invasive geckos are easily overlooked due to their small and cryptic nature, but calls of vocal species offer a potentially important form of detection. The most significant invasive gecko globally is the Asian house gecko (*Hemidactylus frenatus*), which has spread extensively through the tropics and subtropics, including in Australia (Lever 2003; Kraus 2009; Hoskin 2011). In some parts of its introduced range, *H. frenatus* has invaded natural habitats (Cole et al. 2005; Barnett et al. 2017), where impacts on native geckos have been demonstrated (Cole et al. 2005) or inferred (Hoskin 2011; Barnett et al. 2017, 2018). *H. frenatus* remains a constant biosecurity risk to some regions, for example Barrow Island off north-west Australia (Van Der Merwe 2015) and New Zealand, where it makes up 44% of herpetofauna interceptions (Chapple et al.

2016). Key to limiting spread is detecting invaders quickly, because established populations can rapidly achieve densities that preclude eradication (Barnett et al. 2017). *H. frenatus* utters a loud and distinctive multiple-chirp call ('chik, chik, chik…'); however, the effectiveness of using calls for detection requires understanding key aspects of calling behaviour (e.g. who calls, when they call, why they call). Once these key aspects are understood, best practices for acoustic detection methods can be established.

Calling behaviour in *H. frenatus* has received attention but methodological limitations mean that some results are ambiguous. *H. frenatus* utters several call types (Marcellini 1974, 1977; Gramentz 2010; Hoskin 2011), but research has focused on the multiple-chirp call because it is much louder than the other calls and is readily heard by humans. Studies to date suggest the multiple-chirp call is uttered primarily by males but has also been reported occasionally for females (Marcellini 1974; Dame and Petren 2006). However, there is uncertainty regarding the exact caller or sex of the caller in these studies. For example, in the study by Marcellini (1974), the author acknowledges that geckos may have been misidentified as the caller (geckos were free-living rather than captive). Further, *H. frenatus* are difficult to sex, particularly in cooler months when reproductive activity is reduced (due to reduction in the testis bulge). The only reliable way to sex *H. frenatus* is by viewing the male-specific pre-cloacal pores, but even these are difficult to see without careful examination, and are barely discernible in cooler months. Studies that reported calls from captive females did not describe their sexing methods (Marcellini 1974; Dame and Petren 2006), so it is possible calling individuals were incorrectly sexed males.

The function of the multiple-chirp call in *Hemidactylus* species is thought to be related to territorial and/or sexual behaviour (Marcellini 1974; Frankenberg 1982; Regalado 2003). Peaks in calling activity have been recorded at approximately 3 hours before sunrise (Marcellini 1974), 1 hour before sunrise (Frenkel 2006), 30 min before sunset (Frenkel 2006) and at sunset (Gramentz 2010), but this has not been assessed systematically over extended periods. *H. frenatus* males have been observed uttering the multiple-chirp call in a variety of contexts, including in response to conspecific calls, immediately following male–male combats (Marcellini 1974; Gramentz 2010), when approaching a female and without any apparent stimulus (Marcellini 1974). However, once again, these studies are complicated by being observations of free-living animals. It is not known whether *Hemidactylus* calls vary among individuals, and if so, what information this conveys.

In the present study, we assessed calling behaviour in *H. frenatus* using field recordings over extended periods and laboratory-based behavioural trials. We aimed to resolve: (1) when peaks in calling occur; (2) which individuals call (i.e. male, female, juvenile); (3) what causes adult males to call; and (4) variation in calls among individuals. Beyond better resolving calling behaviour in one of the few highly vocal reptiles, we aimed to provide information that can be used to optimise detection of this globally significant invasive species.

Methods

When do Hemidactylus frenatus call?

Patterns of calling were examined in free-living geckos to assess daily and seasonal peaks in multiple-chirp-calling activity of *H. frenatus*. An SM2 songmeter (Wildlife Acoustics, Maynard, MA) sound-recording box was placed on the external deck of a suburban residence in Pallarenda, Queensland, Australia (19.2000°S, 146.7667°E), where *H. frenatus* were abundant both on the dwelling and in the garden. The dwelling was unoccupied throughout the recording periods, with no lighting. Recordings were taken between 1630 and 0730 hours over eight nights in summer from 21 December 2016, and eight nights in autumn from 20 April 2017. A 15-min recording was taken every half hour, and the number of multiple-chirp calls per 15-min recording were scored upon playback. Data for the timing of sunrise and sunset (i.e. when the top of the sun's disk is level with the horizon) were obtained from http://www.ga.gov.au/geodesy/astro/sunrise.jsp (Geoscience Australia, accessed August 2020). To determine whether the amount of calling differed between seasons, we analysed directly comparable timeslots, based on ambient light levels, using a Wilcoxon sign-ranked test. Means were generated for each 15-min timeslot, centred around sunrise and sunset for summer (December) and autumn (April), by averaging across the eight nights. The analysis was run using the 'coin' package (Hothorn et al. 2006) in R 3.6.2 (R Core Team 2016) with RStudio 1.2.5033 (RStudio Team, 2016), and plots were generated using the 'ggplot2' (Wickham 2016) package.

Gecko collection, housing and audio recording methods for laboratory-based trials of calling behaviour

Free-living *H. frenatus* were captured and used in captive experiments to determine which sexes and size classes produce the multiple-chirp call, what causes adult males to call and to assess variation in call among individuals. Between November 2016 and May 2017, geckos were collected at night from buildings and trees around James Cook University (Townsville campus) in tropical north Queensland, Australia (19.3276°S, 146.7581°E). Trials for the different experiments below were interspersed across the sampling period to incorporate any seasonal variation in calling activity. At the time of collection, snout–vent length (SVL) and sex (if mature) were recorded. Because the species can be difficult to sex, a hand lens was used to identify the presence of pre-cloacal pores in males.

Geckos were kept in controlled-temperature rooms on James Cook University campus. Geckos were housed in tetrad containers (four geckos per container) or dyad containers (two geckos per container), depending on the experiment (outlined below). Plastic containers were divided into four equal-sized areas (i.e. tetrad containers) or two equal-sized areas (i.e. dyad). Each area contained a water dish and horizontal tile refuge (Fig. 1). The containers had mesh lids. There was limited air temperature variation in the controlled-temperature rooms (ranging from 26°C to 31°C). Lighting followed a 12 h : 12 h dark : light cycle. A very dull 'night' light (70 lm) was placed in the corner of the room to simulate the low light levels geckos would typically experience in the wild (*v.* complete darkness).

Geckos were placed into separate chambers within either the tetrad or dyad containers immediately following capture and were then undisturbed throughout trials. SM2 songmeter boxes were set to record for 2.25 h following 1730 hours and following 0430 hours (i.e. around 'sunset' and 'sunrise'), and for 15 min every half hour at all other times through the night and day. Each trial was conducted over two nights. The number of multiple-chirp calls produced by geckos in all recordings was scored following playback of recordings. Each individual participated in a single trial, and at the completion of the trial the gecko was injected with an elastomer mark and released. Elastomer marking was used to avoid reusing individuals. Cages and their contents were cleaned with 100% ethanol between each use for sterilisation and to remove pheromones.

