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**Consequences of anthropogenic noise
when conflicting with sexually selected
acoustic signals**

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requirements of Anglia Ruskin University for
the degree of Doctor of Philosophy

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For Ella Grace
& Dexter George

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Abstract

The signalling systems of species throughout the Animal Kingdom are at risk of disruption from human disturbances. Anthropogenic noise is a prominent acoustic pollutant in many environments and is known to conflict with the acoustic sexual signalling systems of both terrestrial and aquatic animals. However, the consequences and causes of such a conflict largely remain unknown, especially in invertebrate species. The experiments I present in this thesis highlight the behavioural and physiological consequences of anthropogenic noise when conflicting with an insect's acoustic signals. Additionally, I also consider the acoustic characteristics necessary in any given stimulus to cause such a disruption. To measure these consequences, I observed the sexual signalling systems of the Mediterranean field cricket, *Gryllus bimaculatus*, under conditions of anthropogenic noise and conducted behavioural, acoustic, and physiological analyses. I found the acoustic signal used to detect the location of a signalling individual (the 'calling song') suffered reduced reception under anthropogenic noise conditions, shown by a reduction in female responses. Additionally, I also found the acoustic signal used during courtship displays (the 'courtship song') sustained reduced signal transmission, as males signalled less, and reduced signal perception, as females failed to distinguish between high and low 'quality' songs. However, aggressive encounters between two males, which feature an acoustic signal (the 'aggressive song'), remained stable under similar acoustic conditions. Physiological analyses revealed potential energetic costs related to acoustic signals that had been altered to combat disruption from anthropogenic noise. Furthermore, the differences I observed between noise conditions highlighted the acoustic characteristics that are important in causing this disruption. The results I present in this thesis detail the deleterious consequences of anthropogenic noise disrupting invertebrate sexual signalling systems, both in terms of behaviour and energetic costs. Additionally, I discuss the variability in signals that may be disrupted by anthropogenic noise conditions and the acoustic characteristics that drive this disruption in the first place. This further cements anthropogenic

disturbances as a new and dangerous selection pressure facing many animal populations.

Keywords

Acoustic Communication, Anthropogenic Noise, *Gryllus bimaculatus*, Sexual Signals

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Copyright Declaration

I declare that this thesis, and the research that it details, are the result of my own work. I acknowledge the helpful guidance of Dr Sophie Mowles and Dr Thomas Ings, and where the work of others was used to inform my research, explicit citations are given. I further state that no part of my work presented here has been submitted for any other qualification, either from Anglia Ruskin University or any other institution.

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Signed.....

Chapter 1

Introduction

Communication in the Animal Kingdom

Animal Signals

Communication systems are processes by which one individual, using an act or structure, changes the behaviour of another individual through adaptive signals and responses (Maynard-Smith and Harper, 2003; but see Scott-Phillips, 2008). These evolved and adapted pathways are so integral and essential to the natural world that the diversity and abundance of communication systems can be found throughout all the biological domains of life. Quorum sensing, for example, is a type of communication system, fitting with the above description, in which prokaryotic cells, including Bacteria (Bassler, 2002) and Archaea (Montgomery et al., 2013), use the release and detection of autoinducers (specific signal molecules) to coordinate certain behaviours with other individuals. Plants have been shown to communicate with not only other plants, but also herbivores and mutualistic species (Karban, 2008), and intraspecific communication that takes place between fungi addresses major biological processes, such as mating, growth and morphology (Cottier and Mühlschlegel, 2012). However, communication outside the human race is arguably mostly recognised in the Animal Kingdom. Communication in animals is known to occur not only throughout different taxa, but also in a variety of modalities, including acoustic (Alexander, 1962; Byers and Kroodsma, 2009), chemical (Shine et al., 2005; Steiger and Stökl, 2014), visual (Salmon and Atsides, 1968; Rutowski, McCoy and Demlong, 2001) and vibrational (Klärner and Barth, 1982; Caldwell et al., 2010). Some signals are even produced in multiple modalities (multimodal), likely as a way to ensure signal transmission (redundant signalling) or because each modality provides the receiver with different information (multiple messages hypothesis; Hebets and Papaj, 2005). Aspects of the signal transmission, including the

modality, will have evolved specifically to ensure signal transmission under the environmental conditions where the signal is utilised.

Sexual Behaviours

A subset of communication in the Animal Kingdom, sexual communication, involves signals that have evolved, at least in part, to maximise reproductive success. These signals, which are exchanged between conspecifics (although heterospecific individuals may eavesdrop for their own purposes; Zuk and Kolluru, 1998; Outomuro et al., 2016), are necessary as potential mates are a limited resource and vary in genetic and phenotypic quality. Thus, they are mostly involved in either attracting and encouraging a potential mate to copulate (Wagner and Reiser, 2000; Rutowski, McCoy and Demlong, 2001; Allen, Barry and Holwell, 2012) or to deter potential competitors from territory, mates or other resources (Alexander, 1961; Clutton-Brock et al., 1979; Caldwell et al., 2010). Individuals attending to these signals can gain information regarding the quality of the signalling individual. This is important for receivers of attraction signals, as they can receive multiple benefits from mating with mates of appropriate quality, such as access to resources (Johnson and Searcy, 1993) or increased offspring attractiveness and survivability (Fisher, 1930; Zahavi, 1975). Additionally, individuals that attend to aggressive signals can alter their behaviour to minimise costs of contests when fighting combatants that are of higher quality (Clutton-Brock et al., 1979). When utilized, these signals can lead to significant boosts to an individual's reproductive success and thus increase their fitness. Fitness is here defined as an individual's ability to impart their genes to the next generation, relative to others in the population (Hamilton, 1964).

Due to the behavioural changes that signals induce, individuals are susceptible to altering their behaviour in response to deceptive information, particularly if another individual would gain something from this. Whilst deceptive signals have been recognised in non-sexual communication, and their consequences discussed (Yom-tov, 1980; Davies, Bourke and de, 1989; Akino et al., 1999), the majority of work regarding the maintenance of honest signalling has been in sexual signals. Here, costs and indices are

two of the main ways that signalling systems are thought to remain reliable (Maynard-Smith and Harper, 2003; Vanhooydonck et al., 2007), although other mechanisms for reliability have also been suggested (Searcy and Nowicki, 2005). Indices are signals that cannot be deceptive due to the nature of what is being signalled, and are thus reliable. For example, certain species of jumping spiders signal using their abdomen, the size of which is affected by nourishment (Taylor, Hasson and Clark, 2000) and thus cannot be faked. Alternatively, costly signals (also known as handicaps; Zahavi, 1975) are signals that can only be produced by an individual of high quality due to the direct (intrinsic) and indirect (extrinsic) cost they incur to produce and/or transmit. A cricket song is an example of a costly signal as it requires energetic input from the signaller (intrinsic cost; Mowles, 2014) and may attract predators to its location (extrinsic cost; Zuk and Kolluru, 1998), so only individuals that can bear these costs can perform this signal for extended periods of time (Zahavi, 1975). These mechanisms allow receivers to gather information on the signaller, as well as ensuring reliability within the signalling systems (on the majority of occasions), and are thus embroiled in dynamic cost-benefit trade-offs (Houslay et al., 2017).

Another aspect of signalling behaviour is the necessity to adapt to the dynamic environment. As many aspects of the environment may change, both regularly and randomly, individuals and populations may need to alter aspects of their signal to ensure successful signal transmission and reception. Specifically, unwanted signal disturbances (signal noise) can come from both biotic and abiotic sources, and may reduce signal efficacy. This effect can be seen throughout different taxonomic groups, and in all signal modalities, such as wind generated motion affecting visual displays (Ord et al., 2007; Ord, Charles and Hofer, 2010), acoustics from heterospecifics affecting conspecific signal perception (Wollerman and Wiley, 2002; Balakrishnan et al., 2014), and rainfall, or lack thereof, can affect the presence of pheromonal (chemical) signals (Sen Majumder and Bhadra, 2015). Often, the individual's behavioural flexibility can help to alleviate the consequences of reduced signal transmission, but both choosing to alter their behaviour or not can come at a cost to the signalling

individual. For example, where wind generated motion may affect the visual signals of anole lizards, certain species have been shown to increase their signalling speed (Ord et al., 2007), whilst other species signal at times with lower wind levels or show no behavioural alterations at all (Ord, Charles and Hofer, 2010). Individuals that increase their signalling effort may need to use up additional energy reserves (Brandt, 2003), whereas individuals that do not signal, or make no signal alterations, may not acquire the benefits of this behaviour, the consequences of which may be costly. This shows how behavioural flexibility of sexual signals can alleviate the effects of a dynamic environment, but it highlights the changes that might occur to the cost-benefit trade-offs of sexual signalling systems. Additionally, such behavioural flexibility is likely only present due to the extended period of time that these signals have evolved over. Thus, individuals may struggle to adapt to an evolutionarily recent selection pressure, which in turn could lead to reductions in both signalling efficacy and signal reliability, increased costs associated with signalling, and thus potential deleterious consequences for the reproductive success of individuals afflicted. Anthropogenic disturbances are one such selection pressure.

Anthropogenic Noise

Human Activity

It is a well-established fact that human populations have a significant effect on the environment. Whilst we have the ability to increase biodiversity and sustainability in habitats, most of our actions involve corrupting and polluting these ecosystems, by both direct and indirect means. These actions lead to environmental pollutants, which, similar to the communication systems previously mentioned, can occur across multiple modalities. Historically, environmental pollution has referred to chemical changes in the environment (Alloway and Ayres, 1998), such as the introduction of heavy metals (Ergül, Varol and Ay, 2013), pesticides (Coppage and Braidech, 1976), and fertilisers (Zhang et al., 1996) into bodies of water. However, more recently, our understanding of pollution has developed to include other polluting modalities, including noise. This invisible pollutant

is often thought of as being an typical component of any urban landscape, due to the amount and diversity of its sources, and is present in both aquatic (McDonald, Hildebrand and Wiggins, 2006) and terrestrial (King et al., 2012) environments. Acoustic pollution, also referred to as anthropogenic noise, has been of concern in human environments for some time (e.g. buildings, cities, transport; Robinson, 1971), but it is only within the last 20 years that we have turned our attention to the effect it has on the natural environment (Ketten and Potter, 1999).

Anthropogenic noise can be produced from two main types of sources: intentional acoustic production and non-intentional acoustic production. However, intentional sources, such as music festivals and events (Mercier, Luy and Hohmann, 2003), are far outweighed by the abundance of non-intentional sources. Examples of these sources include construction projects (Hamoda, 2008), agriculture (Bilski, 2013), and transport networks, such as road (Lee et al., 2014), rail (Janssens and Thompson, 1996), shipping (Andrew et al., 2002), and air traffic (Gasco, Asensio and de Arcas, 2017). Despite this variety in sources, most anthropogenic noise is categorized as being high in amplitude and low in frequency (Berglund, Hassmén and Soames Job, 1996; Waye and Rylander, 2001), although concerns over the abundance of high frequency (ultrasound) anthropogenic noise is increasing (Smagowska, 2013). The louder the acoustic stimulus is, the further from the source it can be perceived, but the low frequency aspect of anthropogenic noise also assists in its spread. Low frequency noise suffers less attenuation (reduction in acoustic power) than higher frequency acoustics (Berglund, Hassmén and Soames Job, 1996) meaning that it can permeate into the environment further from the source. Thus, the combination of its multiple sources and the acoustic characteristics of anthropogenic noise makes it a prevalent component of the modern urban environment.

Aside from the variety of sources and its acoustic characteristics, human population growth is also influencing the prevalence of anthropogenic noise. The global human population has experienced fast and continuous growth over the past few centuries, and this trend is showing no

sign of stopping today. The United Nations has predicted that the global population (approximately 7.6 billion people as of 2017) will increase to nearly 10 billion people by 2050 (United Nations, Department of Economic and Social Affairs, 2017). In terms of anthropogenic noise, this population growth has two main effects. Firstly, urban areas will become more populated, leading to increases in transport networks and construction projects, both of which are very common sources of anthropogenic noise (Berglund, Hassmén and Soames Job, 1996). Thus, it is likely that urban areas, which are already subjected to anthropogenic noise, will suffer higher levels of anthropogenic noise due to this growth (although noise mitigating strategies are becoming more common; Magrini and Lisot, 2016). Secondly, non-urban, or rural, areas will be developed to increase housing, develop infrastructure, and expand on production systems, in a shift known as urbanization. As a result, the surrounding environment of these previously rural areas will be subjected to massive increases in anthropogenic noise. This is a global trend that is seen in both developed and developing countries, with the United Nations predicting that two thirds of the world will be urbanized by 2050 (United Nations, 2014). This effect of population growth on anthropogenic noise, combined with its current abundance and its ability to spread far from the source, makes it a persistent and growing concern within the environment.

Deleterious Effects on Animals

In terms of the applied consequences of anthropogenic noise, the effects on humans have been of concern for a long time. Obvious examples of this include the effect of chronic noise exposure. Human beings are susceptible to hearing loss or damage when subjected to extreme cases of anthropogenic noise (Caciari et al., 2013), and certain noises (e.g. construction noise; Lee, Hong and Jeon, 2015) can lead to disruptions in healthy sleeping patterns (Lee, Shim and Jeon, 2010) and complications with mental health (Hammersen, Niemann and Hoebel, 2016). However, the physiological effects of noise, which may be linked to hearing loss and annoyance, are not as easily noticeable. Studies have shown that chronic anthropogenic noise exposure is linked to physiological stress responses, such as increased blood

pressure and stress hormone release (Evans, Bullinger and Hygge, 1998), which may lead to more serious ailments including cardiovascular diseases (van Kempen et al., 2002). This body of evidence has led to the development of legislation globally, including E.U. directives regarding environmental noise (European Parliament and Council of the European Union, 2002), as an attempt to mitigate the harmful effects that anthropogenic noise can produce. However, this legislation is entirely anthropocentric, with the E.U. directive categorizing harmful effects as “negative effects on human health”, with no mention of the effects on animal populations and their habitats, wild or otherwise.

Soundscapes featuring anthropogenic noises are known to influence animals living in both wild/natural (Chan and Blumstein, 2011) and managed environments (Waynert et al., 1999), as well as throughout terrestrial (Luther and Gentry, 2013) and aquatic environments (Nowacek et al., 2007). Many different species, particularly vertebrates, have been shown to react to anthropogenic noise in a similar physiological and behavioural manner to humans. Cortisol and Corticosterone (CORT) are glucocorticoid hormones, found in vertebrates, released by the hypothalamic-pituitary-adrenal (HPA) axis in response to stress (Chrousos, 1995). These hormones, that work to alter metabolic activity, are widely used as a marker for stress in vertebrate species. A study by Kaiser et al. (2015) used these hormones to determine whether anthropogenic noise elicited a stress response in White’s tree frog (*Litoria caerulea*). The results revealed that individuals exposed to traffic noise stimuli had significantly higher CORT concentrations after exposure, than those in the control group. This is a result that has been reflected throughout different vertebrate taxa (Rolland et al., 2012; Blickley et al., 2012; Wysocki, Dittami and Ladich, 2006), with additional articles noting physiological effects observed in invertebrates (Wale, Simpson and Radford, 2013; Zhao et al., 2016). Furthermore, sperm count and viability were dramatically reduced in tree frogs exposed to traffic noise, likely as a result of the chronic stress (Campbell, Pottinger and Sumpter, 1992).

The abundance and intensity of anthropogenic noise in the environment, coupled with these documented deleterious effects, would be

enough to warrant further investigation into the biological consequences of anthropogenic noise. However, arguably the largest effect of anthropogenic noise on animal populations is due to the conflict between these human generated stimuli and the communication systems used by individuals. This is supported by a large body of literature on this topic, which is concerned with how communication and environmental cues are disrupted in the presence of anthropogenic noise, and the behavioural changes that arise from this.