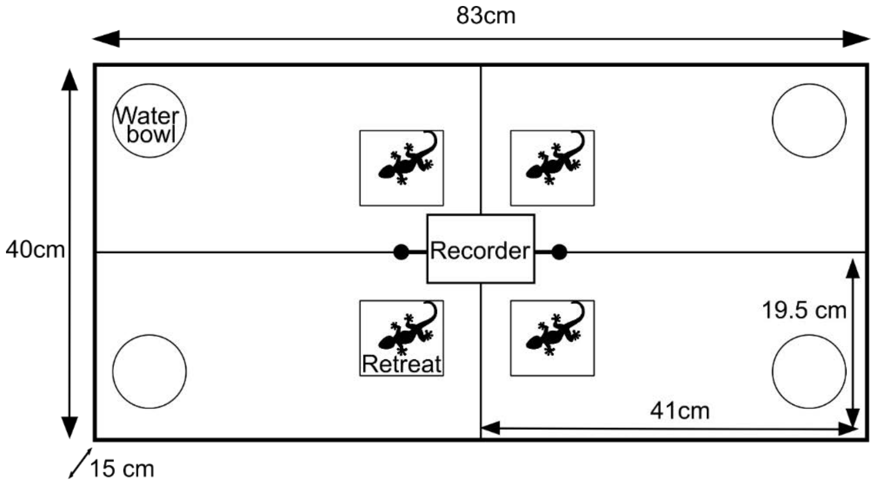

Fig. 1 Experimental chamber configuration used for tetrad trials. The songmeter recorder has two microphones on either side of it (shown here as filled circles attached to the recorder box).

Which individuals call?

Tetrad trials were used to determine which sexes and size classes of *H. frenatus* produce the multiple-chirp call. The four experimental chambers in each container were divided by solid wood, so individuals could not see each other (Fig. 1). A single gecko was placed in each chamber, so that each container ('trial') had four individually housed geckos. *H. frenatus* mature at 40–45 mm SVL (Church 1962; Amey 2013), so we considered geckos to be adults at >44 mm. Geckos were tested in the following sex and size classes: adult males (SVL >44 mm); adult females (SVL >44 mm); large juveniles (SVL 35–44 mm); medium juveniles (SVL 25–34 mm); and small juveniles (SVL <25 mm). We performed three trials of adult males (i.e. 3 × 4 males) and three trials of adult females (i.e. 3 × 4 females). All juvenile size classes were replicated six times each (i.e. 6 × 4 geckos), because sex could not be determined in these classes and we already suspected that only males call (i.e. to include approximately the same number of males and females as the adult trials). An SM2 songmeter recording box was positioned on top of each container to record calls. The calls on the songmeter recordings could not be attributed to individual geckos but instead were pooled for each trial (i.e. container).

What causes adult males to call?

Dyad trials were used to determine what causes adult male geckos to call. Seventeen male–female and seven male–male trials were performed under the same general experimental conditions described above for the tetrad trials. The two geckos in each trial were separated by a cardboard divide with a wire mesh window. This allowed for exchange of visual and chemical signals between paired geckos. The SM2 songmeter box has a directional microphone at each end, and the box was positioned over the central divider with a microphone pointing over each chamber to enable assignment and scoring of calls to each individual (Fig. 2). During the first dyad trials, it was suspected that some calls were carrying through the windowed divider, making it difficult to determine with certainty which gecko within a pair produced the call. We disregarded a total of 28 calls that could not be assigned to an individual (i.e. calls that were detected at equal amplitude on both microphones). We added extra SM2 songmeter boxes (positioned above each side), which resolved the issue in most cases. We then also added video to the trials to resolve the issue completely.

To understand what causes adult males to call, we examined the number of calls produced by males during dyad trials. Once individual call totals were obtained for all males, we assessed the influence of several covariates and fixed factors on call activity using a generalised linear model with a negative binomial distribution. A negative binomial GLM was chosen over a Poisson GLM because the data were overdispersed, and plots of model residuals revealed that a negative binomial GLM was a better fit. The number of calls produced by each male was the dependent variable in the model, and the fixed effects were: the individual's SVL; the paired gecko's SVL; the paired gecko's sex; the month (December, January, March, April, or May – there were no data for February); the interaction between the individual's SVL and the paired gecko's SVL; and the interaction between the individual's SVL and the paired gecko's sex. Backward stepwise selection was used to assess the importance of interactions and select the best model for testing the significance of covariates and fixed effects. We assessed the importance of interaction terms by examining the z-values and corresponding P-values generated in the model summary and dropped non-significant interactions. Residuals were inspected to ensure a robust model fit. Using the best-selected model, the significance of each covariate or fixed effect was assessed by, again, examining z-values and P-values. Analyses were run in using the *glm.nb* function in the 'MASS' package (Venables and Ripley 2002) in R with RStudio. Plot values were generated using the 'effects' package (Fox 2003), which calculates fitted values and standard errors of y based on a fixed effect from the model, assuming covariates are at their mean values. Values were plotted using the 'ggplot2' package.

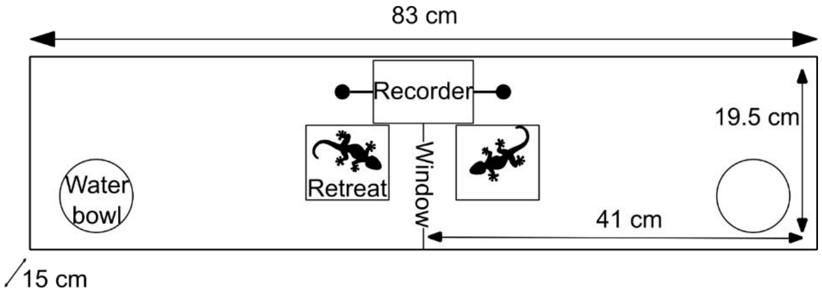

Fig. 2 Experimental chamber configuration during dyad trials. The songmeter recorder has two microphones on either side of it (shown here as filled circles attached to the recorder box).

What causes call property variation?

Using multiple-chirp calls produced during dyad trials, measurements of call properties were taken to determine what covariates and factors influence call properties, and to assess the degree of among-individual call variation. Using Raven Lite version 2.0 (Center for Conservation Bioacoustics)) with a spectrogram window size of 256, the following traits were measured for up to 10 calls per individual: call duration (beginning of first pulse to end of last pulse); number of chirps per call; and chirp rate (chirps per second). Call dominant frequency was also measured; however, these measurements were imprecise. For many individuals, entire calls and individual chirps within calls do not contain a single, clear dominant frequency peak in the spectrum and spectrogram visualisations (e.g. Fig. 3). They instead contain densely packed harmonics that vary in structure and energy distribution among, and sometimes within, individuals. In most *H. frenatus* calls, the frequency peak is broad, making it difficult to score a dominant frequency. We measured dominant frequency with a window size of 256, and then repeated the measurements with window sizes of 512 and 1024. The larger window sizes did not give clearer dominant frequency measurements. Measurements taken with a window size of 256 are, we believe, the best possible measurements that can be obtained from *H. frenatus* calls, and a level of inaccuracy in the quantification of call pitch (dominant frequency) has to be accepted due to the structure of *H. frenatus* calls.

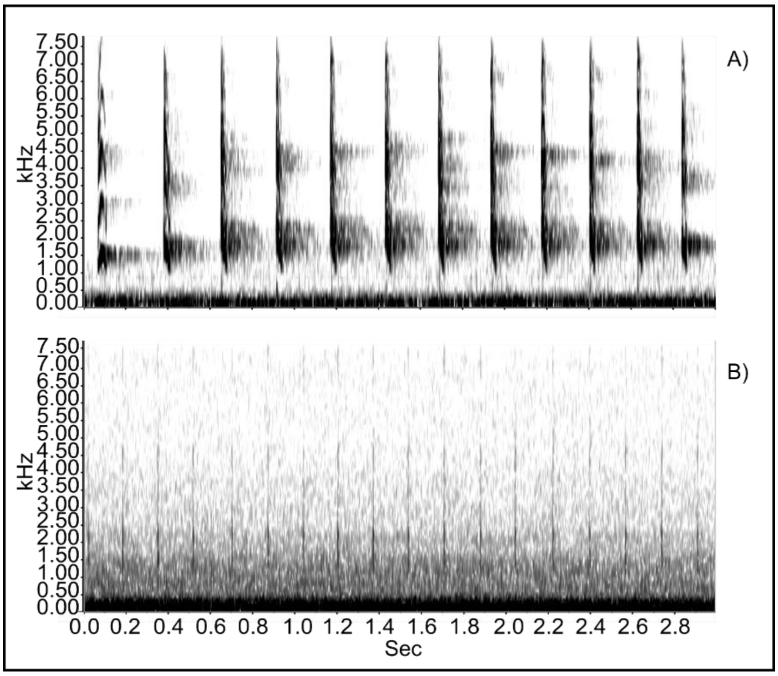

Fig. 3 A) normal multiple chirp ‘chik chik chik...’ call; B) low amplitude ‘click’ call detected in this study.

We tested whether call traits were dependent upon covariates and fixed effects using linear mixed effect models with Gaussian distribution with the *lmer* function in the ‘lme4’ package (Bates et al. 2015) in R. Call duration, number of chirps per call and chirp rate were each analysed separately as the dependent variable in a model, with the following fixed effects: the individual’s SVL; the paired gecko’s SVL; the paired gecko’s sex; the month; the interaction the individual’s SVL and the paired gecko’s SVL; and the interaction between the individual’s SVL and the paired gecko’s sex. Individual was included as a random effect. Model selection, and covariate and fixed-effect significance testing methods, were the same as described for the number of calls, except we used the ‘lmerTest’ package to examine *t*-values and *P*-values in the model summary, rather than *z*-values. Due to the inaccuracy of dominant frequency measurements (see above and Fig. 3), the relationship between SVL and call dominant frequency was assessed using a non-parametric Kendall’s rank correlation analysis in R rather than a linear model.

Using the raven measurements described above, variation in call among individuals was examined with a canonical discriminant analysis on call traits, including only individuals from dyad trials from which we could measure at least 10 calls. We performed two separate analyses: one that included dominant frequency measurements and one that omitted them (due to recognised inaccuracy in scoring dominant frequency). We also ran an additional discriminant analysis (including dominant frequency measurements) that included a single call produced by a juvenile gecko during the tetrad trials, and the calls of males that produced at least 10 calls during dyad trials, to assess differences between juvenile and adult calls. Discriminant scores were calculated in SPSS version 24.0 (IBM Corp., 2016), with calls grouped by individual. Discriminant score group centroids with 95% confidence were then calculated for each gecko in R and plotted using the ‘ggplot2’ package.

Results

When do Hemidactylus frenatus call?

In both the summer (December) and autumn (April) sampling periods, calling activity in the wild clearly peaked at 30 min before sunrise and at sunset (Fig. 4). Calling rate during these peaks was three to 10 times the background rate through the nights, with the peak at 30 min before sunrise in summer being particularly high (Fig. 4). Although the daily calling pattern was similar in summer and autumn, the amount of calling was significantly lower in the autumn sampling period ($z = 4.54$, $n = 27$, $P < 0.001$), which was also on average 3–4°C cooler at night than in the December sampling period.

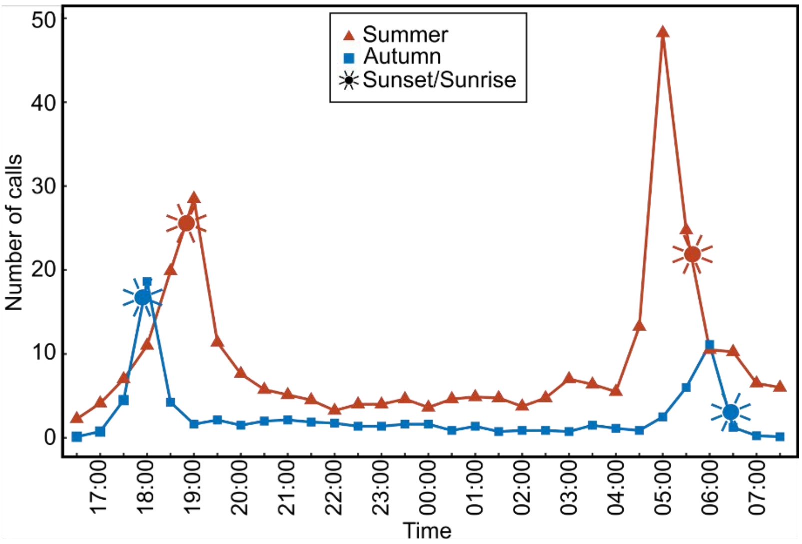

Fig. 4 Calling peaks at sunset and 30 min before sunrise in *Hemidactylus frenatus*. Mean number of calls produced during each 15-min sampling period for the summer (December) and autumn (April) recording periods in the wild.

Which individuals call?

The tetrad trials revealed that the multiple-chirp call was produced by at least one adult male in each trial (n = 12; three independent trials of four males per trial) and never produced by adult females (n = 12; three independent trials of four females per trial) (see Table 1). A single call from the medium juvenile size class (SVL 25–34 mm) was recorded (out of 24 individuals), with this being the first reported case of a juvenile *H. frenatus* multiple-chirp call. This individual was presumed to be a male (based on the adult trials), but *H. frenatus* cannot be externally sexed at this size. No calls were recorded from the large juvenile (SVL 35–44 mm) or small juvenile (SVL <25 mm) class trials (24 individuals in each of the size classes; Table 1).

What causes adult males to call?

For the number of calls produced by males, model selection revealed that the best model included no interactions (SVL × paired gecko's SVL, z = –0.512, P = 0.609; SVL × paired gecko's sex, t = 0.376, P = 0.707). The best model had a dispersion parameter of 0.98 (1 being ideal). Consistent with the wild sampling data above, the GLM revealed that the number of multiple-chirp calls produced by males in dyad trials was significantly influenced by month, with fewer calls being produced in cooler months (z = –3.946, P < 0.001; Fig. 5). The individual male's SVL influenced the number of calls produced, with large males calling the most (z = 3.587, P < 0.001; Fig. 6). The number of multiple-chirp calls was also marginally dependent upon the paired gecko's sex (z = –1.954, P = 0.051), with males calling more when they were paired with a female compared with when paired with another male (Fig. 7). The number of calls uttered by a male was not by influenced by the SVL of the paired gecko (z = –0.410, P = 0.682; Table 2).

Table 1 Total number of calls produced by *Hemidactylus frenatus* individuals of different sexes and size classes in tetrad trials

	Adult		Juveniles		
Sex and size class (SVL)	Males (>44 mm)	Females (>44 mm)	Large (35–44mm)	Medium (25–34 mm)	Small (<25 mm)
No. independent trials (containers)	3	3	6	6	6
No. individuals trialled	12	12	24	24	24
Total no. calls from all individuals	71	0	0	1	0

Table 2 Significance tests derived from models testing for significance of covariates and factors

Trait	SVL			Paired gecko's SVL			Paired gecko's sex			Month			SVL × Paired gecko's SVL		
	Test statistic	d.f.	*P*-value	Test statistic	d.f.	*P*-value	Test statistic	d.f.	*P*-value	Test statistic	d.f.	*P*-value	Test statistic	d.f.	*P*-value
Number of calls	3.587	–	<0.001	–0.410	–	0.682	–1.954	–	0.051	–3.946	–	<0.001	–	–	–
Duration	2.417	19	0.026	2.329	19	0.031	0.190	15	0.852	–0.564	17	0.580	–2.471	19	0.023
Number of chirps	–0.624	17	0.541	–0.889	19	0.386	–0.575	15	0.574	0.160	18	0.875	–	–	–
Chirp rate	–0.016	16	0.987	–0.458	17	0.653	–1.198	16	0.249	1.716	16	0.105	–	–	–

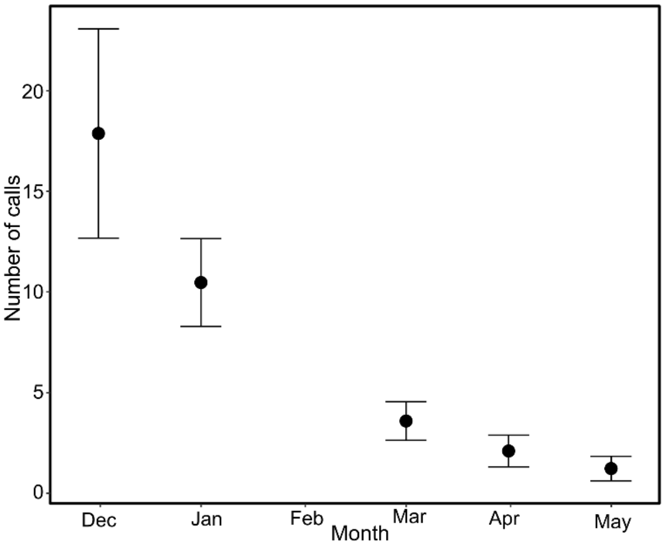

Fig. 5 Negative binomial GLM fitted values +/- SE of number of calls produced by males in dyad trials, predicted by effect of month. Male Hemidactylus frenatus call more in the hotter mid-summer months of December and January.