The Conflict

Sources of anthropogenic disturbances are well known to conflict with the biological processes of animal populations, particularly when regarding their sensory systems (Chan and Blumstein, 2011; Naguib, 2013; Halfwerk and Slabbekoorn, 2015). For example, human generated changes in environmental chemistry can lead to reductions in pheromone based mate location (Griesinger, Evans and Rypstra, 2011) or alter resource detection and selection (de la Haye et al., 2011), whilst night-time light pollution affects navigation and/or attraction of nocturnal species (van Langevelde et al., 2011). Anthropogenic noise is no exception. Studies have documented the effect it has on the efficiency of tactile and chemosensory signals and cues (Walsh, Arnott and Kunc, 2017). However, anthropogenic noise is most notably documented for its conflict with acoustic signals and cues in the Animal Kingdom (Cynx et al., 1998; Brumm et al., 2004; Melcón et al., 2012; Lampe et al., 2012; Holt and Johnston, 2014; Kern and Radford, 2016; Kleist et al., 2016; Klett-Mingo, Pavón and Gil, 2016; Lucass, Eens and Müller, 2016; Nemeth and Brumm, 2016; Orci, Petróczki and Barta, 2016). Acoustic communication is likely to be most susceptible to disruption (reductions in signal efficacy and reliability) from anthropogenic noise due to the shared modality. Any signalling system is susceptible to conflict with stimuli that share that modality (Partan, 2017), although different modalities and combinations of modalities can also have a disrupting affect (Halfwerk and Slabbekoorn, 2015), and this conflict is also present with natural stimuli, as previously discussed (Wollerman and Wiley, 2002; Ord et al., 2007; Balakrishnan et al., 2014). However, unlike natural

acoustic stimuli, anthropogenic noise represents a rapid environmental change in an evolutionary timescale and often occurs at very high amplitudes (Berglund, Hassmén and Soames Job, 1996; Waye and Rylander, 2001). The presence of disruptive anthropogenic noise can lead to shifts in behavioural strategies, to ensure signal transmission and reception, either where individuals possess some degree of behavioural flexibility, or over time through natural selection. For example, common urban song birds, such as the great tit (*Parus major*) and the blackbird (*Turdus merula*), are known to sing at a higher pitch and amplitude in urban areas, a behavioural shift that can increase the detectability of the song under conditions of anthropogenic noise (Nemeth and Brumm, 2016). Similarly, *Cyprinella venusta*, a species of acoustically signalling cyprinid fish, increase the amplitude of their signals under noisy conditions, a phenomenon termed the “Lombard effect” (Holt and Johnston, 2014). These results act as evidence that these changes are a necessary adaptive response to anthropogenic noise. Additionally, the perception of acoustic environmental cues, which function on the same sensory mechanisms as signal detection, is also known to be reduced when anthropogenic noise is present. European eels (*Anguilla anguilla*) are known to have reduced behavioural responses when stimulated with predator cues under conditions of anthropogenic noise (Simpson, Purser and Radford, 2015). Finally, as anthropogenic noise shows a high degree of spatial variation within the environment (Job et al., 2016), some species will avoid foraging or breeding in areas with high levels of noise (Francis, Ortega and Cruz, 2009). Many other behavioural changes have been documented for a variety of taxa, likely due to the pressures of anthropogenic noise conflicting with acoustic communication. These taxa include birds (Díaz, Parra and Gallardo, 2011; Chen and Koprowski, 2015; Luther, Phillips and Derryberry, 2016), amphibians (Cunnington and Fahrig, 2010), fish (Sebastianutto et al., 2011), mammals (Brumm et al., 2004; Melcón et al., 2012), and invertebrates (Lampe et al., 2012; Orci, Petróczki and Barta, 2016; but see Costello and Symes, 2014). Interestingly, even species which are closely related may, under anthropogenic noise conditions, alter their behaviour differently from each other (Francis, Ortega

and Cruz, 2011), making it difficult to make general predictions about the response of animals to anthropogenic noises source.

The conflict between anthropogenic noise and the perception of acoustic stimuli works on many levels, in the sense that there are a number of mechanisms for how anthropogenic noise may disrupt signal transmission, reception or perception. Some of these pathways are detailed in Naguib's (2013) paper detailing the 'indirect effects' of this conflict. Firstly, the most obvious mechanism is known as masking. This can be defined as "the amount or the process by which the threshold of detection for a sound is increased by the presence of the aggregate of other sounds" (Barber, Crooks and Fristrup, 2010), here relating to the detection of a signal in the presence of anthropogenic noise. This mechanism functions on not only a noise-to-signal ratio, with higher ratios leading to decreased detectability (Wiley, 2006), but likely also on a frequency scale, which would explain the amplitude and frequency changes in songbirds when under anthropogenic noise conditions (Nemeth and Brumm, 2016). Distraction, or attention, is another mechanism by which this conflict could occur. Animals have a finite attention span, or cognitive performance ability, and this 'resource' is divided up between stimuli, such as those necessary in foraging and vigilance (Dukas, 2004), which maximises productivity and minimises risk. Thus, the introduction of anthropogenic noise adds another stimulus that may require attention that could otherwise be used to process acoustic signals from a potential mate or competitor. This mechanism has been reported in other species (Chan and Blumstein, 2011) and is an aspect that is beginning to receive greater consideration throughout the field. Furthermore, unlike masking, distraction mechanisms are multimodal, meaning that different modalities of sensory inputs can affect responses to other inputs (Chan et al., 2010; Walsh, Arnott and Kunc, 2017). These masking and attentional mechanisms are not mutually exclusive, so a combined or additive mechanism is possible. Some of these definitions make reference to the importance of acoustic characteristics when conflicting with signals (i.e. frequency is important in masking stimuli), but this is an aspect that is not reflected well in the literature of communication

disruptions due to anthropogenic noise, and the perception of the acoustic environment under anthropogenic noise conditions requires much deeper analysis (Rosa and Koper, 2018). By understanding more about what aspects of disruptive acoustic stimuli lead to conflict with acoustic signal, we may be able to better predict, and thus avoid, how a given group of animals may be affected by anthropogenic noise.

The majority of studies regarding the effects of anthropogenic noise on animals focus on the benefits of adaptive or flexible behaviours and mostly do not consider the potential costs that conflicting with a complex signalling system may have (Read, Jones and Radford, 2014), and thus the reason that these adaptive responses are necessary in the first place. However, it is possible to predict the potential costs of signalling under conditions of anthropogenic noise. For example, individuals that produce co-operative signals, such as the alarm calls produced by silvereyes, *Zosterops lateralis* (which have been shown to alter their acoustics in noise conditions; Potvin, Mulder and Parris, 2014), may suffer a reduction in survivability under anthropogenic noise conditions due to a reduction in the perception of alarm calls, food calls, or other important social stimuli. Individuals utilising sexual acoustics on the other hand, which may already suffer a decrease in likelihood of survival due to the nature of costly signals (Zahavi, 1975), could risk a reduction in reproductive success, and thus fitness, if they do not alter their signal to combat the disruptive effects of anthropogenic noise, and could face more costs if they do. For example, acoustic signals that are used to attract and encourage individuals to mate, such as the vocalisations of anurans (which have been shown to change under conditions of anthropogenic noise; Cunningham and Fahrig, 2010), may not be received under noisy conditions, resulting in no fitness benefits, but still incurring the associated production costs. Alternatively, if the signals are received, they may be partially disrupted by anthropogenic noise, which could lead to misinformed mate choices (Huet des Aunay et al., 2013) if the signaller is believed to be of a higher or lower quality than they actually are. Similarly, if acoustic signals that are used in aggressive contests, such as the roars produced by red deer stags, *Cervus elaphus*

(Clutton-Brock et al., 1979), are misinterpreted or not received due to anthropogenic noise, this could lead to an increase in potentially injurious, and usually avoidable, combat. Thus, the consequences of anthropogenic noise conflicting with acoustic signals, particularly those based in sexual behaviours, and the costs related to altered signalling effort, is a current gap in our knowledge that requires further investigation.

To summarize, human generated noises are ubiquitously present in many environments, and increasing in abundance. This has deleterious consequences to various aspects of animal biology and is known to conflict with the acoustics signals and cues utilized by many species. However, the consequences of this conflict remain largely unexplored, particularly when regarding sexual acoustics involved in the attraction and defending of potential mates. Furthermore, the mechanisms of conflict (i.e. why anthropogenic noise disrupts) and the costs of altering acoustic behaviour in response to noise also require further research to better understand the consequences, and potentially predict the impacts it might have. Thus, using a model organism, this thesis will detail the consequences of signalling under conditions of anthropogenic noise, throughout various signalling systems, as well as the important characteristics of noise that lead to this disruption.

Gryllus bimaculatus: A Model System

Invertebrates

Much of the literature regarding acoustic signalling and anthropogenic noise that is cited in the previous sections regards vertebrate systems. This is intentional, as it reflects the relative lack of literature concerning invertebrate populations and the effects that anthropogenic noise has on them. However, this is not representative of the diversity of species that are likely to be affected by the presence of anthropogenic noise. Invertebrates make up approximately 97% of all animal species on the planet, and they are responsible for countless ecosystem services. This includes, but is not limited to, pollination (McGregor, 1976), decomposition (Graça, 2001) and soil maintenance (Lee, 1985), as well as their importance as both prey (food

source) and predators (biological control) in any given ecosystem (Schoenly, Beaver and Heumier, 1991). Thus, it is important to document this diverse and important group of organisms, and how anthropogenic noise affects them. Many invertebrates are known to signal acoustically, a necessary requirement for this research, and often through different mechanisms. This includes the stridulations of crickets (Alexander, 1961), muscular vibrations of cicadas (Pringle, 1954), wing vibrations in bees (Hrncir, Barth and Tautz, 2005) and rapid air movement (hissing) in cockroaches (Nelson and Fraser, 1980), as well as those that utilize ultrasonic frequencies (e.g. moths; Jang and Greenfield, 1996) or use acoustics in aquatic environments (Aiken, 1985). The abundance and diversity of acoustic signalling in invertebrates is further evidence of the risk that anthropogenic noise may be severely affecting them. Furthermore, the short generation times and small size of certain invertebrates makes them a highly suitable study organism in a laboratory setting, where they require less space and resources than a vertebrate counterpart would.

Orthopterans

The order Orthoptera is a taxonomic group of insects (Class: Insecta) containing crickets, katydids (bush crickets) and grasshoppers. These globally occurring animals are well known for the production of acoustic signals through the process of stridulation (rubbing two body parts together; Alexander, 1961). For this reason, they have attracted much attention from the scientific community regarding the function and evolution of these ‘songs’. Like many of the species discussed here, they have been documented in environments where anthropogenic noise is present and have been shown alter their behaviour in response to this human-generated stimulus (Lampe et al., 2012; Lampe, Reinhold and Schmoll, 2014). The genus *Gryllus* has attracted a lot of interest in particular, due to their conspicuously structured sexual behaviour (Alexander, 1961; Popov and Shuvalov, 1977; Simmons and Ritchie, 1996; Hall, Beck and Greenwood, 2000). Alexander (1962) listed several different “types” of song produced by cricket species, of which three are now widely recognised in *Gryllus* species as sexually selected signals (Figure 1.1). These signals, produced

only by the males, are; the calling song (used to attract females from a distance), the courtship song (used to encourage females within range to copulate), and the aggressive song (used during male-male conflicts). Because these signals are necessary for reproductive success, and are also known to be costly to the signaller both in terms of energetics (Mowles, 2014) and survival (Zuk and Kolluru, 1998), it is rational to consider them in a trade-off of costs and benefits, which may be disrupted when in the presence of anthropogenic noise. This makes *Gryllus* species a good model for looking specifically at the cost of conflicting acoustics, and the results can be applied and extrapolated past this taxonomic group to the wider Animal Kingdom. Specifically, I used the species *Gryllus bimaculatus* (De Geer, 1773) throughout the experiments presented in this thesis. Whilst field crickets share many characteristics, their acoustic stimuli differ significantly (which is necessary for species recognition; Bailey, Moran and Hennig, 2017), so it is important to identify signalling at the species level (Figure 1.1).

Mature male individuals of the species *G. bimaculatus* produce acoustic signals to attract mature female from a distance known as a “calling song” (Alexander, 1962, Figure 1.1a). In this species, the signal is a repetitive stimulus that features relatively low frequency pulses (4-6 kHz) which make up short trills (3-5 pulses). This signal is known to vary between individuals, informing receivers about the signaller’s condition (Zhemchuzhnikov and Knyazev, 2015) and lineage (Ferreira and Ferguson, 2002), and females are known to be selective over males based on this acoustic signal (Verburgt, Ferreira and Ferguson, 2011; Hirtenlehner et al., 2013). Females detect this signal and move towards its source in a behaviour known as phonotaxis (Hedwig and Poulet, 2005), but do not produce their own acoustic signal.

Following the success of the calling song, males switch to a conspicuously different acoustic signal in an attempt to encourage the now approached female to mate, known as a “courtship song” (Figure 1.1b). Whilst still produced through the stridulation of wings, this song is instead made up of high frequency singular ticks (13 - 18 kHz) and low frequency

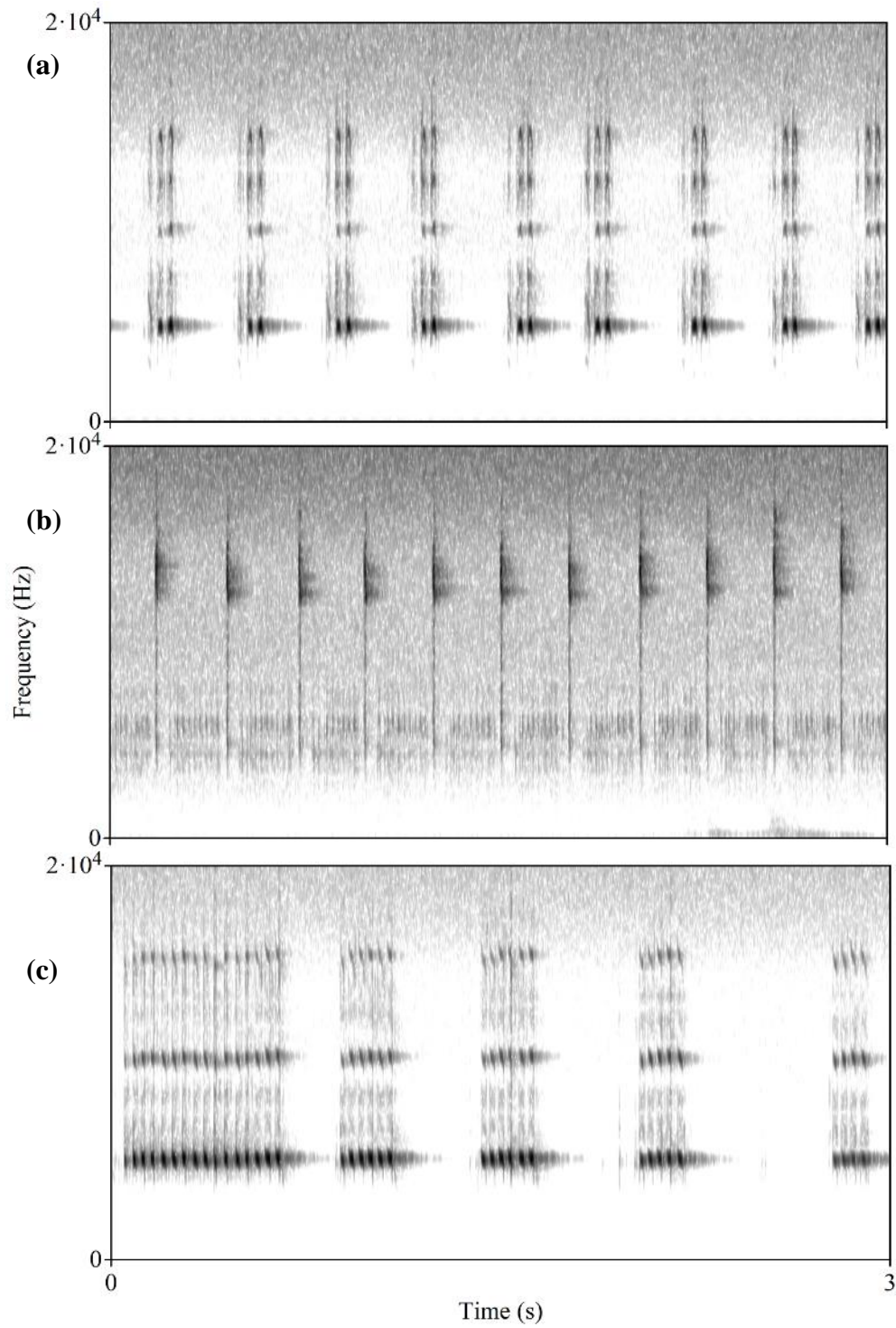


Figure 1.1. Frequency spectrograms showing the structural and frequency differences between the three distinct songs types used by *Gryllus bimaculatus*; (a) calling song, (b) courtship song and (c) aggressive song. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0–3s); frequency range: 0–20000Hz.

pulses (4 - 6 kHz). Similar to the calling song, the courtship song is also known to vary based on individual condition (Rantala and Kortet, 2003), but less research has focused on this. Females respond to the courtship song by mounting the males and initiating mating, but there is a degree of variation in this based on female preference (i.e. mate choice; Shestakov and Vedenina, 2015).