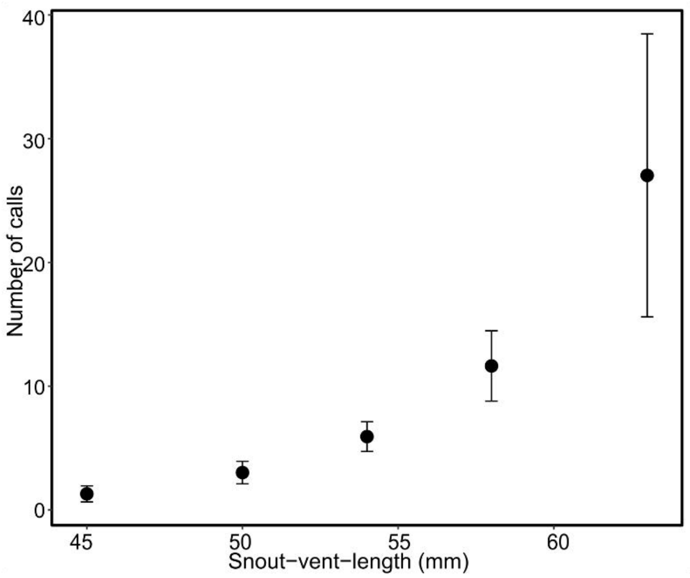

Fig. 6 Negative binomial GLM fitted values +/- SE of number of calls produced by males in dyad trials, predicted by effect of SVL. Larger male *Hemidactylus frenatus* call more than smaller males.

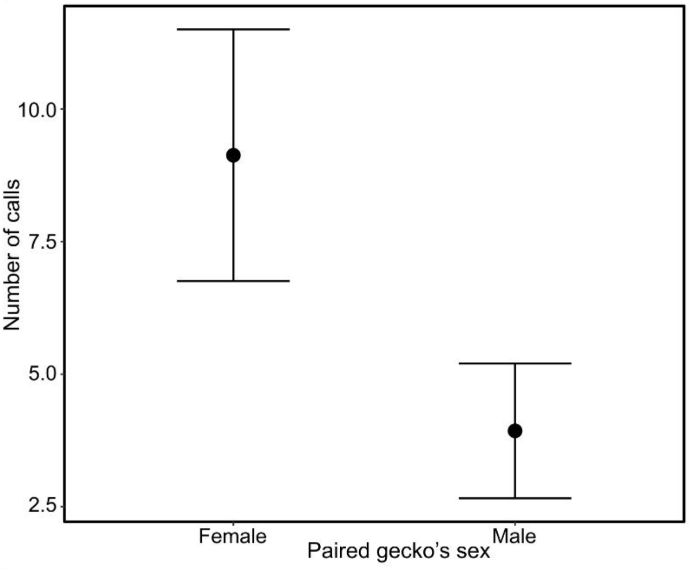

Fig. 7 Negative binomial Generalised Linear Model (GLM)-fitted values ± s.e. of number of calls produced by males in dyad trials, predicted by effect of the paired gecko's sex. Male calling in *Hemidactylus frenatus* is dependent on the sex of the gecko they are paired with, with males calling significantly more when paired with females than with other males.

What causes call property variation?

Model selection revealed that the best linear model of call duration excluded the interaction between the individual's SVL and the sex of the paired gecko ($t_{14} = 1.139$, $P = 0.273$), and included the interaction between the individual's SVL and the SVL of the paired gecko. Males produced significantly longer calls if they were small ($t_{19} = 2.417$, $P = 0.026$), or if the paired gecko was small ($t_{19} = 2.329$, $P = 0.031$). Large males also produced longer calls when they were paired with relatively smaller individuals, and the duration of small individuals' calls was less affected by the paired gecko's size than large individuals' calls were (i.e. the interaction; $t_{19} = -2.471$, $P = 0.023$; Fig. 8).

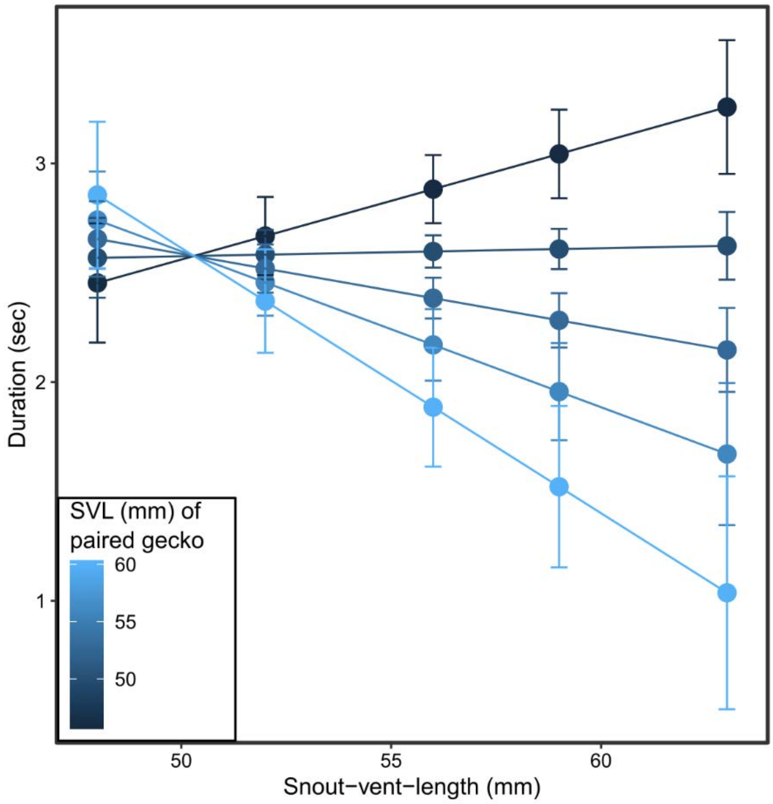

Fig. 8 Linear model fitted values ± s.e. of call duration, predicted by the interaction. Call duration in male *Hemidactylus frenatus* is dependent upon the interaction between an individual's snout–vent length (SVL) and the SVL of the gecko it is paired with, with large males producing significantly shorter calls when paired with another large gecko, than with a small gecko. The duration of small male calls is less affected by the SVL of the gecko it is paired with.

Model selection revealed that the interaction terms were non-significant for models of the number of chirps per call (SVL × paired gecko's sex, $t_{14} = 0.874$, $P = 0.396$; SVL × paired gecko's SVL, $t_{19} = -1.769$, $P = 0.093$) and for chirp rate (SVL × paired gecko's sex, $t_{14} = -0.517$, $P = 0.614$; SVL × paired gecko's SVL, $t_{16} = 0.851$, $P = 0.407$). Accordingly, the final models for the number of chirps per call and chirp rate did not include interactions. We found no relationship between number of chirps per call or chirp rate and the fixed effects of: SVL of the individual, SVL of the paired gecko, the sex of the paired gecko or the month (Table 2).

Despite our inability to precisely measure dominant frequency, the Kendall's rank correlation analysis showed that dominant frequency was significantly negatively associated with SVL in adult males ($r = -0.223$, $P < 0.001$) (Fig. 9).