Finally, males also produce a signal used in competitive male-male interactions, termed as an “aggressive song” (Figure 1.1c). Whilst spectrally similar to the calling song of this species, due to shared frequency and temporal patterns, this song is distinct due to the lengthened trills (3-20 pulses) and its use after intra-specific physical combat. Following a stereotypical set of aggressive behaviours, the signal is most usually produced by the winner at the end of an aggressive encounter as a type of “victory song” (Alexander, 1961). The exact purpose of this signal is still debated, but most likely it functions either to reduce the likelihood of subordinate males from re-engaging or to advertise the outcome of the encounter to other conspecifics (i.e. browbeating or advertising; Rook, Fitzsimmons and Bertram, 2010). Furthermore, whilst females do not produce their own acoustic aggressive signals, they do produce all other behaviours seen in an aggressive encounter, particularly when multiple females are in the presence of a low number of males (Rillich et al., 2009).

Thesis Aim and Structure

I designed the experiments presented in this thesis to investigate the consequences that arise when anthropogenic noise conflicts with sexually selected acoustic signals, both from signal disruptions and from acoustic behavioural alterations. I observed the behaviours associated with the transmission, reception and perception of sexually selected signals in the Mediterranean field cricket *G. bimaculatus*, under different acoustic conditions. Previous studies have focused on the fitness benefits of behavioural flexibility in an anthropogenic environment, but the initial fitness consequences remain largely un-investigated (Read, Jones and Radford, 2014). Thus, the overall aim of this thesis is to provide information

on the deleterious effects that anthropogenic noise causes, specifically when conflicting with the sexual communication present in many species, whilst also developing our understanding of why this conflict occurs at all.

Chapter 2

The first data chapter focuses on anthropogenic noise conflicting with acoustic mate searching communication, specifically detailing a signal receiver's ability to detect and approach the signaller under anthropogenic noise conditions. An individual's ability to find a potential mate will heavily affect their reproductive success, resulting in a potential reduction in fitness should anthropogenic noise conflict with these signals. I hypothesize that unfavourable noise conditions conflict with the detection of mate searching acoustics, and thus predict that there will be a difference in phonotactic behaviour between acoustic conditions. To test this hypothesis, I used arena-based behavioural assays to observe the behaviour of female *G. bimaculatus* when presented with the "calling song" of conspecific males and under different acoustic conditions.

Chapter 3

In this data chapter, the focus shifts towards anthropogenic noise conflicting with acoustic courtship signals, specifically looking at behavioural differences in both the signaller and receiver when courtship interactions occur under anthropogenic noise conditions. In many species, a courtship ritual is adhered to in order to initiate mating, and thus these behaviours are highly sexually selected, which could lead to potential reductions to fitness should anthropogenic noise conflict with it. I hypothesize that unfavourable acoustic conditions conflict with the detection of acoustic courtship signals, and thus predict a reduction in successful courtship interactions under anthropogenic noise conditions and/or alterations in courtship behaviours performed. To test this hypothesis, I used arena-based behavioural assays of the acoustic courtship interactions of a male and female *G. bimaculatus* under different acoustic conditions. Furthermore, I also implemented physiological analyses to quantify the energetic costs of altered courtship signalling under anthropogenic noise conditions.

Chapter 4

In this data chapter, the conflict between acoustic courtship communication and anthropogenic noise is revisited. The focus, however, is shifted towards a signal receiver's ability to discern information about a signaller's quality from their signal, and using this to make an informed decision. An individual's ability to detect differences in quality between potential mates can lead to them selecting fitter partners, which in turn will increase their reproductive success. This enables anthropogenic noise to reduce an individual's fitness by conflicting with the signals, leading to a non-optimal choice in mates. I hypothesize that unfavourable acoustic conditions disrupt the perception of acoustic courtship signals, and thus predict that females will show altered preferences to courtship songs under different acoustic conditions. To test this hypothesis, I used no-choice behavioural tests where females are presented with "muted" males coupled with either high quality, low quality or no courtship song. Again, these trials were staged under different acoustic conditions to observe how preferences change.

Chapter 5

The last data chapter focuses on the conflict between anthropogenic noise and acoustic aggressive signals specifically examining the costs to both combatants when aggressive contests occur under anthropogenic noise conditions. Dominant individuals that emerge from intra-specific aggressive encounters are likely able to acquire more resources, such as territory, food or mates, leading to an increase in their reproductive success. This enables anthropogenic noise to potentially alter an individual's fitness by conflicting with the acoustic signals used in these encounters. I hypothesize that unfavourable acoustic conditions conflict with acoustic "victory displays" and thus predict an increase or alteration in the aggressive behaviours displayed in interactions under anthropogenic noise conditions. To test this hypothesis, I use arena-based behavioural assays of the stereotypic aggressive contests between male *G. bimaculatus* under different acoustic conditions. Furthermore, I implemented physiological analyses to quantify the energetic costs of aggressive signalling under anthropogenic noise conditions.

Chapter 6

This final chapter synthesises the findings detailed in the previous chapters to compose a final discussion on the conflict between anthropogenic noise and sexually selected acoustic communication, and the resulting behavioural consequences and disruptions to energetic trade-offs. I also give further consideration to the acoustic characteristics necessary for causing any disruptions observed and discuss potential future projects that have arisen from this thesis.

Thus, this thesis details the consequences to fitness when anthropogenic noise conflicts with multiple different acoustic sexual signals, including mate searching, courtship and aggressive behaviours. Furthermore, my research offers insight into the mechanisms that cause this conflict to occur and the costs associated with changing behaviours to combat disruption from anthropogenic noise.

Chapter 2

Mate Searching under Conditions of Anthropogenic Noise

Abstract

Many animals use acoustic communication as a means of sending important biological information, such as their location, to potential receivers.

However, anthropogenic noise is known to affect the ability of some species to either produce or receive signals, which may influence their reproductive success. This chapter investigates the effect of anthropogenic noise on the mate searching behaviours of the field cricket *Gryllus bimaculatus*. To accomplish this, I conducted phonotaxis trials with female field crickets under different acoustic conditions, and assessed their ability to detect and move towards conspecific male calls. The presence of traffic noise reduced the likelihood that the female would approach the male calls and also reduced the time that the female spent attending to the calling stimulus before making her decision. However, the presence of white noise did not reduce the likelihood of approaching the calling speaker, indicating that the average amplitude of anthropogenic noise is, alone, not enough to cause this conflict, but frequency and fluctuations in the stimulus or other characteristic might be. This study supports the hypothesis that anthropogenic noise does indeed influence the detectability of acoustic mate location signals, thus disrupting mate searching behaviour.

Introduction

Many animal communication systems are heavily influenced by sexual selection due to their importance to reproductive success (Andersson, 1994). Such reproductive communication can fall into a number broad categories: i) mate attraction signals, ii) courtship signals and iii) agonistic displays. Both courtship and agonistic displays are well studied in the context of sexual selection (Andersson, 1994). Here, signals are assumed to advertise

some aspect of the individual's quality either to encourage intercourse with a conspecific mate (Scheuber, Jacot and Brinkhof, 2004) or to reduce unnecessary contest costs when fighting for resources (Clutton-Brock et al., 1979). Mate attraction signals, on the other hand, are signals that have evolved, at least in part, to convey information about the location of the signaller so that a conspecific receiver can locate them and mating can occur (Tyack, 1981; Rutowski, McCoy and Demlong, 2001; Shine et al., 2005; Allen, Barry and Holwell, 2012). Species that are solitary, or dispersed over large home ranges, utilize these signals to ensure they can accurately locate a conspecific mate and that meetings are not left entirely to chance. This can be seen in territorial male butterflies of the species *Asterocampa leilia*, which use visual cues (colour, spots and patterns on the wing) from conspecific females to detect them and locate them (Rutowski, McCoy and Demlong, 2001). However, these signals are also observed in species which live socially or in close proximity to conspecifics. Garter snakes (Genus: *Thamnophis*), which undergo brumation in large communal dens, are a good example of this. Directly after emerging from brumation, the males will follow trails of pheromones left by the females in order to locate them, and potentially mate with them (Shine et al., 2005). Thus, mate location can occur though different social structures but also though different modalities (e.g. visual and pheromonal). Yet perhaps the most well-known modality associated with mate location is acoustic communication. Acoustic communication is widespread throughout the Animal Kingdom and is used by both vertebrates (e.g. birds, Byers and Kroodsma, 2009; and marine mammals, Tyack, 1981), and invertebrates (e.g. insects such as crickets, Hirtenlehner and Römer, 2014). As such, acoustic behaviours may be essential in allowing conspecifics to find each other in order to mate. A reduction in the ability to send or receive these signals could be detrimental to an individual's reproductive success and population stability (Griesinger, Evans and Rypstra, 2011).

The evolution and flexibility of acoustic behaviours allows species to exploit particular acoustic niches and adapt to environmental changes in order to maximise signal transmission. For example, many communication

systems are subjected to, and indeed adapted to, high levels of background noise (Wollerman and Wiley, 2002). However, due to the heavy development and urbanisation that our planet is undergoing (United Nations, 2014), anthropogenic noise is increasingly recognised as a new selection pressure on acoustic signalling systems (See Chapter 1). Research into the acoustic behaviours of certain species has shown that anthropogenic noise conflicts with their acoustic signals, and they alter their behaviours to minimise the conflict and ameliorate potential costs. For example, Cunnington and Fahrig (2010) researched this phenomenon in anurans, a group of animals that have globally declining populations and are heavily affected by road traffic, a common source of anthropogenic noise. In their study, they observed a number of different species in either high or low traffic noise breeding grounds and compared their vocalizations within each species. They found that individuals of the same species had differing song characteristics between the two breeding grounds. To demonstrate that the traffic noise was the main factor influencing this difference, they broadcast traffic noise at the low noise site and noted that the vocalizations of the individuals changed almost immediately and were more similar to the high traffic individual's vocalizations. Responses to anthropogenic noise through signal alterations are found throughout other taxa, including birds (Luther, Phillips and Derryberry, 2016), fish (Holt and Johnston, 2014), mammals (Melcón et al., 2012), and invertebrates (Orci, Petróczki and Barta, 2016). Interestingly, different types of acoustic alterations are also observed both within and between signalling systems. Examples include pitch modulation to avoid conflicting with similar frequency bands of anthropogenic noise (Cunnington and Fahrig, 2010; Luther, Phillips and Derryberry, 2016), shifts to different signal modalities (Partan, 2017), increases in amplitude to increase signal to noise ratio (Cunnington and Fahrig, 2010; Holt and Johnston, 2014; Luther, Phillips and Derryberry, 2016), as well as changes to the temporal (Melcón et al., 2012; Orci, Petróczki and Barta, 2016) and spatial use of signalling (Cunnington and Fahrig, 2010) to avoid competition with anthropogenic noise. This is strong supporting evidence that unwanted noise can lead to communication difficulties, necessitating changes in behaviour. However, these signalling alterations are constrained by the

behavioural flexibility of the species or signalling system in question, so signalling individuals may not be able to alter aspects of their acoustic behaviour to avoid conflicting with anthropogenic noise. For example, many avian species can change the frequency of their song (e.g. *Zonotrichia leucophrys nuttalli*; Luther, Phillips and Derryberry, 2016) due to their muscular vocal organs and ability to sing at multiple frequencies, whereas mature crickets (e.g. *Gryllus bimaculatus*) should be unable to do this due to the fixed structures used for stridulation (Bennet-Clark, 1989), although there is now emerging evidence of long-term changes occurring in other Orthopterans (Lampe et al., 2012).

The finding that signalling individuals will adjust their acoustic behaviours in response to competition from anthropogenic noise is now a well-documented phenomenon. Due to the necessity of mate location signals for the discovery of potential mates, these alterations compensate for the presence of anthropogenic noise are strongly associated with ameliorating potential costs to an individual's fitness. However, a review by Read, Jones and Radford (2014) highlights the extent to which studies on anthropogenic noise specifically fail to address the associated costs to the reproductive success of the signaller, whilst instead concentrating on the 'benefits' of apparently adaptive responses, even though empirical evidence of these benefits is scarce (Slabbekoorn, 2013). Studies exploring the sexual consequences of anthropogenic noise are now becoming more familiar (Schmidt, Morrison and Kunc, 2014; Gurule-Small and Tinghitella, 2018) but these mostly fail to address the mechanisms behind these consequences, demanding further investigation in this area.

This chapter aims to address this gap in knowledge by investigating the conflict between acoustic mate location communication and low frequency anthropogenic noise. Specifically, I examined the ability of individuals to detect conspecific sexual signals under noise conditions, along with the mechanisms behind this conflict, to quantify the potential costs of signalling under these conditions. To investigate this, I conducted an experiment using *G. bimaculatus*, a model species chosen for their well-documented acoustic communication (Alexander, 1961; Simmons, 1988)

but also due to the necessity for more invertebrate research when discussing anthropogenic disturbances (Morley, Jones and Radford, 2014). The males of this species produce a long range signal, known as a “calling song”, to attract potential mates to their location (Alexander, 1961). Conspecific females perceive this signal using specialized organs on their front tibia, known as the tympana, and navigate their way towards the signal in a process known as phonotaxis (Hirtenlehner and Römer, 2014). Furthermore, the auditory tuning of female *G. bimaculatus* is well understood (Popov, Markovich and Andjan, 1978), meaning a more comprehensive conclusion on the different aspects of anthropogenic noise can be inferred. In this experiment, I exposed females to three acoustic conditions with distinct characteristics (ambient, traffic or white noise) and observed their ability to locate the calling songs of a group of males. If anthropogenic noise conflicts with acoustic signals due to average amplitude alone, then we would expect to see compromised phonotaxis under both traffic noise and white noise conditions. However, if the critical component causing interference is another characteristic, such as the frequency or signal fluctuations of this stimulus, then we would expect to see compromised phonotaxis only under one of these noise conditions.

Methods

Study Organisms

I set up breeding colony of the species *G. bimaculatus* in July 2016 using individuals purchased from a local entomological retailer (Cambridge Reptiles, Cambridge, U.K.). Individuals were housed in multiple plastic terraria (30 x 17 x 20cm) in a temperature controlled room at 26°C and at an ambient noise level (44.3 ± 3.8 dBA, measured through a CEM DT-8852 IEC 61672-1 class 2 compliant handheld sound level meter over a period of 1 hour). A 12:12h light cycle was used, with “Sunrise” and “Sunset” at 06:00 and 18:00 local time respectively. I provided sustenance in the form of fresh vegetables, rabbit food pellets and water *ad libitum*. Each terrarium was also equipped with newspaper to create a 3D environment to enable crickets to moult successfully. Prior to final moult, I separated juveniles into male and female tanks to ensure that I only used virgin crickets in

behavioural trials, as mating status can alter phonotaxis response behaviour in *Gryllus* spp. (Lickman, Murray and Cade, 1998). I only observed final moult females, free from obvious damage or disease, of three week post moult or younger, as they become less receptive to male calls after this time (Adamo and Hoy, 1994). Prior to behavioural trials, I weighed females (in grams) and measured them by taking the width of their pronotum (in mm). I only used females once each to avoid a reduction in stimuli response or habituation to acoustic conditions.