Substantial call variation was evident among individuals. The call from the single recorded juvenile from the tetrad trials was obviously different to adult male calls from dyad trials analysed in this study, with this difference being driven primarily by higher dominant frequency and higher chirp rate (Fig. 10, Table 3). Significant call variation was also found among the adult males recorded. In the canonical discriminant analysis that included dominant frequency, 53% of calls were classified to the correct individual, with the biggest drivers of among-individual differences being dominant frequency and chirp rate (Fig. 11a; Table 4). Because dominant frequency was influenced by a gecko's SVL (outlined in the correlation analysis above), it is likely the gecko's size influenced this result. In the canonical discriminant analysis without dominant frequency, among-individual variation was less, but 35% of calls could still be classified to the correct individual (Fig. 11b) driven primarily by differences in chirp rate (Table 5). Because chirp rate was not significantly influenced by any of the covariates or factors outlined above in the linear model results, the among-individual variation observed in the discriminant analyses was unlikely to be influenced by these covariates and factors.

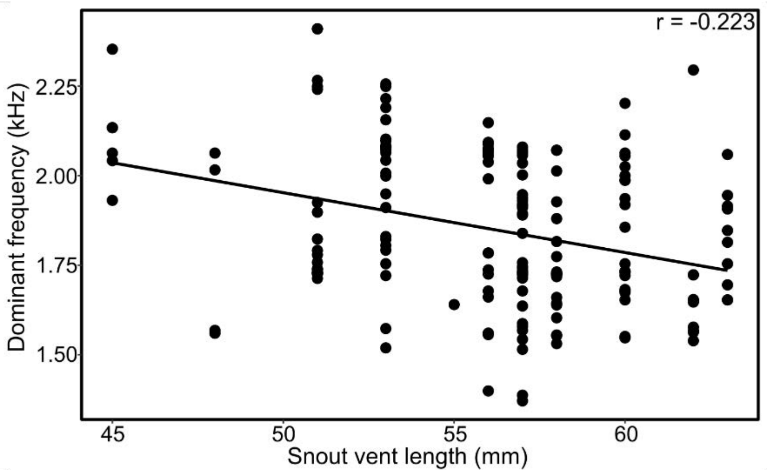

Fig. 9 Call dominant frequency is negatively correlated with body size (SVL) in Hemidactylus frenatus, with smaller males producing higher pitched calls and vice versa (r = -0.223, N = 171, P < 0.001).

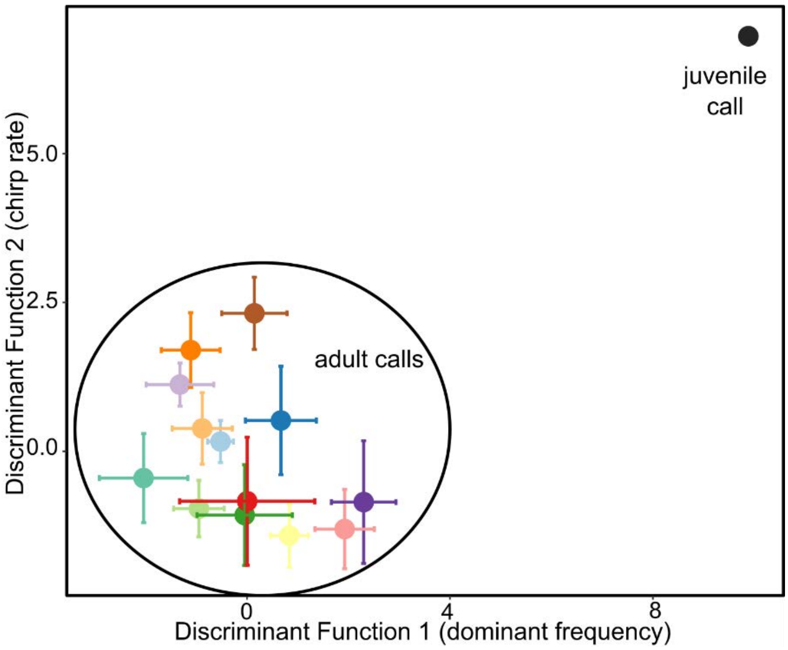

Fig. 10 The single call produced by a juvenile Hemidactylus frenatus in this study was extremely different from calls produced by 13 adult males. Canonical discriminant score centroids with 95% confidence for individual geckos, calculated from 10 calls, with the addition of the single juvenile call recorded during this study.

Table 3 Call trait loadings on canonical discriminant functions for analysis that included juvenile gecko. Note duration has been dropped from the analysis.

	Function 1	Function 2	Function 3
% of variance	50.8	39	10.2
Number of chirps	0.573	-0.040	0.935
Chirp rate	-0.702	0.844	-0.047
Dominant frequency	0.688	0.671	-0.312

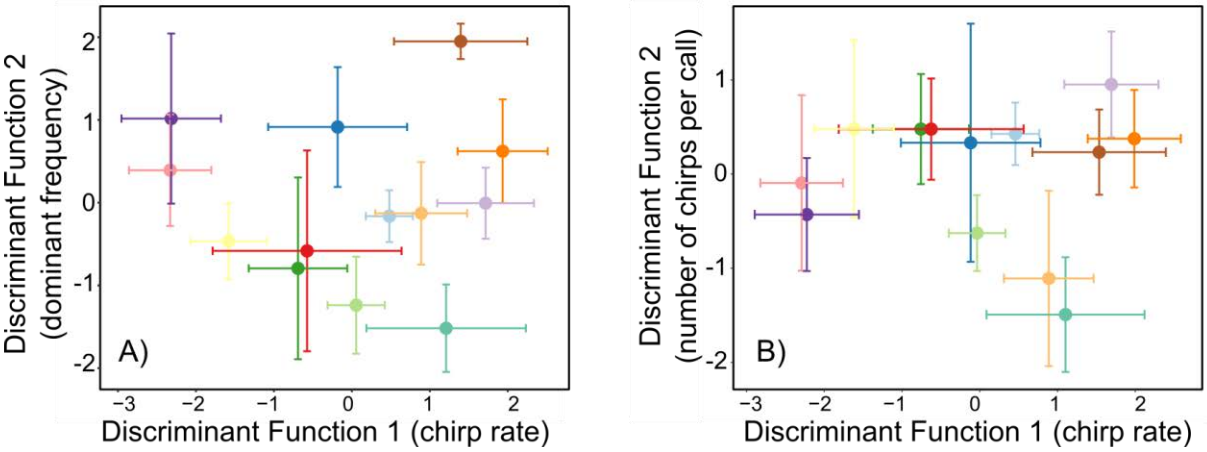

Fig. 11 Canonical discriminant score centroids, with 95% confidence intervals, calculated for each gecko with more than 10 recorded calls. Individual *Hemidactylus frenatus* produce different, but overlapping, multiple-chirp calls. Discriminant scores in (*a*) are calculated from dominant frequency, duration, number of chirps per call and chirp rate, and in (*b*) are calculated from the same variables, except dominant frequency.

Table 4 Call trait loadings on canonical discriminant functions for analysis that included dominant frequency. Note duration has been dropped from the analysis.

	Function 1	Function 2	Function 3
% of variance	59.7	26.6	13.7
Number of chirps	-0.453	0.283	0.958
Chirp rate	1.084	0.174	-0.040
Dominant frequency	-0.076	0.977	-0.246

Table 5 Call trait loadings on canonical discriminant functions for analysis that excluded dominant frequency. Note duration has been dropped from the analysis.

	Function 1	Function 2
% of variance	80.4	19.6
Number of chirps	-0.442	1.001
Chirp rate	1.094	-0.002

Incidental findings

The ‘churr’ call was detected during both male-only and female-only tetrad trials, meaning that both males and females produce this call. This call type had only previously been recorded for males (Marcellini 1974). Additionally, a low amplitude, fast-paced, long-duration ‘clicking’ call (Fig. 3) was commonly detected in sound files from both male–female and male–male dyad trials. The call was detected as louder on the male side of the enclosure during male–female trials. It is therefore possible that this soft clicking call, like the multiple-chirp call, is produced exclusively by males.