Creating Playback Stimuli

The three main acoustic stimuli I used in this study were *G. bimaculatus* calling song (Figure 1.1a), road traffic noise, and generated white noise (Figure 2.1), and the ambient noise conditions of the room were also utilized (44.3 ± 3.8 dBA). For the male calling song, I recorded a group of mature virgin males (approximately 20 individuals, varying in quality indicators such as age and size) using a RØDE NTG4+ shotgun microphone connected to a TASCAM DR-07 MKII linear PCM recorder (.wav format, 16-bit resolution and 48 kHz sampling rate). A group was chosen as opposed to a single calling individual in order to negate any effect of quality perception or individual preference on male calls. Recordings were conducted adjacent, at a distance of 15cm, to a 30 x 17 x 20 cm plastic tank that held the individuals being recorded. To encourage naturalistic singing behaviour, the tank was also equipped with crumpled newspaper to create a 3-D environment with burrows, from which males naturally sing (Simmons, 1988). I obtained a 30 minute recording between 11:00 - 13:00 local time and between 24 - 28°C to match the conditions used in the phonotaxis trial. Simultaneously, I measured the Sound Pressure Level (SPL) of the calling individuals using a CEM DT-8852 IEC 61672-1 class 2 compliant handheld sound level meter (56.2 ± 6.1 dBA). I edited the acoustic recording using Audacity 2.1.2 (<http://www.audacityteam.org/>, last accessed 7th March 2019) to remove extended periods of silence (2+ seconds), which is not a necessary component of the song for recognition (Meckenhäuser, Hennig and Nawrot, 2011), and excessive courtship singing, resulting in a 20 minute long “song” stimulus.

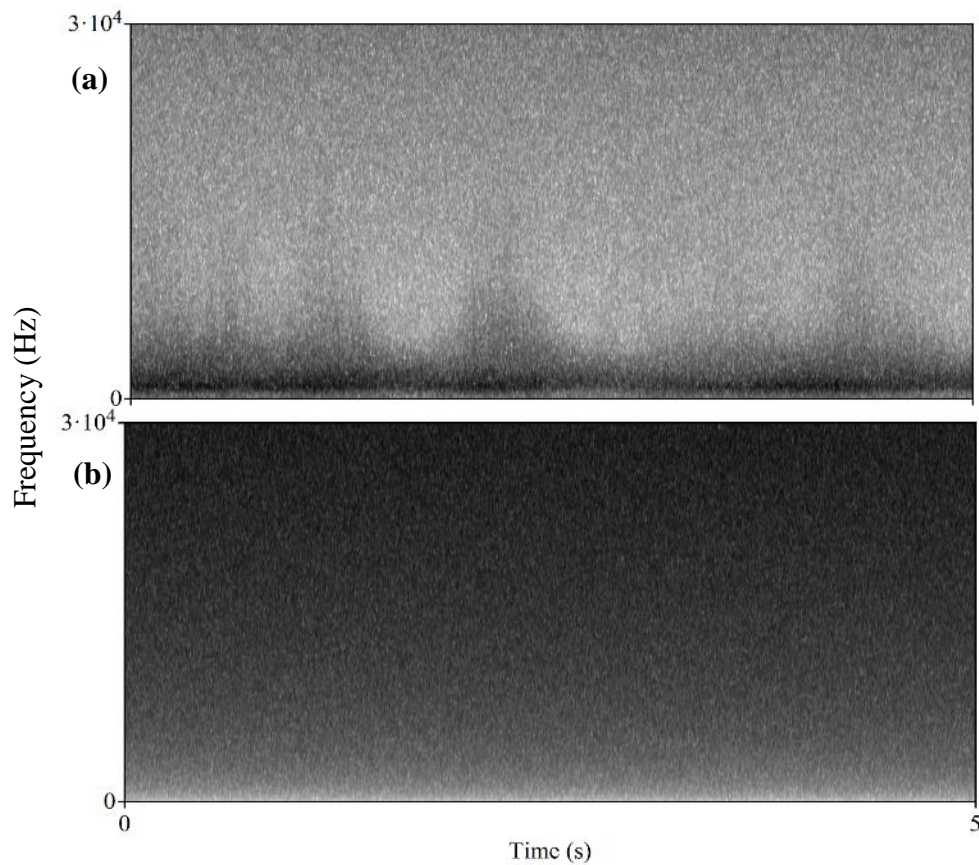


Figure 2.1. Frequency spectrograms of recordings used showing (a) typical traffic noise, and (b) computer generated white noise, with darker region showing which frequencies have the most energy at any given time. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0–5s); frequency range: 0–30000Hz.

I used two novel acoustic stimuli, traffic noise and white noise, to measure the effects of different noise characteristics on female behaviour. Traffic noise was selected as a suitable representation of anthropogenic noise as it is low in frequency (approximately 0.5 - 5 kHz), has fluctuating characteristics (such as amplitude), and is common in urban areas (Lee et al., 2014). Traffic noise was recorded using the same equipment as the calling song recording. I obtained a 30 minute recording during rush hour (16:00-18:00 local time) and at a five metre distance from the A14 road (South Cambridgeshire, U.K.), with SPL being measured simultaneously using the handheld sound meter (79.1 ± 3.5 dBA). I removed excessive periods of quiet (5+ seconds) using Audacity, which produced a 20 minute

long “traffic noise” stimulus (Figure 2.1a). White noise was selected as an alternative noise stimulus as it is an electronically generated stimulus that covers all frequency bands at the same level of intensity. I generated a 20 minute “White noise” stimulus using Audacity 2.1.2, with the same average amplitude as the “Traffic noise” stimulus (79.1 dBA) so that average amplitude was the only characteristic the stimuli shared (Figure 2.1b). The relative acoustic power of both of these stimuli was also calculated using output from the `ama()` function (50 kHz sampling frequency, absolute amplitude envelope) from the package `seewave` (Sueur, Aubin and Simonis, 2008) in Rstudio (Rstudio Team, 2016; R Development Core Team, 2017). At 4.5 - 5.5 kHz (the frequency band which is most sensitive to female *G. bimaculatus*; (Popov, Markovich and Andjan, 1978), the traffic and white noise stimuli had similar relative power (2.7% and 2.2% respectively).

Behavioural Trials

Phonotaxis trials took place in a 70cm x 49cm behavioural arena constructed from corrugated plastic (Figure. 2.2). The arena was surrounded by a 10cm high wall and with a speaker embedded into each end wall, both surrounded by foam cushioning in order to reduce any structural vibration caused by sound production. I checked playback from these speakers was the same average amplitude (from the centre circle) as each initial recording by measuring SPL through the same handheld sound level meter as before. The inside of the arena was visibly split into several areas; a central circle (diameter = 6cm), 4 ‘main’ areas (top left, top right, bottom left, bottom right) and two half circles by each speakers (radius = 8cm, >1% of the overall area of the arena) to designate where individuals has made a “choice”. A video camera (SONY HDR-CX625 HANDYCAM®) was secured above the arena to record each observation. I conducted all observations between 11:00 - 13:00 local time to minimize time of day affecting phonotaxis behaviour, as it does in other *Gryllus* behaviours (Zuk, 1987), and at 24 - 28°C.

I used *A priori* sample size calculations using G*Power (<http://www.gpower.hhu.de/en.html>, last accessed 7th March 2019) on the

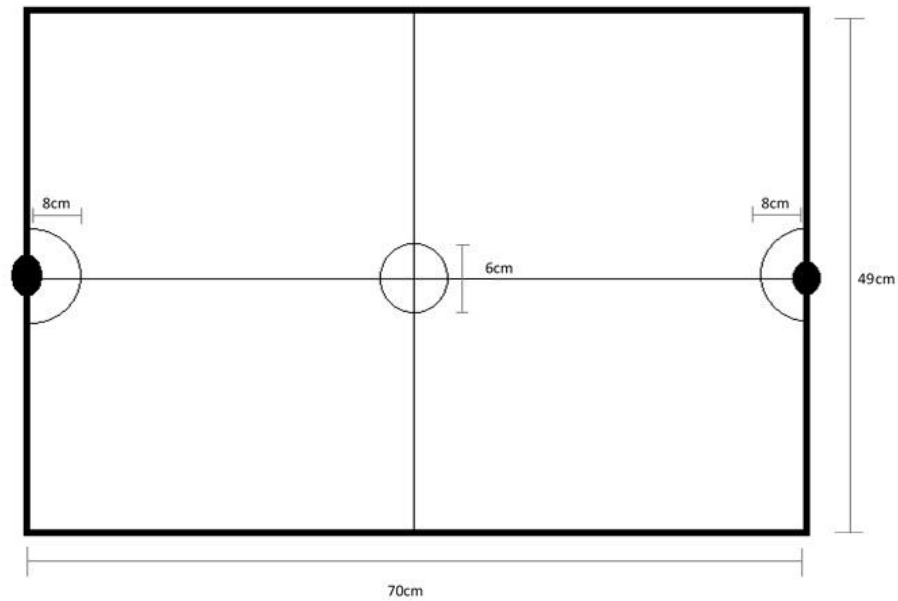


Figure 2.2. Behavioural arena used in choice trials. Figure shows speakers embedded in both ends (black ovals) and the visible sections of the arena.

first four trials of each noise condition. This was to calculate the sample size necessary to achieve a particular level of power. This led to 30 replicate trials for each acoustic condition, resulting in a total of 90 trials (ambient noise $n = 30$, traffic noise $n = 30$, white noise $n = 30$). For each trial, I placed a single virgin adult female *G. bimaculatus* under a plastic cup (diameter = 6 cm) on the central circle, and left them to acclimatise for five minutes under ambient noise conditions. After this acclimatisation period, the “song” stimulus was played from one of the speakers (switched pseudo-randomly between trials so that there were the same number of trials involving calls being produced from the left and right speaker). Simultaneously, I broadcast the acoustic condition (traffic noise or white noise) from both speakers, or not at all for ambient noise conditions, so choice was not linked to condition preference. The cup was then immediately removed, allowing the female to move freely around the arena. I continued each trial for a period of ten minutes or until the individual made a ‘choice’ (enters the half circle next to either speaker). The individual was then removed and the arena was cleaned with 70% ethanol and wiped dry to remove any olfactory cues ahead of subsequent trials. For each

behavioural trial I conducted, a control counterpart was also carried out ($N = 90$; ambient noise control $n = 30$, traffic noise control $n = 30$, white noise control $n = 30$) to measure possible spatial biases (i.e. side preference), and to compare behavioural results with standard trials. These trials were interspersed with the main trials and featured the same setup as them, but no calling song was broadcast, so females reacted to the non-directional acoustic condition alone.

Behavioural Analysis

I coded the footage of each trial through the use of B.O.R.I.S. (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). For each trial, I recorded the female ‘choice’ (an individual made a ‘correct’ choice if she entered the half circle next to the calling speaker, and an ‘incorrect’ choice if she entered the half circle next to the non-calling speaker). In addition, an individual’s choice latency (make a choice from the start of the trial), latency to move (leave the starting area) and latency reach a speaker (time taken from leaving starting area to making a ‘choice’) were also measured.

Statistical Analyses

I conducted all statistical analysis with the statistical package RStudio 1.0.143 (Rstudio Team, 2016; R Development Core Team, 2017) with the packages ‘dunn.test’ (Dinno, 2015), ‘Hmisc’ (Harrell Jr., 2006), and ‘multcomp’ (Hothorn, Bretz and Westfall, 2008). All graphs and plots were created using base R and with the package ‘ggplot2’ (Wickham, 2016). To prevent outliers from skewing the analysis, I removed statistical outliers from the dataset (ambient noise $n = 1$, traffic noise $n = 2$, white noise $n = 0$, ambient noise control $n = 2$, traffic noise control $n = 2$, white noise control $n = 4$) using a generalized extreme Studentized deviate (GESD) test to the level of $k = 5$ for each acoustic condition. This did not change the outcome of primary analysis, but did allow for clearer post-hoc analysis. I used a generalized linear model (GZLM) using a binary logistic function to test for difference in choices between acoustic conditions (‘correct’ versus ‘incorrect’), and coupled this with a TukeyHSD multiple comparison

analysis to test the difference between each pair of groups. Presented effect sizes (Log odds ratio \pm SE) were acquired from the model summary. I also visually assessed plots of residuals vs. fitted values to ensure that the model fit the data well, and that the data did not violate the model's assumptions. I used one-way Chi-Squared tests to determine if choice ratios were different to a random distribution in each acoustic condition. All scale data used in analysis were tested for normality using a Shapiro-Wilk tests, and suitable non-parametric analysis were conducted when necessary. I used Kruskal-Wallis tests to check for differences in weight and size between acoustic conditions. Differences between the three different measures of latency between acoustic conditions were analysed using a Kruskal-Wallis test. I coupled these with Dunn's test post hoc tests with Bonferroni corrections to identify between-group differences. I also checked arena side biases were checked in the control trials using Chi-squared tests, and the effect of the calling song, female pronotum width (mm), female mass (g), and ambient temperature ($^{\circ}\text{C}$) on latency was checked using Spearman's rank correlation tests and Mann-Whitney U tests.

Results

Size and Weight Differences

I found no difference between the size (Kruskal-Wallis: $X^2_2 = 0.171$, $n_1 = 29$, $n_2 = 28$, $n_3 = 30$, $P = 0.918$) or weight (Kruskal-Wallis: $X^2_2 = 5.114$, $n_1 = 29$, $n_2 = 28$, $n_3 = 30$, $P = 0.078$) of individuals between acoustic conditions. There was also no difference between the size (Kruskal-Wallis: $X^2_2 = 3.929$, $n_1 = 28$, $n_2 = 28$, $n_3 = 26$, $P = 0.140$) or weight (Kruskal-Wallis: $X^2_2 = 3.175$, $n_1 = 28$, $n_2 = 28$, $n_3 = 26$, $P = 0.204$) of the individuals between acoustic conditions in the control trials.

Mate Location Choices

Females did not show any side preference within any of the acoustic control conditions (Ambient Noise: $X^2_1 = 1.286$, $N = 28$, $P = 0.257$; Traffic Noise: $X^2_1 = 0.571$, $N = 28$, $P = 0.449$; White Noise: $X^2_1 = 0.154$, $N = 26$, $P = 0.695$), or any difference between acoustic conditions ($X^2_2 = 0.26$, $N = 82$, $P = 0.878$). Individuals did show a significant difference in the amount of

‘correct’ choices made between the three acoustic conditions (GLZM{b}:Likelihood $X^2_2 = 7.462$, $N = 86$, $P = 0.024$). Fewer individuals approached the calling speaker in both traffic noise (-1.57 ± 0.49) and white noise trials (-1.16 ± 0.62) compared to those under ambient noise conditions. TukeyHSD post-hoc pairwise comparisons also revealed differences between in the amount of ‘correct’ choices made between some of the acoustic conditions (Figure. 2.3). Females made significantly more ‘correct’ choices under ambient noise conditions than under traffic noise conditions ($N = 57$, $P = 0.031$). There was no difference in the amount of ‘correct’ choices made between ambient noise conditions and white noise conditions ($N = 58$, $P = 0.142$), and there was no difference in the amount of ‘correct’ choices made between traffic noise conditions and white noise conditions ($N = 59$, $P = 0.724$).

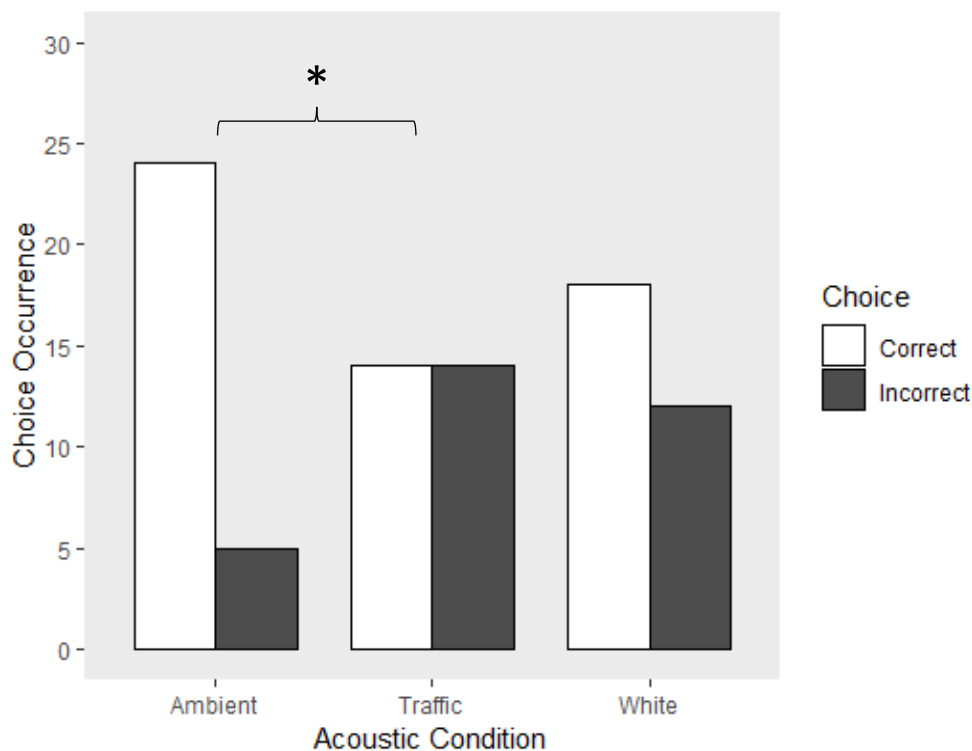


Figure 2.3. The number of ‘correct’ and ‘incorrect’ choices between ambient noise ($n = 29$), traffic noise ($n = 28$), and white noise ($n = 30$) trials. Brackets with an asterisk show a significant result from pairwise analysis.