Discussion

The present study presents the most comprehensive assessment of calling behaviour in *H. frenatus*. Our results show peak calling at 30 min before sunrise and at sunset, and in warmer months. The loud, multiple-chirp calls are produced almost exclusively by adult males but can also be uttered by juveniles (presumably also only males). Large males call more than small males, and males call more in the presence of females than of other males. Males can also alter properties of the call depending on social environment. We found that large males produce shorter calls when they are paired with another large individual, as opposed to a small individual. The duration of small males’ calls is less affected by the paired gecko’s size. Males also produce a soft, clicking call, which differs from the multiple-chirp call, when in the presence of both males and females.

Daily peaks of wild *H. frenatus* multiple-chirp call activity occur at 30 min before sunrise and at sunset. These times are associated with the beginning and ending of nocturnal activity, and movement between daytime refugia and evening feeding areas (Marcellini 1974). Frenkel

(2006) found similar daily peaks in Costa Rican *H. frenatus*, and suggested this was due to warmer air and surface temperature at sunrise and sunset. However, temperature is an unlikely explanation in our data because our largest call peaks were at 30 min before sunrise, when temperatures are lowest. Natural light cues are a more likely explanation of this trend. In both the summer and autumn study periods, peak calling activity was tightly associated with 30 min before sunrise and at sunset, despite nearly an hour's difference between the summer and autumn sunrise and sunset times. Samples of peak calling periods at 30 min before sunrise in summer also coincided with the start of cicada choruses, suggesting they were responding acoustically to the same first light cue.

Calling activity of free-living and recently captive *H. frenatus* was significantly higher in mid-Summer (December and January) than in Autumn (March, April, May). This may be explained by the higher average temperature (on average 3–4°C warmer at night) and hence gecko activity. The body temperature of *H. frenatus* closely matches air and substrate temperature (Marcellini 1976). The mid-summer calling peak may also reflect seasonal breeding behaviour. Peak calling in our study was at 30 min before sunrise in summer (December) (Fig. 4; Fig. 5). Increased general activity or breeding activity in summer may drive males to call more to defend territories or advertise to potential mates, particularly as they return to their diurnal refuges. We noticed a decline in reproductive condition in the autumn period (smaller hemipenes in males, and fewer gravid females). Calling activity in *Gekko gecko* has been shown to peak with androgen levels and gonadal mass during the height of their mating season (Tang et al. 2001).

The multiple-chirp call of *H. frenatus* is uttered regularly by adult males and never by adult females. This finding is consistent with previous studies examining calling behaviour of congeners *H. mabouia* (Regalado 2003) and *H. turcicus* (Frankenberg 1982). However, two previous studies of *H. frenatus* concluded that females also utter the multiple-chirp call, albeit rarely compared with males (Marcellini 1974; Dame and Petren 2006). Marcellini (1974) acknowledged the difficulty in determining exactly which gecko uttered a call, as the geckos were wild and often called from secluded locations out of direct sight. It is plausible that Marcellini (1974) misidentified some females as the calling gecko, while a nearby male went undetected. Marcellini (1974) also reported three calls from captive females, and Dame and Petren (2006) similarly reported a call from a captive female. However, neither study described their method for identifying sex, and it is possible calling individuals were males incorrectly sexed as females. *H. frenatus* is difficult to sex due to a small testicular bulge (especially when not in peak breeding condition), small hemipenes and male pre-anal pores that are inconspicuous without use of a hand lens or macro photography. Given the complete absence of calls from females in our study, despite a large sample size (n = 29), compared with the abundance of calls from males, we believe previous records of females calling are likely mis-identified males. Our study suggests that, as for all congeners, only male *H. frenatus* produce the multiple-chirp call.

We found that juveniles can utter the multiple-chirp call, but do so very rarely. Of the 72 juveniles, recorded for over 64 h each, we only recorded one call from one individual. This individual was one of four geckos that ranged from 26 to 32 mm SVL and couldn't be sexed, but we assume it was a male based on the results for adults. This suggests that *H. frenatus* males are capable of calling from a young age but do so rarely.

Both males and females in our study were recorded uttering the 'churr' call described by Marcellini (1974). This vocalisation was previously thought to be a male-only trait in *Hemidactylus* species (Marcellini 1974; Regalado 2003; Dame and Petren 2006; Gramentz 2010), and is thought to be uttered in aggressive encounters (Marcellini 1974; Regalado 2003). We clearly recorded females uttering this call.

The main objective of the dyad trials was to test whether male geckos call more when in the company of a female or another male. We found a borderline significant result that showed males paired with a female call more than those paired with another male. We believe that the paired female stimulated males to call more. The alternative is that males in the male–male pairs inhibited each other's calls. However, we do not believe that is the case because one of our results is that larger males call more, and we would expect the large male in each pair to not be inhibited by the presence of a smaller male. The result may suggest that the call is directed mostly at females and plays a role in sexual advertisement. However, the call could be directed mostly at males (i.e. territoriality), with males calling more in the presence of a female to ward off males that may be present but out of sight. *H. frenatus* multiple-chirp calls can be heard up to 150 m away (Marcellini 1974). A function in territoriality also fits the observed call peaks at sunset and 30 min before sunrise in the wild, when males are moving from and to their diurnal refuge sites.

We found that larger males called significantly more than smaller males. Once again, this result is difficult to interpret in terms of male–male aggression versus sexual advertisement. Male–male aggression seems an intuitive conclusion, but studies of other ectotherms (frogs and insects) generally show that females prefer higher call rates and have been shown to produce more surviving offspring when they mate with those males (Gerhardt and Huber 2002; Forsman and Hagman 2006). Calling may be directed at both males and females and function in both aggression and mating. Behavioural studies testing the response of each sex to the multiple-chirp call are required to further resolve the function of this call.

H. frenatus displayed significant variation in call rate, as discussed above, and it also displayed significant variation in call properties. We found that dominant frequency of *H. frenatus* calls was negatively correlated with body size, suggesting a receiver could assess body size from the call. In several frogs and insects (Gerhardt and Huber 2002; Hoskin et al. 2009), and the gecko *Ptenopus garrulus* (Hibbitts et al. 2007), calls are known to convey information about the caller, through negative correlations between spectral aspects of the calls body size. It is likely that *H. frenatus* also use dominant frequency to assess the size of potential rivals and mates.

We also found that call duration was highly variable in large males – they produced longer calls when paired with small males, and shorter calls when paired with other large males. The call duration of smaller males, however, was less variable and less dependent on the size of the paired gecko. This finding indicates that large males display plasticity in call duration and shorten calls in the presence of other large individuals, male or female. Doing so may be an indicator of fitness, with short calls used by larger males looking to assert their dominance, particularly so when another large gecko is nearby. It is also possible that smaller males display lower vocal plasticity in call duration due to physiological constraints. More work is needed to resolve the function of call duration plasticity and physiological constraints associated with call duration.

We found significant variation in the multiple-chirp call among individuals. Using multivariate analyses, we were able to assign ~50% of calls to an individual. The most important traits in call assignment were chirp rate and dominant frequency (even though this is difficult to measure accurately in *H. frenatus*). For reasonably accurate determination of individuals, chirp rate, dominant frequency and potentially other spectral measurements, such as minimum and maximum frequencies, should be measured and analysed.