Behavioural Latencies

Females showed no difference in their latency to make a choice from the start of the trial between each acoustic condition and their respective control counterpart (ambient noise: $U = 365$, $N = 57$, $P = 0.521$; traffic noise: $U = 306$, $N = 56$, $P = 0.162$; white noise: $U = 491$, $N = 56$, $P = 0.099$). Choice latency was not affected by individual weight or size, or the ambient temperature, within each condition in both standard and control trials (Table 2.1). There was a difference in choice latency between different noise conditions (Table 2.2). Post-hoc tests revealed that individuals under traffic noise conditions ‘chose’ more quickly than those in ambient and white noise conditions (Figure 2.4). The driving factor here was the latency to leave the starting area, not the latency to reach a speaker. Individuals under traffic noise conditions left the starting area more quickly than individuals in either ambient or white noise conditions (Figure 2.5), but there was no significant difference between the latency to reach a speaker between the acoustic conditions (Table 2.2). Individuals showed a similar pattern in their latency to choose in the control conditions (Figure 2.6), and in their latency to leave the starting area, although this was not significant (Table 2.2).

Table 2.1. Output from Spearman’s Rank Correlation test on the effect of weight, size and temperature on latency to choose, within each acoustic condition. r_s and P values are stated, along with sample sizes for both standard and control trials.

		Ambient ($N = 29$, $N_{\text{control}} = 28$)		Traffic ($N = 28$, $N_{\text{control}} = 28$)		White ($N = 30$, $N_{\text{control}} = 26$)	
		r_s	P	r_s	P	r_s	P
Weight	Standard	0.133	0.493	0.017	0.932	0.205	0.277
	Control	-0.105	0.594	0.106	0.591	-0.173	0.397
Size	Standard	-0.138	0.476	-0.07	0.722	-0.097	0.61
	Control	-0.287	0.139	0.105	0.595	-0.258	0.202
Temperature	Standard	0.182	0.345	0.178	0.364	-0.061	0.75
	Control	-0.067	0.735	0.234	0.23	0.177	0.387

Table 2.2. Output from Kruskal-Wallis tests and pairwise post hoc tests (including test statistic, sample size and P-value) on different levels of latency for standard trials and control counterparts. χ^2_2 and z test statistics are reported for Kruskal-Wallis tests and Dunn's test respectively. A *n*, T *n*, and W *n* show the sample size of each noise conditions (ambient, traffic, and white, respectively) used in each test. Bold P-values indicate a significant result.

	Statistic	A <i>n</i>	T <i>n</i>	W <i>n</i>	P	Figure
Latency to choose	10.89	29	28	30	0.004	Figure 2.4
Ambient-Traffic comparison	3.095	29	28	-	0.003	
Ambient-White comparison	0.557	29	-	30	0.886	
Traffic- White comparison	-2.569	-	28	30	0.015	
Latency to choose control	8.224	28	28	26	0.016	Figure 2.5
Ambient-Traffic comparison	2.261	28	28	-	0.036	
Ambient-White comparison	2.646	28	-	26	0.012	
Traffic- White comparison	0.427	-	28	26	1	
Latency to move	9.672	29	28	30	0.008	Figure 2.6
Ambient-Traffic comparison	3.018	29	28	-	0.004	
Ambient-White comparison	0.858	29	-	30	0.586	
Traffic- White comparison	-2.192	-	28	30	0.043	
Latency to move control	5.787	28	28	26	0.055	-
Latency to choose from movement	1.934	9	28	30	0.38	-

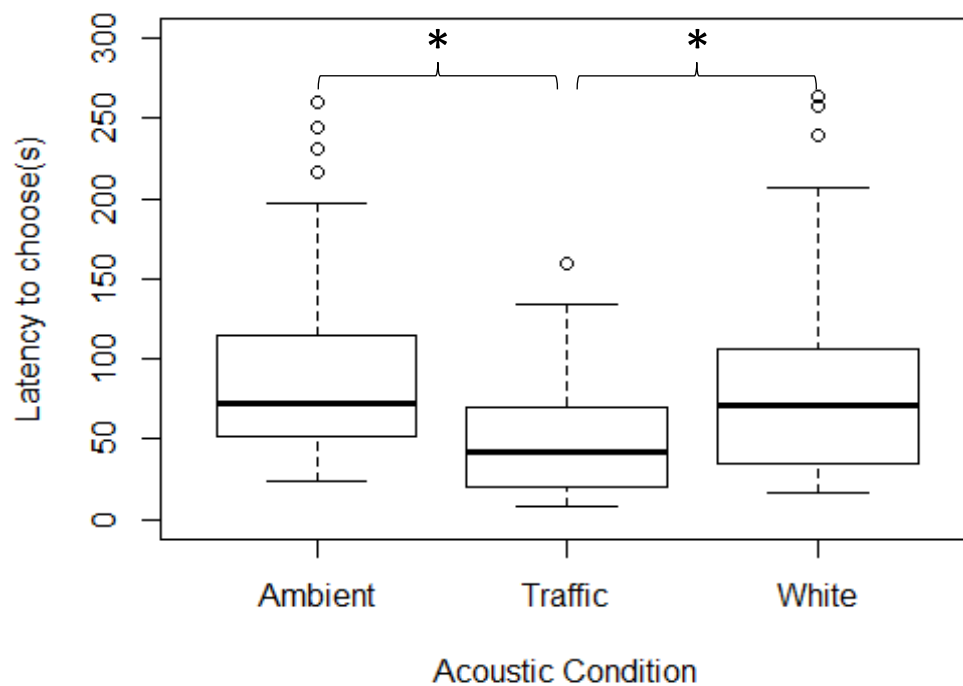


Figure 2.4. Boxplot of choice latency (amount of time from start of trial to choice) between the different acoustic conditions. Brackets with an asterisk show a significant results from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

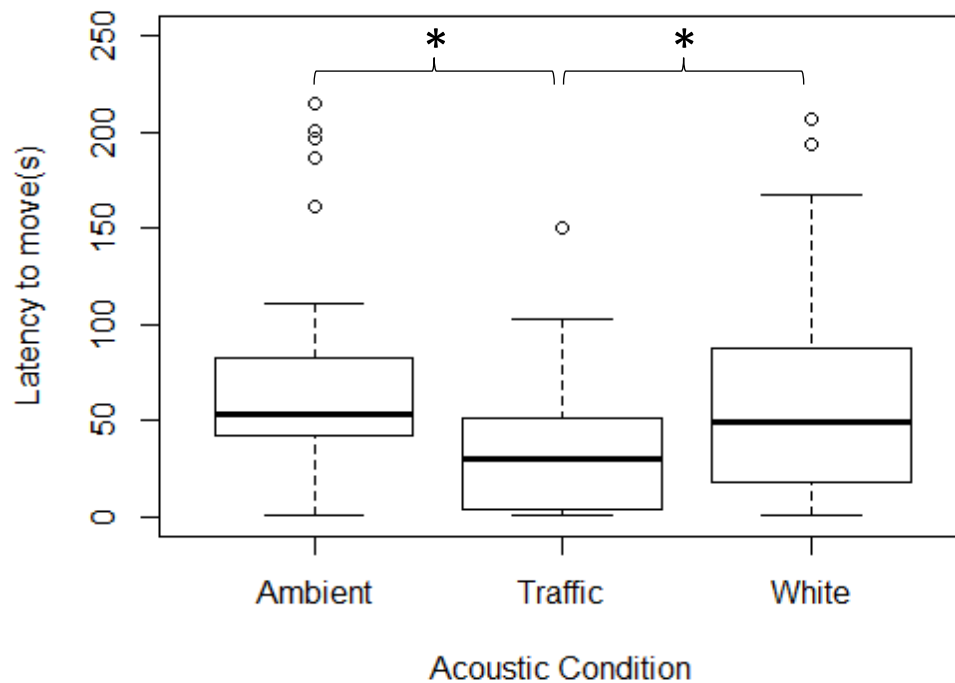


Figure 2.5. Boxplot of movement latency (amount of time from start of trial to initial movement) between the different acoustic conditions. Brackets with an asterisk show a significant results from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

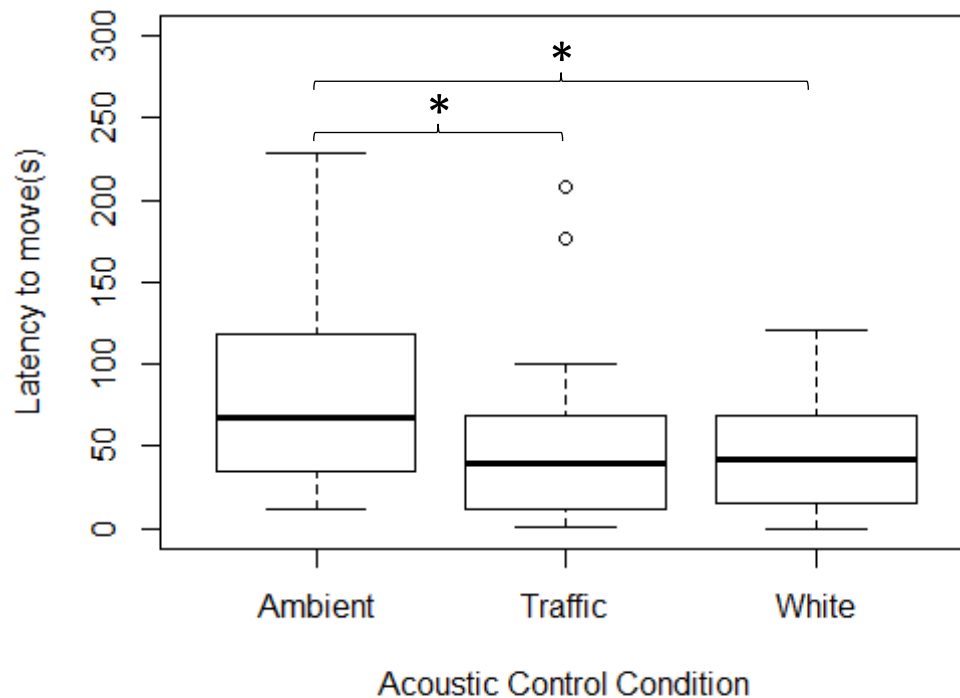


Figure 2.6. Boxplot of choice latency (amount of time from start of trial to choice) between the different acoustic control conditions. Brackets with an asterisk show a significant results from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

Discussion

My results demonstrate that anthropogenic noise can affect the ability of mate-searching individuals to locate potential mates via acoustic signalling behaviours. Furthermore, this acoustic interference is not driven simply by the average amplitude of the noise in the environment, but is potentially due to the dominant frequency or fluctuating characteristics of the stimulus, or by a combination of these factors. Traffic noise significantly affected the mate-locating behaviour of female *G. bimaculatus*, both in terms of finding calling males and in their latency to do so. These results support those of a previous study that show reduced phonotaxis capabilities of *G. bimaculatus* under anthropogenic noise conditions (Schmidt, Morrison and Kunc, 2014), whilst revealing more about the acoustic mechanisms driving the changes, which may have profound implications for an individual's fitness.

There was a clear difference in the amount of 'correct' choices made between the three acoustic conditions. This significance lies between ambient noise (where the majority of individuals chose 'correctly') and traffic noise (where only half the individuals chose 'correctly'). The results of the traffic noise trials could indicate three possible outcomes. First, it could show that half the individuals failed to make the correct choice. Second, it could show that all individuals could not locate the mate but some approached the correct speaker by chance. Or third, it could show any number of mixtures between these two. Conversely, white noise trials showed no significant difference in choice ratios between either ambient noise or traffic noise conditions. These observations thus provide evidence in support of the hypothesis that anthropogenic noise is conflicting with these signals due to its frequency characteristics. Both *G. bimaculatus* calls and anthropogenic noise have relatively low frequencies (<5 kHz, approximately 4 - 5 kHz and 0.5 - 6 kHz respectively; Figure 1.1a, Figure 2.1). This phenomenon is known as masking, and occurs when irrelevant noise decreases the likelihood of detecting or recognising the desired signal (reviewed by Naguib, 2013). By altering the frequency components of an acoustic signal, individuals can avoid the effects of masking from anthropogenic noise, a behaviour that has been observed in anurans

(Cunnington and Fahrig, 2010) and birds (McMullen, Schmidt and Kunc, 2014; Potvin, Mulder and Parris, 2014), providing further evidence that the conflict occurs due to sharing similar frequencies. Similar short term adaptations are difficult to observe in orthopterans due to their fixed signal producing structures. However, long term effects have been documented showing similar changes in frequency (Lampe et al., 2012; Lampe, Reinhold and Schmoll, 2014). Interestingly, some animals adopt alternative, or a combination of, strategies for competing with masking background noise, including increased signalling effort (Cunnington and Fahrig, 2010; Holt and Johnston, 2014), which would suggest that frequency is not the only factor causing this acoustic conflict.

Evidence for another characteristic (such as modulations/fluctuations of a stimulus) being the driving factor is also present in these results. Due to the narrow auditory tuning of *G. bimaculatus* (Popov, Markovich and Andjan, 1978), females are much more sensitive to frequency bands around 4.5 - 5.5 kHz. Interestingly, both stimuli used in this experiment had a similar relative acoustic power at this frequency band (2.7% and 2.2% respectively). As these stimuli were played at the same average amplitude, then the power at this frequency band would have also been similar, suggesting that the amplitude and frequency of the noise may not be the main factor of this acoustic conflict. An alternative way that these two stimuli differ is in the fluctuations within the signal. The traffic noise stimulus has fluctuations in both its amplitude and frequency distribution, which is a common characteristic also seen in other forms of anthropogenic noise. Comparatively, the white noise stimulus is constant in frequency spectrum and amplitude, meaning it differs from anthropogenic noise notably in this regard. Through exposure to this predictable, persistent noise, individuals may be able to habituate or increase their tolerance to such noises (Nedelec et al., 2015; Kern and Radford, 2016). Thus, the amount and intensity of fluctuations in an acoustic stimulus could be a key factor in the conflict with acoustic signals.

A further acoustic effect that may compromise communication systems is that of 'distractions', which use up an individual's finite

attentional capabilities (Naguib, 2013). This is mostly dependent on cognition and the neural processing of different sounds (i.e. many different frequencies), and fluctuating noises, such as the passing of vehicles, are less detrimental to attentional capabilities than constant noises (Vélez and Bee, 2011). As such, it is unlikely that this is the effect seen with traffic noise in this study, as it has a small dominant frequency range (0.5 – 6 kHz) and fluctuations in amplitude (79.1 ± 3.5 dBA) and other acoustic characteristics (Lee et al., 2014). However, this could explain the difference, or lack thereof, we see between white noise and ambient noise or traffic noise. White noise, which is a continuous non-fluctuating broadband noise, may have affected the processing capabilities of the females used in this study, resulting in a reduced, but not complete loss, reaction to male calls. As these hypotheses are not mutually exclusive, it is plausible that the conflict between anthropogenic noise and acoustic signals is due to a mixture of factors, such as frequency, amplitude, and the modulation of the acoustic characteristics. Incorporating electrophysiology methods for song attraction, as seen in studies such as Stout et al. (2011), could discern the main effect causing this difference.

Interestingly, studies in *Oecanthus* tree crickets have shown that females suffer no reduction in their phonotaxis abilities when in the presence of road noise (Costello and Symes, 2014), making them an intriguing comparison to the *Gryllus* species used in this study. Potentially, the difference in outcomes between these studies could be due to the active auditory tuning present in *Oecanthus* species (Mhatre, Pollack and Mason, 2016). This ability to alter their hearing under different environmental conditions, for which there is no evidence in *Gryllus* species, would offer a strong selective advantage in rapidly changing environments were the plasticity of behavioural traits are utilized, such as those affected by anthropogenic disturbance.

The other distinct behavioural difference shown in this experiment is the contrast in choice latency (amount of time from start of trial to choice) between the different acoustic conditions. Females took significantly less time to make a decision in the traffic noise trials than either the ambient

noise trials or white noise trials. The main aspect of this was due to latency of initial movement, where females under traffic noise conditions were much quicker to initiate movement and thus approach a speaker sooner than those in either white or ambient noise conditions. There was no difference in the time spent actually in motion between the acoustic conditions, meaning that females did not move quicker but actually spent less time attending to the acoustic stimuli present. Mate choice in *Gryllus* spp. is an important factor affecting fitness, as females will choose mates based on their various qualities indicators (Scheuber, Jacot and Brinkhof, 2003). In reducing the time spent assessing the resource, females risk making error-prone decisions, resulting in the choice of lesser quality males (Mowles, Jennions and Backwell, 2018). This effect of anthropogenic noise on resource assessment (the resource here being the signalling male) can also be seen in species using chemical and visual cues (Walsh, Arnott and Kunc, 2017). A possible explanation is that the lower latencies are a coping strategy for a reduced antipredator response. Anthropogenic noise has been shown to reduce an individual's antipredator response, in comparison to those under standard acoustic conditions (Chan et al., 2010; Kern and Radford, 2016), and also alter anti-predator behaviours due to its presence. For example, great tits, *Parus major* Linnaeus, maximize vigilance behaviours and reduce feeding behaviours during aircraft noise (Klett-Mingo, Pavón and Gil, 2016), suggesting that predator detection is reduced in these conditions. *Gryllus* spp. are also known to acoustically detect predators (Miller and Surlykke, 2001; Pollack and Martins, 2007). By acting more quickly, the females in these observations may have altered their strategy to find shelter (Hedrick and Kortet, 2006) or a quieter area, in order to compensate for the reduced likelihood of predator detection. Interestingly, a similar result can be seen in the latency to choose and latency to move in control conditions, where a conspecific call was not present. Here, the latency to choose (amount of time from start of trial to choice) of individuals was faster in both traffic noise and white noise conditions, compared to those in ambient noise conditions. This would suggest that the difference in latencies is primarily affected by acoustic conditions, and not whether a conspecific male call was present.

When choice latency was first plotted, it was clear there were some extreme values present. Through the use of the Generalized ESD it was possible to identify the statistical outliers. All the removed points of data were individuals that took much longer to make a decision in comparison to the majority of individuals observed. One possible explanation for these outliers is the presence of predator response behaviour. Freezing (immobility) is an anti-predator response behaviour present throughout many taxa (Chelini, Willemart and Hebets, 2009; Niemelä, DiRienzo and Hedrick, 2012). It is possible that some females perceived being released as a predation attempt, and thus froze in order to reduce the chance of a predator detecting them. This would mean extra time before the individual actually started to respond to the call song, which fits with the outlier occurring later than the majority of the data. Another possible explanation to these outliers is the effect of age. Built in to the methodology for this experiment is a cap for age to control for changes in response at an older age. However, there was no control for females of a younger age after eclosion. Individuals of other *Gryllus* species have shown to have an optimal phonotaxis response period around 10 days post eclosion (Pacheco et al., 2013). This opens up the methods for the possibility that some individuals in this experiment were below this age, thus their phonotaxis responses were not optimal, leading to increased time taken to choose. Whilst this last option would not explain outliers in the control trials (due to the lack of male calls), the first option may. If either of the possible explanations are the cause, they are not mutually exclusive so it may be a combination of the two.

Conclusions

The results in this chapter have shown that the presence of anthropogenic noise affects phonotaxis abilities, leading to potential consequences for an individual's reproductive success. In this case, traffic noise reduced the phonotaxis abilities of female *G. bimaculatus* compared to similar individuals in ambient noise conditions. Furthermore, the inclusion of a white noise trial demonstrated that average amplitude alone is not enough to offset phonotaxis behaviour significantly, and other acoustic characteristics,

such as the frequency of the stimulus, fluctuations in the signal or a combination of characteristics, are responsible for this conflict. The presence of traffic noise also reduced the time individuals spent assessing the calling song, which could lead to selecting a lower quality mate. As similar acoustic behaviours are used by a variety of taxa, these findings have important implications for mate location systems throughout the animal kingdom. Further research is required to understand the whole spectrum of effects in relation to sexually selected acoustic communication and its competition with anthropogenic noise (Chapter 3, 4 and 5).

Chapter 3

Acoustic Courtship Interactions under Conditions of Anthropogenic Noise

Abstract

Many animals use acoustic signals to advertise their willingness to mate during courtship interactions. However, due to the global increase in anthropogenic noise, the ability of some species to either produce or receive signals has been heavily affected, which may have important consequences for their reproductive success. In this study, I investigate the fitness costs incurred when anthropogenic noise interferes with acoustic courtship signals by observing the courtship behaviours of the Mediterranean field cricket, *Gryllus bimaculatus*. The presence of white noise significantly reduced the likelihood of successful courtship in staged courtship interactions. This effect was driven by a reduction in male courtship behaviour under white noise, not by reduced female response. Furthermore, under white noise conditions, the characteristics of male songs varied in several ways, including dominant frequency, stridulation rate and signal escalation. The comparison of typical anthropogenic noise (low-frequency traffic) and white noise (broadband frequencies) in this experiment allows me to discern the importance of specific characteristics (i.e. frequency, amplitude etc.) of the acoustic pollutant in causing this disruption. Differences in haemolymph metabolites were also detected between acoustic conditions and in relation to the changes in song characteristics, revealing potential physiological trade-offs. These results highlight the extent to which anthropogenic noise conditions may disrupt acoustic courtship signals and interactions.

Introduction

Sexually selected signals are ubiquitous throughout the Animal Kingdom as a result of the advantages they offer to an individual's reproductive success. Consequently, the variety of these signals, both in terms of function and modality, is incredibly diverse. For example, female *Hierodula masjuscula*,

a relatively understudied praying mantis species, use airborne pheromones in an attempt to attract potential mates, a behaviour correlated with mating success (Allen, Barry and Holwell, 2012). Similarly, male red-collared widowbird (*Euplectes ardens*) possess red collars which display an individual's fighting ability and aggressiveness. Males with larger collars often possess larger territories (Pryke, Lawes and Andersson, 2001), which in turn gives the male access to more females, boosting their reproductive success. These examples highlight two of the main functions of sexually selected signals (mate location signals and agonistic displays), along with two possible signal modalities (chemical and visual). However, in many biological systems an individual may also signal to a conspecific in order to encourage them to mate. These courtship displays may be costly to produce, which ultimately reveals information to the potential mate regarding the courting individual. For example, in many invertebrate systems, males provide their mates with a nuptial gift (such as a prey item or other nutritionally valuable material) in an attempt to gain or prolong mating privileges (Vahed, 1998). In the scorpion fly *Bittacus apicalis*, females will assess the value of the nuptial gift offered and only mate if the item is big enough, and thus of more nutritional value (Thornhill, 1976), although evidence for paternal investment correlated with their nuptial gifts is scarce (Vahed, 1998, 2007). Similarly, male sword tail fish, *Xiphophorus cortezi*, present themselves for inspection (lateral presentations) as part of their courtship display, allowing females to assess the symmetry of the male, which is associated with the male's ability to cope with genetic/environmental stress (Morris and Casey, 1998).

In many of these systems, these courtship behaviours are crucial for mating to occur, and are part of complex cost-benefit trade-offs. The "benefits" here relate to potential boosts in fitness (here used to describe the amount of genetic material passed on to the next generation, relative to others in the population; Hamilton, 1964) gained through increased reproductive success. On the other hand, the "costs", or potential reductions to fitness, involved in the production of these signals fall into two categories; extrinsic (indirect costs) or intrinsic (direct costs). Extrinsic costs

include the increased chances of attracting predators or reduction of other essential activities such as foraging (Zuk and Kolluru, 1998), whilst intrinsic costs include the physiological and energetic costs of an individual's ability to signal (Vehrencamp, Bradbury and Gibson, 1989; Mappes et al., 1996). To quantify the courtship intensity or quality of the signaller, intrinsic (energetic) costs are often observed through metabolic measurements (but see Clark, 2012) such as oxygen consumption (Zollinger, Goller and Brumm, 2011), CO₂ production (Vehrencamp, Bradbury and Gibson, 1989), or concentrations of circulating glucose (Matsumasa and Murai, 2005) and lactic acid (Mowles, 2014). The evolutionary balance of these trade-offs could be significantly altered if disruptions to courtship behaviours results in increased intrinsic costs and reduced fitness benefits.

Acoustic courtship signals are a common modality seen in courtship interactions throughout different taxa, including mammals (Alberghina et al., 2016), birds (Clark and Mistick, 2018), fish (De Jong et al., 2016), insects (Rybak, Sureau and Aubin, 2002), and crustaceans (Salmon and Atsides, 1968), and in both terrestrial and aquatic environments. Similar to other types of courtship signals, these acoustic stimuli are thought to display some aspect of signaller quality. For example, in male field crickets (*Gryllus bimaculatus*), which produce a courtship song to encourage females to mate, higher signalling rates are correlated with immunocompetence and females prefer signals with this aspect (Rantala and Kortet, 2003). We also know that these signals are energetically costly to produce (Mowles, 2014), suggesting that this is an 'honest' signal that reflects the overall condition of the signaller. Unfortunately, akin to other types of acoustic signals, these courtship signals are open to interference from a human generated acoustic pollution, also commonly known as anthropogenic noise (See Chapter 1 and 2). Anthropogenic noise is known to conflict with courtship interactions by reducing the amount of acoustic signals produced (De Jong et al., 2016; Smith et al., 2016). Additionally, many species have been shown to change aspects of their acoustic signal when under anthropogenic noise conditions, such as alterations in frequency (Cunnington and Fahrig, 2010; Potvin, Mulder and Parris, 2014) and amplitude (Brumm, 2004; Holt and Johnston,

2014). These changes to an acoustic signal may alter the physiological costs associated with the signalling behaviour (Zollinger, Goller and Brumm, 2011). With increases in urbanisation leading to increases in anthropogenic noise sources and spread (Chapter 1), it is important to investigate how anthropogenic noise conflicts with acoustic courtship signals, and the behavioural and physiological consequences that might arise from signalling disruptions.

This chapter aims to identify the behavioural differences and energetic consequences of anthropogenic noise when it conflicts with acoustic courtship interactions, whilst also identifying important characteristics of the noise stimulus that leads to this conflict. To investigate this important and potentially far reaching topic, I observed the acoustic courtship behaviours of the Mediterranean field cricket, *G. bimaculatus*, as a model system. Males belonging to the genus *Gryllus* produce a number of different acoustic sexual signals through the stridulation of their wings (Alexander, 1961). This includes a courtship “song” (Figure 1.1b), which is very typical of courtship display in that it is necessary for mating to occur and that the song itself has been linked to various aspects of a cost-benefits fitness trade off, including both extrinsic (Zuk and Kolluru, 1998) and intrinsic costs (Mowles, 2014). Furthermore, characteristics of the song have been shown to correlate with different quality aspects of males (but see Gray and Eckhardt, 2001), such as immunocompetence (Rantala and Kortet, 2003), meaning females can use the song to assess the signallers “quality”. In this experiment, I staged courtship interactions between a single male and female under different acoustic conditions, and conducted analyses on the behaviour and physiology of individuals involved. If anthropogenic noise does have a cost to fitness due to its competition with acoustic courtship signals, individuals should differ in their ability to court, or be courted, between the different acoustic conditions, leading to behavioural alterations in the individuals involved. Additionally, such alterations may lead to differences in circulating metabolites, which may highlight the physiological trade-offs of altered acoustic signals. Furthermore, differences between the effects of different acoustic conditions, or lack of, will reveal

the important characteristics of anthropogenic noise that are integral in causing acoustic conflict.

Methods

Study Organisms and Playback Stimuli

The crickets used in this study were reared as in Chapter 2, but here both males and females were utilised in the behavioural trials. Additionally, the noise stimuli used were the same as those used previously (Chapter 2, Figure 2.1).

Courtship Interactions

I conducted all courtship interactions in a transparent plastic terrarium (15 x 8 x 10cm) with sand as a substrate and an opaque middle partition (Figure 3.1). The speakers for condition playback were positioned 20cm above each end of the area, pointing towards the centre of the arena. I staged these courtship interactions during the “light” stage in the light:dark cycle (09:00 - 12:00 local time) and at 24 - 28°C. Interactions were set up to investigate the effect of the acoustic environment on courtship interactions by subjecting the individuals to one of three acoustic conditions; ambient, traffic or white noise. Furthermore, to observe natural lengths and set lengths of courtship singing, I used one of three female conditions in each interaction; no female, free female, or tethered female. Females that were tethered had a thread attached to their pronotum, using cyanoacrylate glue (Loctite, Munich, Germany), and the other end was attached to small piece of cardboard (placed under the substrate to act as an anchor), all of which could be removed after the encounter. By doing this, females were unable to mount the male, which would normally terminate the courtship display, and the interaction could continue for a set length of time (five minutes). By generating set length courtship displays, I could better compare the physiological costs associated with changes in acoustic behaviour, as length of courtship is variable in natural conditions and is known to affect the costs of signalling (Mowles, 2014). These conditions also allowed for female behaviour to be controlled, leading to a more in depth examination of male

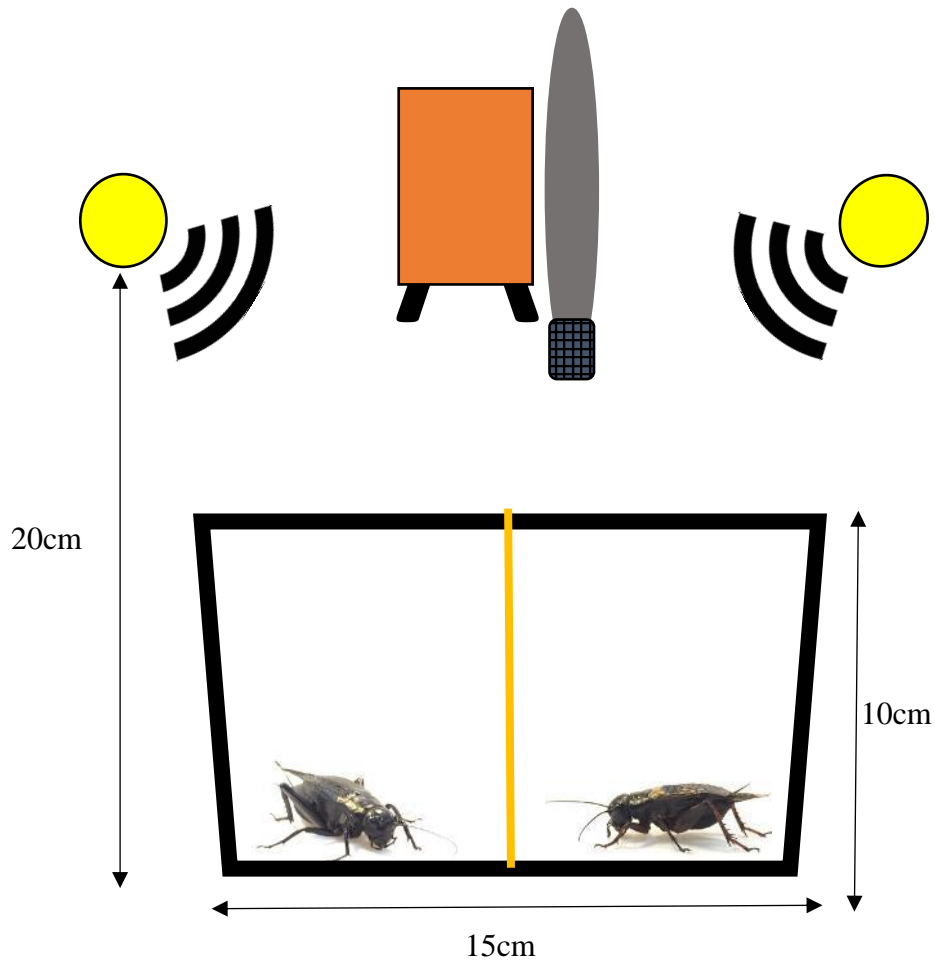


Figure 3.1. Cross section of the experimental arena used for courtship interactions. The large trapezoid represents the behavioural arena with speakers on either side (circles). The camera (small rectangle) and microphone (oval) were positioned above.

behavioural and acoustic responses. The combination of these two sets of factors produced nine separate testing conditions.