Using calls for detection of H. frenatus

Hemidactylus frenatus is the world's most invasive reptile, in terms of rate of international spread (Lever 2003; Hoskin 2011). Spread through urban areas may have limited impacts on biodiversity, but of concern is that it is increasingly being detected in natural habitats (e.g. Hoskin 2011; Barnett et al. 2017, 2018). In these areas *H. frenatus* may outcompete native geckos for food or refuges, or directly prey on their hatchlings, and may potentially impact communities more broadly (Cole et al. 2005; Hoskin 2011; Barnett et al. 2017, 2018). There are no current control options for large established populations, but rapid detection is the key to stopping establishment in new areas. *H. frenatus* has been identified as a high priority biosecurity threat in some areas, including New Zealand (Chapple et al. 2016), and Barrow Island and other conservation islands off northern Australia (Van Der Merwe 2015). Although the species is cryptic in the sense of being small and nocturnal, its loud multiple-chirp call is conspicuous and readily identified. Call has therefore been identified as the best means of detection, but the effectiveness of using calls for detection relies on understanding calling behaviour.

Conclusion

Although our study was conducted in a long-established area of high *H. frenatus* density, our results are of relevance to early detection for several reasons. First, our experiments are insightful for a newly introduced population because we took individuals from the wild, placed them in small numbers (four or less) in novel conditions in a constant-temperature room and recorded their calls. Second, knowing the calling behaviour of males, females and juveniles is fundamental knowledge in regards to who you can detect on arrival of geckos in a new location. The calling ability of males, females and juveniles is not likely to change based on density (although the propensity to do so likely does). Third, even though our wild recordings of temporal calling activity patterns involve a high-density population, the basic calling behaviour (in this case, greater tendency to call at dusk and dawn) is likely to hold for individuals in a newly introduced area.

Our results have the following implications for detecting newly establishing populations of *H. frenatus*. Geckos are most likely to be detected by call (either by humans or machines) in the warmer summer months and, at any time of the year, during the pre-sunrise and sunset calling peaks. Conversely, geckos are most likely to go undetected in a new area during the cooler months when calling activity is reduced. It is also important to realise that acoustic detection will be effective for detecting adult males but ineffective for detecting females, and unreliable for detecting juveniles. Therefore, visual surveys should also be performed to detect females and juveniles. A new population could start without the introduction of adult males if it is founded from juveniles or females with stored sperm. Our results also suggest that calls could potentially be used to estimate the number of individuals in recordings. Juvenile calls appear to be obviously different to those of adults and, with sufficient recordings, it is possible to assign adult male calls to multiple individuals. This could be valuable for assessing whether *H. frenatus* calls detected on acoustic listening devices represent a single, or multiple, individual(s) that need to be manually located and removed.

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Appendix S2

Supplementary material for Chapter 3

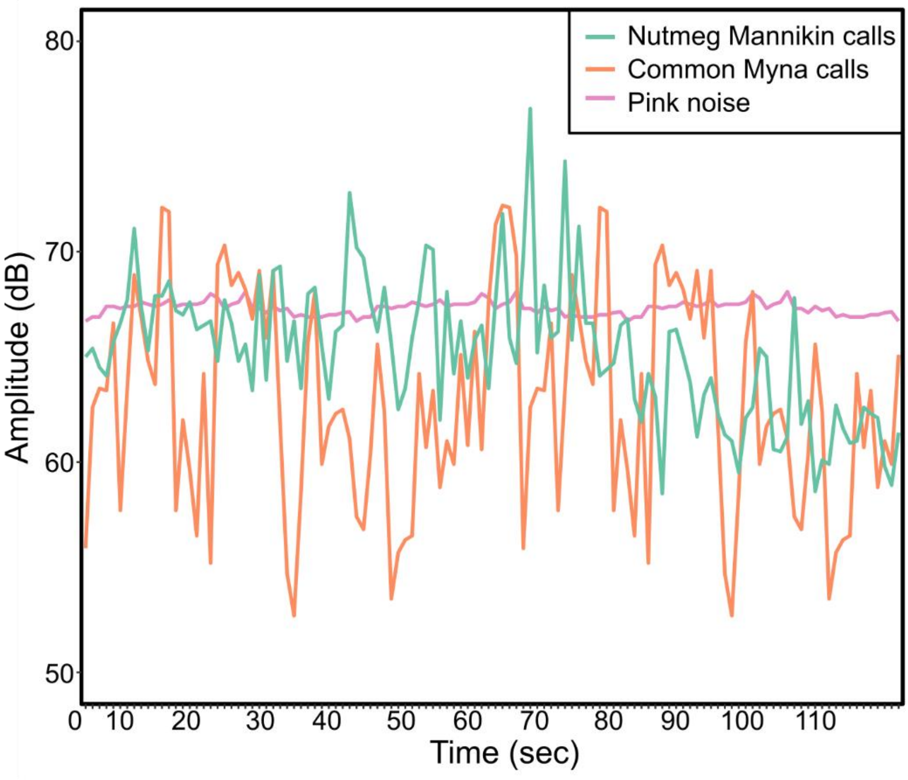

Fig. S2.1 Amplitude measurements of stimulus noises. Measurements taken 1 m from speaker with sound-pressure metre.

Appendix S3

Supplementary material for Chapter 4

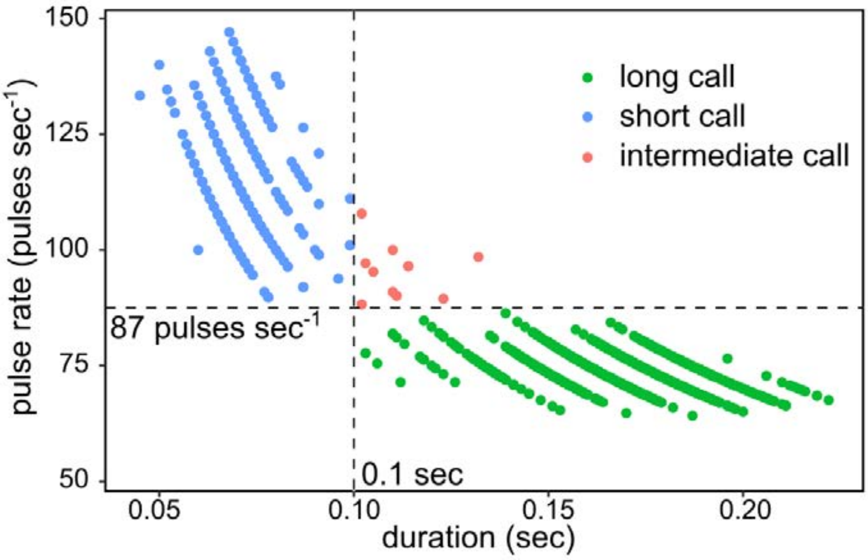

Fig. S3.1 Classification of different call types based on call pulse rate and duration.