The protocol for these interactions involved me placing a single male into one half of the arena, after being weighed (in grams to two decimal places), and left to acclimate overnight (sixteen hours minimum). Twenty minutes prior to starting the interaction, I set up the female condition in the other half of the arena (no female, free female, or tethered female). I started the encounter by removing the partition and broadcasting the acoustic stimulus into the arena. For no female conditions, the male was allowed to explore the arena and the trial ended five minutes after the

partition was removed. In free female conditions, the male was allowed to court the female and the encounter was terminated when the female mounted the male, or after fifteen minutes in the absence of mounting (as over 80% of successful encounters occurred in under five minutes). In interactions where females mounted the male, I separated individuals before mating occurred so that any physiological costs to the male were due to courtship alone. Finally, in tethered female conditions, the encounter lasted for five minutes after the male started courtship signalling behaviours, or for fifteen minutes if the male did not court. There were twenty separate encounters in each condition, resulting in 180 encounters in total. In all conditions, males were placed into 7.0ml plastic tubes immediately after the end of the interaction, and then humanely euthanized by submersion in liquid nitrogen. These frozen samples were then kept at -80°C until analyses of haemolymph glucose and lactic acid concentrations could be carried out. Females were added to the breeding colony and not used for any future trials.

I recorded all interactions visually and acoustically (Figure 3.1) for later analysis. Video recordings were conducted as in Chapter 2. Acoustic recordings were conducted using a RØDE NTG4+ shotgun microphone (positioned directly above the arena) connected to a TASCAM DR-07MKII Linear PCM recorder (.wav format, 16-bit resolution, 48 kHz sampling rate).

Acoustic and Behavioural Analysis

I coded the video footage of each trial using the software B.O.R.I.S. (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). Courtship interactions were scored on multiple events, including counts of successful signalling (if the male sang) and courtships (if the female mounted), latencies and lengths of courtship behaviours (e.g. latency to start courting, length of song), and occurrences and intensities of other behaviours, such as judders, which are used by male *G. bimaculatus* in courtship interactions as a form of mate guarding (Parker and Vahed, 2010).

For courtship song analysis, I cut acoustic files (to where the song started) and ran them through a noise reduction feature twice using Audacity 2.1.2 (<http://www.audacityteam.org/>, last accessed 7th March 2019). To get the mean dominant frequency of the courtship song, I imported files into R (Rstudio Team, 2016; R Development Core Team, 2017) and analysed them using the ‘dfreq’ function in the package ‘seewave’ (20% threshold; Sueur, Aubin and Simonis, 2008). This was run twice, once with the bandpass at 3-9 kHz and another time with the bandpass at 9-20 kHz, in order to analyse the low frequency “pulses” and high frequency “ticks” of the song separately. Furthermore, I calculated signal escalation by plotting the interval between signal components (≤ 750 ms, so as to not include inter-signal intervals) against the time at which they occurred during the interaction, in order to quantify courtship intensity (escalation).

Physiological Assays

I defrosted frozen specimens at room temperature for twenty minutes before attempting to extract haemolymph samples. Spermatophore presence was recorded by gently squeezing the abdomen to expose the spermatophoric pouch. To extract the haemolymph, I removed a single middle leg from the thorax (approximately at the trochanter) and encouraged the haemolymph to form a globule at the cut. A 10 μ l sample was collected using a glass capillary tube and thoroughly mixed with 0.5ml Biosen Glucose/Lactate System Solution. Within two hours of this, samples were run through a Biosen C-line glucose and lactate analyzer (EKF Industrie, Elektronik GmbH, Barleben, Germany), providing results on haemolymph glucose and lactic acid concentrations to 0.01 mmol per litre (Max: 40mmol L⁻¹, Min: 0.05mmol L⁻¹).

Statistical Analysis

I carried out all statistical analyses in the statistical package R studio (Rstudio Team, 2016; R Development Core Team, 2017) with the packages ‘multcomp’ (Hothorn, Bretz and Westfall, 2008), ‘dunn.test’ (Dinno, 2015), ‘rcompanion’ (Mangiafico, 2018), and ‘Hmisc’ (Harrell Jr., 2006). All graphs and plots were created using base R and with the package ‘ggplot2’

(Wickham, 2016). Where necessary, data were tested for normality using a Shapiro-Wilk test, and non-parametric tests were used when appropriate. On all occasions where data from free female and tethered female conditions could be combined to boost statistical power (e.g. occurrence of male song), differences between the conditions were tested to see if they had any significant effect on the outcome variable. If they did, then the data were tested separately. Where I used generalized linear models (GLZMs), I also visually assessed plots of residuals vs. fitted values to ensure that the models fitted the data well, and that the data did not violate the model assumptions.

I tested the differences in courtship success between acoustic conditions using a GZLM using a binary logistic function. GZLMs with a binary logistic function were also used to test for differences in male signalling behaviour and female responsiveness between acoustic conditions. In statistically significant models, I coupled analyses with a TukeyHSD multiple comparison analysis to test the differences between each pair of groups. GZLMs with a binary logistic function, Kruskal-Wallis and Mann Whitney U tests were conducted to test for difference in the occurrence and intensity of juddering behaviours performed by males under different categorical conditions. Presented effect sizes (Log odds ratio \pm SE) were acquired from the model summary.

I used Kruskal-Wallis tests to test for differences in movement latency (for both males and females), latency to signal (from first contact), and duration of courtship (latency to mount) between the three acoustic conditions. Where appropriate, post-hoc Dunn's tests were carried out with Bonferroni corrections to identify between-group significant differences.

I used one-way ANOVAs were used to test for differences in courtship intensity between acoustic conditions. Courtship intensity was measured using stridulation rate (average amount of stridulations per minute) and mean interval (mean amount of time between each individual stridulation). I also tested the differences in signal escalation between acoustic conditions with a two-way Chi-Squared test, paired with Bonferroni corrected post-hoc pairwise tests. Kruskal-Wallis tests were used to test for differences in dominant frequency, both at high (9-20 kHz) and

low levels (>9 kHz), between acoustic conditions. Finally, all of these acoustic aspects were tested to see if they affected courtship success using Kruskal-Wallis tests, or chi-squared tests where appropriate.

Using Kruskal-Wallis tests, I tested for differences in haemolymph respiratory metabolites between acoustic conditions. The differences between respiratory metabolites based on whether or not the male courted was tested for each individual acoustic condition using Mann-Whitney U tests. The relationship between respiratory metabolites and stridulation rate, dominant frequency, mean inter-stridulation interval, and courtship duration were tested using regression analyses (where the data met parametric criteria or did so when log transformed) and GLZMs paired with a gamma error structure and log-link function (where data did not meet parametric criteria and was right skewed; Fitzsimmons and Bertram, 2013; Mowles and Jepson, 2015). I also tested the differences in haemolymph metabolites between males that escalated their song or not, in each acoustic condition using one-way ANOVAs and one-way t-test (where the data met parametric criteria or did so when log transformed) or Mann-Whitney U and Kruskal-Wallis tests (where data did not meet parametric criteria and were right skewed). In tests that had more than two categories in the predictor variable, and were found to be statistically significant, suitable pairwise post-hoc tests were conducted with adjusted P-values.

Results

Courtship Success

Overall, I found the acoustic conditions affected whether courtship interactions were successful or not (GLZM(b): Wald $X^2_2 = 7.470$, $N = 60$, $P = 0.024$; Figure 3.2), with fewer successful interactions occurring under traffic noise (-2 ± 0.87) and white noise (-1.79 ± 0.87) conditions. However, Tukey multiple comparisons did not show any significant differences between acoustic conditions in pairwise analyses (Ambient to White: $N = 40$, $P = 0.099$; Ambient to Traffic: $N = 40$, $P = 0.055$; Traffic to White: $N = 40$, $P = 0.945$). This can be split into two separate behaviours that can lead to unsuccessful courtship interactions; whether males chose to signal, and

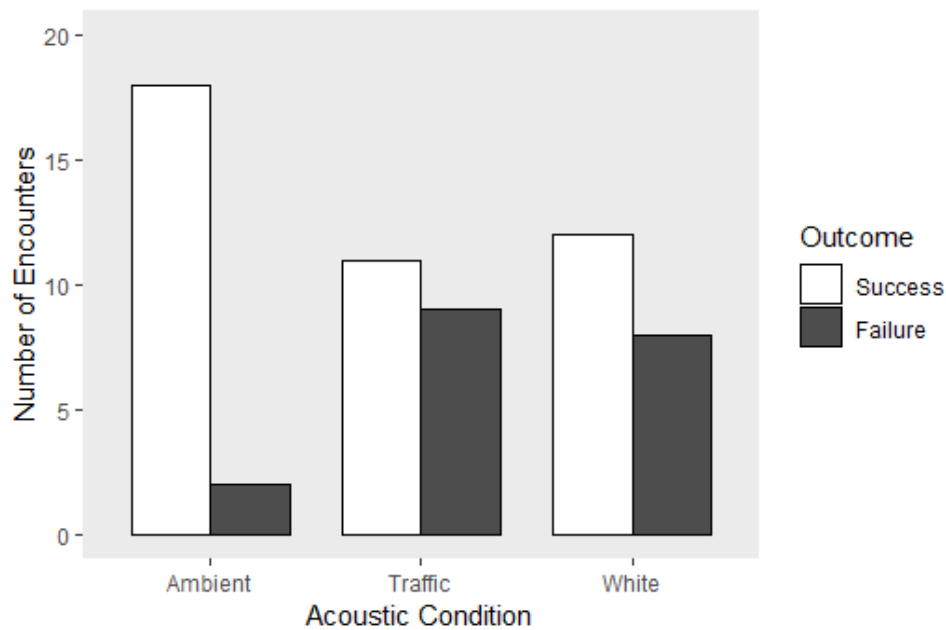


Figure 3.2. The number of successful courtship encounters (where the female mounted the male) between ambient noise ($n = 20$), traffic noise ($n = 20$) and white noise ($n = 20$) conditions.

whether females responded. Acoustic condition affected whether males choose to signal (GLZM(b): Wald $X^2_2 = 13.98$, $N = 120$, $P = <0.001$, Figure 3.3), with the largest reduction in males signalling in white noise conditions (-2.43 ± 0.8). A Tukey multiple comparisons post-hoc test showed that the significance lay between white and ambient noise conditions (ambient to white: $N = 80$, $P = 0.006$; ambient to traffic: $N = 80$, $P = 0.089$; traffic to white: $N = 80$, $P = 0.307$). However, acoustic condition did not significantly change the amount of females responding to courting males (GLZM(b): Wald $X^2_2 = 5.746$, $N = 50$, $P = 0.057$).

Juddering Behaviour

Neither acoustic condition (GLZM(b): Wald $X^2_1 = 1.8913$, $N = 120$, $P = 0.169$), female condition (GLZM(b): Wald $X^2_2 = 2.1282$, $N = 120$, $P = 0.345$) nor whether male chose to signal or not (GLZM(b): Wald $X^2_1 = 0.463$, $N = 120$, $P = 0.496$) affected whether males performed mate guarding juddering behaviours. Also, acoustic conditions (Kruskal–Wallis: $X^2_2 = 2.064$, $n_1 = 40$, $n_2 = 40$, $n_3 = 40$, $P = 0.356$) and female conditions (Mann–Whitney U: $U = 1106.5$, $n_1 = 60$, $n_2 = 60$, $P = 0.766$) did not affect the

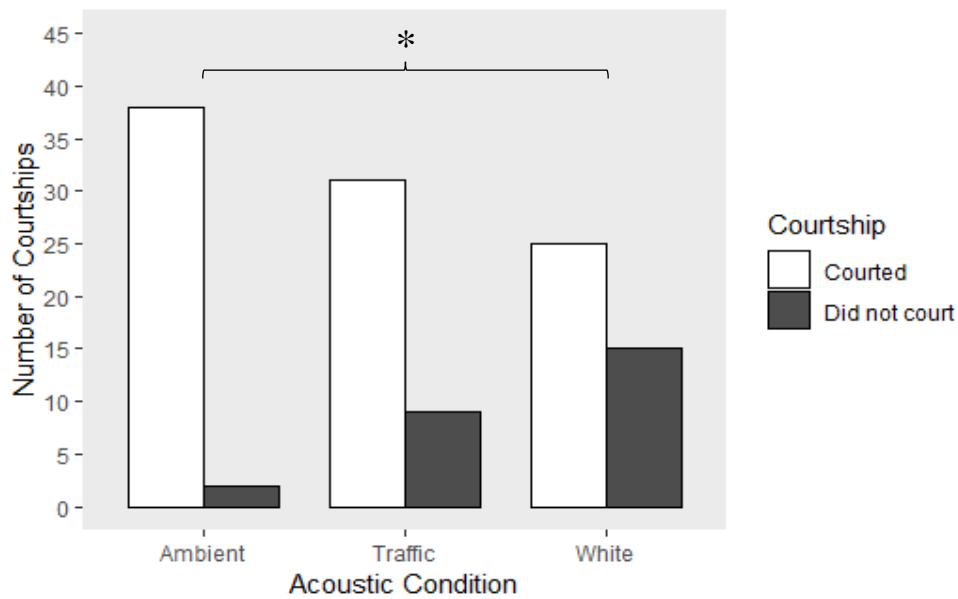


Figure 3.3. The number of males that courted or not in courtship interactions between ambient ($n = 40$), traffic ($n = 40$) and white noise ($n = 40$) conditions. Brackets with an asterisk show a significant result from pairwise analysis.

intensity (amount) of juddering behaviour occurring in males that displayed the behaviour. However, males that did not court showed a higher amount of juddering behaviours, compared to individuals that did court (Mann–Whitney U test: $U = 257$, $n_1 = 94$, $n_2 = 26$, $P = <0.001$, Figure 3.4).

Timings and Latencies

Neither female movement latency nor male movement latency were significantly affected by the acoustic condition in any of the female testing conditions (Table 3.1). Acoustic condition also did not influence male latency to start signalling or the duration of successful courtship displays (Table 3.1).