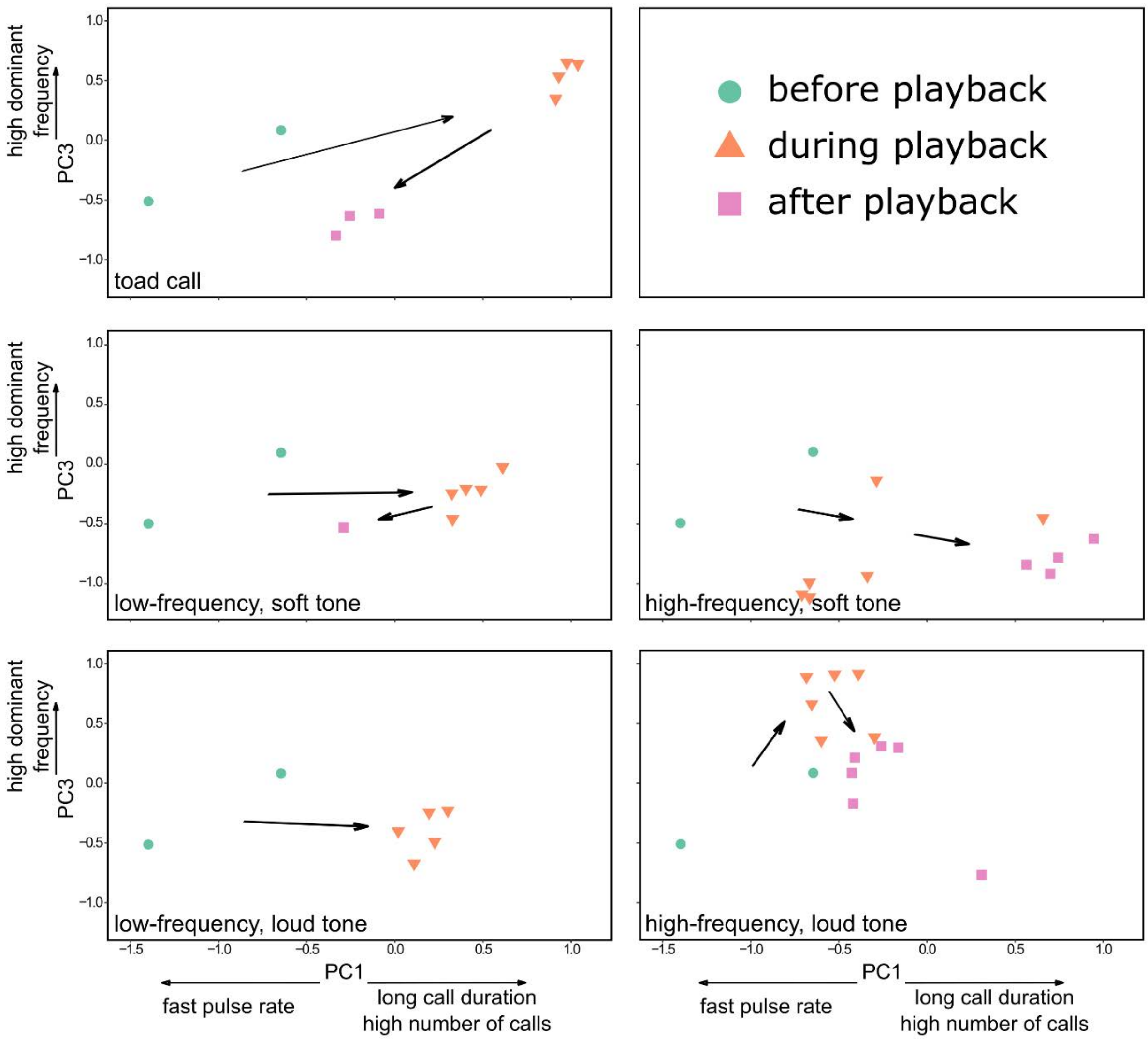

Fig. S3.2 PC1 and PC3 scores for floodplain toadlet short call property measurements. Points are the fitted values derived from the models, standardised (i.e., z-scores) by individual. Arrows indicate the shift in calls from "before" to "during" noise playback, and from "during" to "after" noise playback.

Table S3.1 Pairwise comparisons of the before and during periods, and during and after periods for each of the stimulus noises for models where the effect of treatment is significant. Significant results are bolded.

		Toad call	High-frequency, soft tone	High-frequency, loud tone	Low-frequency, soft tone	Low-frequency, loud tone
Total number of calls	Before – During	t = -2.0256 p = 0.6316	t = -1.7621 p = 0.7997	t = -0.9547 p = 0.9969	t = -2.2322 p = 0.4867	t = -2.8264 p = 0.1602
	During – After	t = 1.7175 p = 0.82394	t = -0.0896 p = 1.0000	t = 0.3766 p = 0.9999	t = 1.6880 p = 0.8389	t = 2.9435 p = 0.1213
<u>Long calls</u>						
Intercall interval	Before – During	t = -1.2576 p = 0.9753	t = 2.0615 p = 0.6054	t = -0.4932 p = 1.0000	t = -0.2353 p = 1.0000	t = 0.6249 p = 0.9999
	During – After	t = 0.7716 p = 0.9995	t = -1.4513 p = 0.9348	t = 1.0070 p = 0.9955	t = -0.9000 p = 0.9982	t = -0.4968 p = 1.0000
Duration	Before – During	t = -2.1109 p = 0.5696	**t = -3.4971** **p = 0.0210**	t = -0.5598 p = 1.0000	t = -2.0779 p = 0.5935	**t = -3.8711** **p = 0.0054**
	During – After	t = -0.8271 p = 0.9991	t = 1.8887 p = 0.7248	t = 1.5121 p = 0.9159	t = 1.9429 p = 0.6887	t = 2.2261 p = 0.4866
Pulse rate	Before – During	t = 2.7864 p = 0.1646	**t = 3.9440** **p = 0.0041**	t = 2.7096 p = 0.1968	t = 2.8230 p = 0.1507	**t = 5.3405** **p < 0.0001**
	During – After	t = -1.7284 p = 0.8207	t = -0.3447 p = 1.0000	t = -0.3507 p = 1.0000	t = -1.0565 p = 0.9934	t = -2.3597 p = 0.394
Dominant frequency	Before – During	t = 1.5398 p = 0.9061	t = -1.0414 p = 0.9941	**t = 5.3749** **p < 0.0001**	t = 0.5179 p = 1.0000	**t = 4.3091** **p = 0.0009**
	During – After	**t = 3.4126** **p = 0.0278**	**t = 3.6537** **p = 0.0122**	t = -1.9317 p = 0.6962	t = 1.5399 p = 0.9061	t = -1.0534 p = 0.9936
<u>Short calls</u>						
Intercall interval	Before – During	t = -2.0638 p = 0.5545	t = -1.8655 p = 0.6923	t = -1.4271 p = 0.9181	t = -1.6507 p = 0.8219	t = -0.2645 p = 1.0000
	During – After	t = -1.0795 p = 0.9864	t = 0.9314 p = 0.9953	t = -1.3551 p = 0.9396	t = -2.1210 p = 0.5139	NA
Duration	Before – During	t = -2.1095 p = 0.5218	t = -0.5069 p = 1.0000	t = -0.8885 p = 0.9968	t = -1.3904 p = 0.9297	t = -1.0574 p = 0.9883
	During – After	t = 2.9829 p = 0.0876	t = -3.0210 p = 0.0790	t = -0.1906 p = 1.0000	t = -0.4133 p = 1.0000	NA
Pulse rate	Before – During	t = 1.3895 p = 0.9300	t = 1.0418 p = 0.9895	t = 0.5002 p = 1.000	t = 0.9701 p = 0.9937	t = 0.3102 p = 1.0000
	During – After	t = -0.5356 p = 0.9999	t = 1.5355 p = 0.8771	t = 0.8393 p = 0.9979	t = -1.3408 p = 0.9435	NA
Dominant frequency	Before – During	t = -1.2621 p = 0.9613	t = 1.2019 p = 0.9718	t = -2.3061 p = 0.6531	t = 0.7292 p = 0.9993	t = 1.9234 p = 0.6531
	During – After	**t = 3.6333** **p = 0.0116**	t = 0.3752 p = 1.0000	t = 2.1302 p = 0.5072	t = 0.6673 p = 0.9997	NA

Table S3.2 Significance of treatment in models on short call counts and measurements. (*) indicates statistically significant results ($P < 0.05$).

Call property	Test Statistic	P-value
Number of calls	$\chi^2_{10} = 14.97$	0.13
Intercall interval	$LRT_9 = 44.78$	< 0.01*
Duration	$LRT_9 = 24.79$	< 0.01*
Pulse rate	$LRT_9 = 18.24$	0.03*
Dominant frequency	$LRT_9 = 72.57$	< 0.01*

Table S3.3 Weightings of short call property measurements along principal components one, two and three.

	PC1	PC2	PC3
% Explained variance	37.1%	31.1%	19.3
Number of calls	1.1851	-0.9714	0.5243
Intercall interval	0.1038	1.5321	-0.0071
Duration	1.5455	0.1739	0.3850
Pulse rate	-1.1209	-0.9001	0.4250
Dominant frequency	-0.5025	0.5681	1.4714