Courtship Song Characteristics

In free female conditions, signal intensity (stridulation rate) was decreased in males signalling under white noise conditions (one-way ANOVA: $F_{2,44} = 4.637$, $P = 0.015$, Figure 3.5), but this effect was not seen in tethered female trials (one-way ANOVA: $F_{2,38} = 0.873$, $P = 0.873$). Males had a significantly lower stridulation rate in white noise conditions when compared to ambient noise ($N = 32$, $P = 0.037$), and

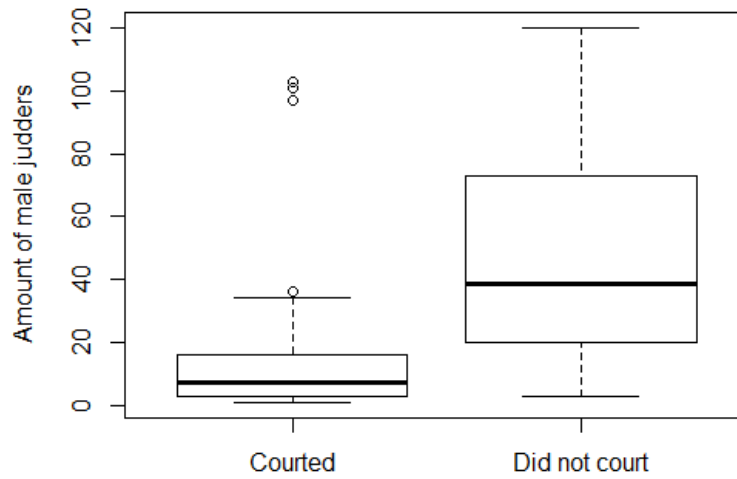


Figure 3.4. Differences in the amount of juddering behaviour in males that courted and those that did not. Only males that showed this behaviour at least once were included in this analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

traffic noise conditions ($N = 28$, $P=0.025$), but no difference was seen when comparing ambient and traffic noise conditions ($N = 36$, $P = 1$). However, stridulation rate did not affect courtship success in any of the acoustic conditions (Table 3.2). Mean interval was found not to be significantly different between acoustic conditions (one-way ANOVA: $F_{2,84} = 0.367$, $P = 0.694$) and did not affect courtship success in any of the acoustic conditions (Table 3.2). Individuals in different acoustic conditions had altered ratios of signal escalation type (two-way Chi-squared: $X^2_4 = 12.126$, $N = 94$, $P = 0.016$, Figure 3.6), although Bonferroni adjusted post-hoc pairwise analyses did not find a statistically significant result between any groups (ambient to traffic: $N = 66$, $P = 0.115$; ambient to white: $N = 59$, $P = 0.088$; traffic to white: $N = 49$, $P = 0.258$). When compared to a 1:1:1 ratio, the ratio of escalation type in ambient and white noise conditions was significantly different (Ambient one-way Chi-squared: $X^2_2 = 14.263$, $N = 38$, $P < 0.001$; White one-way Chi-squared: $X^2_2 = 18$, $N = 21$, $P < 0.001$), but the ratio in the traffic noise condition was not (Traffic one-way Chi-squared: $X^2_2 = 2.643$, $N = 28$, $P = 0.267$). The low frequency components (pulses and trills) of the courtship songs were not affected by the acoustic condition (Kruskal–Wallis: $X^2_2 =$

3.8649, $n_1 = 38$, $n_2 = 24$, $n_3 = 20$, $P = 0.145$), but the high frequency components (ticks) were (Kruskal–Wallis: $X^2_2 = 11.017$, $n_1 = 30$, $n_2 = 20$, $n_3 = 13$, $P = 0.004$, Figure 3.7). Males signalled at a higher frequency (kHz) in white noise conditions when compared to both ambient ($N = 43$, $P = 0.011$) and traffic noise conditions ($N = 33$, $P = 0.002$), but no difference was found between ambient and traffic noise conditions ($N = 50$, $P = 0.572$). However, the dominant frequency of the high frequency ticks did not affect courtship success (Table 3.2).

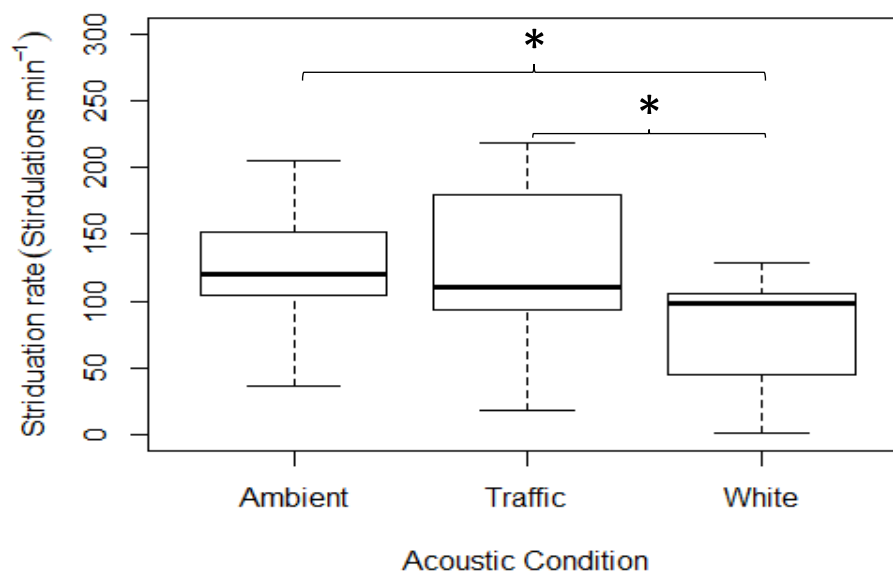


Figure 3.5. Differences in stridulation rate (average number of stridulations per minute) between acoustic conditions in free female trials. Brackets with an asterisk show a significant result from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively.

Table 3.1. Output for Kruskal-Wallis tests on measures of behavioural latency and duration (in seconds). A_n , T_n and W_n show the sample size for each noise condition (ambient, traffic and white noise, respectively) and the population means and standard error of the mean are also shown.

	X^2	A_n	\bar{x}	S.E.	T_n	\bar{x}	S.E.	W_n	\bar{x}	S.E.	P
Latency to male movement											
No female	1.999	17	156.53	17.08	20	103.38	12.82	18	98.92	13.68	0.368
Free female	1.682	20	99.07	14	20	73.51	12.85	20	81.77	24.82	0.431
Tethered female	1.46	20	138.82	20.82	20	101.61	46.42	20	111.74	22.82	0.482
Latency to female movement											
Free female	2.066	20	86.53	16.21	20	83.48	27.2	20	90.04	25.62	0.356
Tethered female	0.835	20	107.07	12.93	20	88.16	12.28	20	132.77	28.02	0.659
Latency to signal	3.416	38	51.14	10.37	31	42.09	13.98	25	60.09	11.48	0.181
Duration of courtship	1.525	18	92.91	13.91	11	125.4	31.87	12	79.05	10.27	0.467

Table 3.2. Output for Mann-Whitney U on the effect of signal characteristics (stridulation rate, mean interval and dominant frequency) on courtship success for each acoustic condition. The population means and standard error of the means are also shown.

	U	n_1	\bar{x}	S.E.	n_2	\bar{x}	S.E.	P
Stridulation rate (ticks min ⁻¹)								
Ambient	18	18	118.72	10.5	1	204.9	-	0.105
Traffic	35	10	115.44	19.18	5	155.59	24.6	0.254
White	11	11	78.14	10.13	2	64.89	63.63	1
Mean Interval (ms)								
Ambient	4	18	290.07	7.69	1	264.48	-	0.523
Traffic	19	10	287.62	10.06	5	271.91	11.51	0.514
White	0	11	296.57	11.94	1	256.7	-	0.167
Tick Dominant frequency (kHz)								
Ambient	14	18	14.76	0.31	1	15.42	-	0.526
Traffic	10	7	14.74	0.37	5	13.92	0.46	0.268
White	5	11	14.39	1.52	2	13.09	2.43	0.308

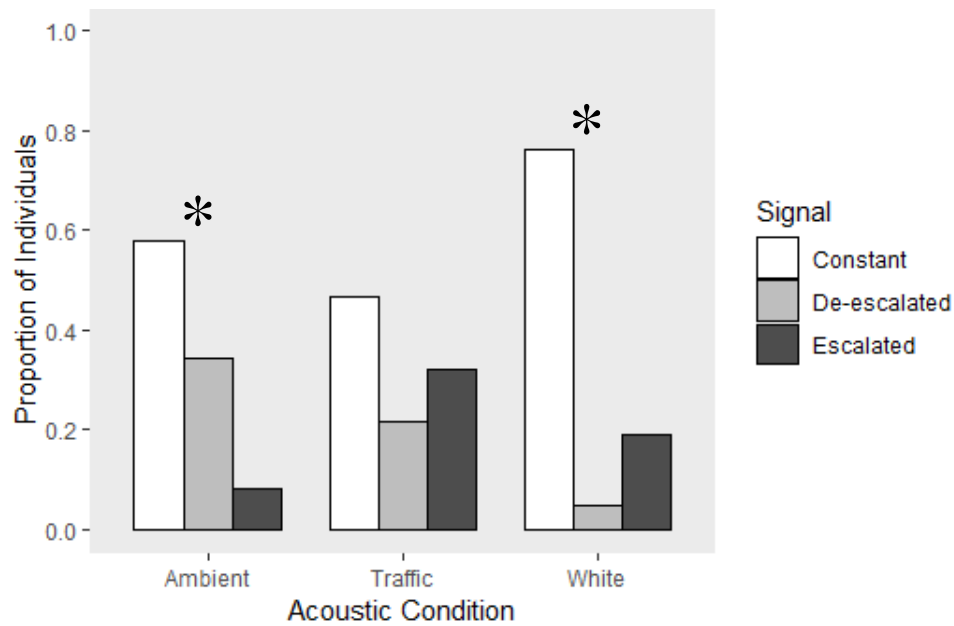


Figure 3.6. Differences in the proportion of signalling males that escalated, de-escalated their signal, or held a constant rate. Proportion was used to visualize that data over true counts as populations differed in sample size. Asterisks indicate acoustic conditions were ratios differed significantly from a 1:1:1 expected ratio.

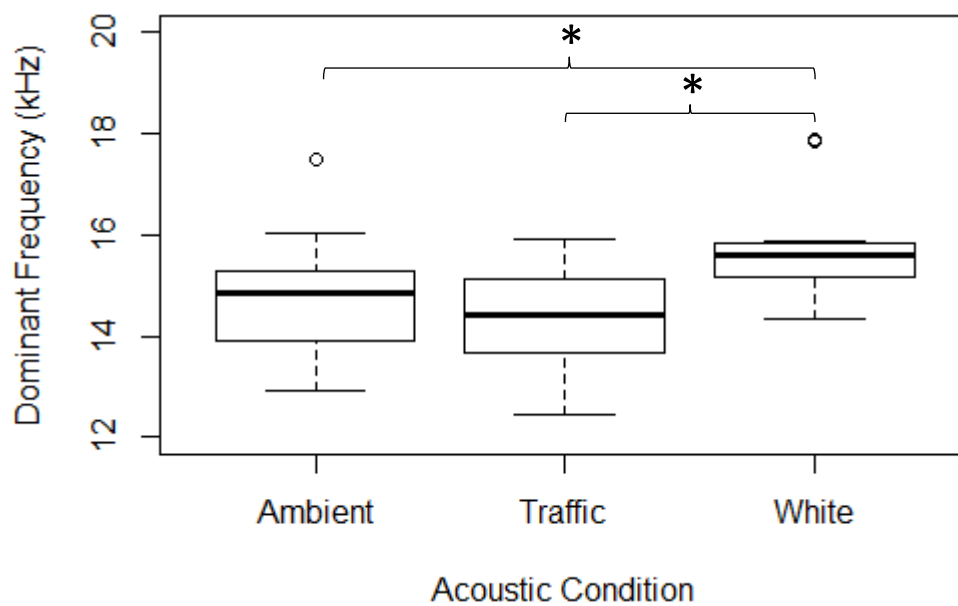


Figure 3.7. Differences in high tick (9-20 kHz) dominant frequency between acoustic conditions. Brackets with an asterisk show a significant result from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function boxplot() in R are shown as o.

Physiological Analyses

There was no relationship between male weight and haemolymph metabolites in no female conditions (glucose: $r_s = -0.076$, $N = 29$, $P = 0.695$; lactic acid: $r_s = -0.304$, $N = 29$, $P = 0.108$). In no female trials, males in ambient noise conditions had higher concentrations of haemolymph glucose (Figure 3.8, Table 3.3), with significance lying between ambient and traffic noise conditions ($N = 24$, $P = 0.014$), but not ambient and white noise conditions ($N = 18$, $P = 0.664$) or traffic and white noise conditions ($N = 16$, $P = 0.328$). There was no differences in glucose concentrations between acoustic conditions in free or tethered female trials (Table 3.3).

Haemolymph lactic acid concentrations did not differ between acoustic conditions in either no female, free female or tethered female trials (Table 3.3). In each noise condition, there was no difference in glucose or lactic acid concentrations between males that courted and those that did not court

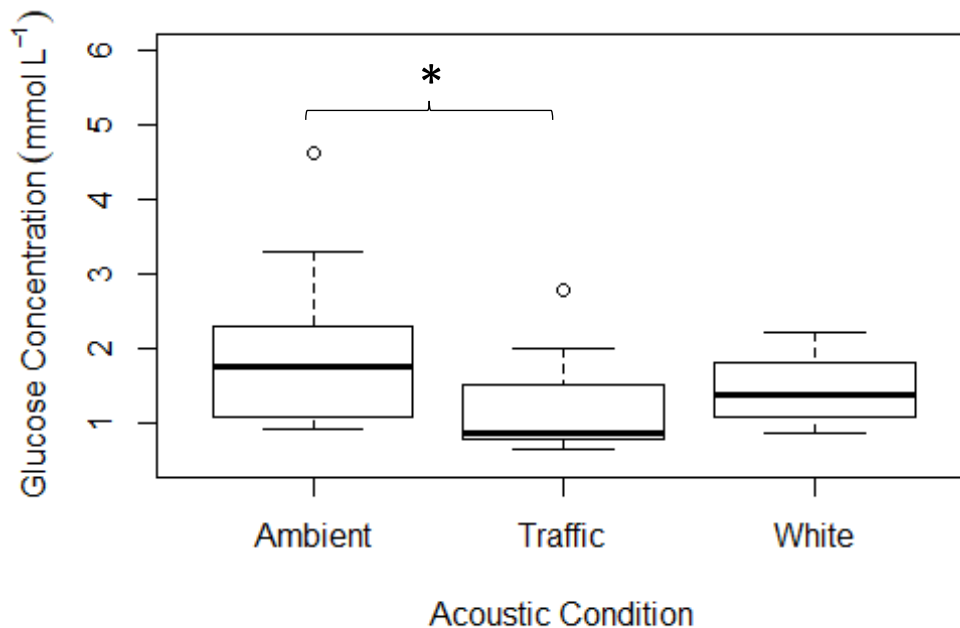


Figure 3.8. Differences in haemolymph glucose concentration between acoustic conditions in no female trials. Brackets with an asterisk show a significant result from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

(Table 3.4). Out of the scale characteristics of the male courtship song, only three relationships were found to be significant, and only under white noise conditions; lactic acid concentration and stridulation rate, lactic acid concentration and courtship duration, and glucose concentration and dominant frequency (Table 3.5, Table 3.6, Figure 3.9). All other regression analyses were non-significant. Finally, song escalation type only affected lactic acid concentration, and again only in white noise conditions (Log Transformed t-test: $t_8 = 3.424$, $P = 0.028$, Figure 3.10). All other differences between escalation type were non-significant (ambient glucose log transformed one-way ANOVA: $F_{2,18} = 0.017$ $P = 0.984$; traffic glucose log transformed one-way ANOVA: $F_{2,10} = 1.442$ $P = 0.282$; white glucose Mann–Whitney U test: $u = 9$, $n_1 = 5$, $n_2 = 3$, $P = 0.786$; ambient lactic acid Kruskal–Wallis: $X^2_2 = 0.79$, $n_1 = 13$, $n_2 = 7$, $n_3 = 1$, $P = 0.674$; traffic lactic acid log transformed one-way ANOVA: $F_{2,10} = 0.6$ $P = 0.568$). Finally, spermatophore presence did not differ significantly between acoustic conditions (two-way Chi-squared: $X^2_2 = 3.903$, $df = 2$, $P = 0.142$).

Table 3.3. Output for Kruskal-Wallis tests on differences in glucose and lactic acid concentrations (mmol L⁻¹) between acoustic conditions. *A n*, *T n* and *W n* show the sample size for each noise condition (ambient, traffic and white noise, respectively) and the population means and standard error of the means are also shown. Bold P-values indicate a significant result.

	X ²	<i>A n</i>	\bar{x}	S.E.	<i>T n</i>	\bar{x}	S.E.	<i>W n</i>	\bar{x}	S.E.	P
Glucose concentration											
No female	6.817	13	1.98	0.3	11	1.21	0.2	5	1.48	0.25	0.033
Free female	2.455	9	3	0.75	12	1.95	0.41	5	6.17	4.28	0.293
Tethered female	0.592	13	2.29	0.33	7	4.36	1.69	8	6.05	2.86	0.744
Lactate concentration											
No female	5.138	13	1.01	0.19	11	1.14	0.1	5	0.94	0.06	0.077
Free female	4.001	9	1.04	0.14	12	0.93	0.06	5	1.1	0.04	0.1352
Tethered female	0.664	13	0.97	0.11	7	0.91	0.09	8	0.98	0.07	0.718

Table 3.4. Output for Mann-Whitney U on the effect of courtship (presence or absence) on Haemolymph glucose and lactic acid concentrations (mmol L^{-1}) for each acoustic condition. The population means and standard error of the means are also shown.

	U	n_1	\bar{x}	S.E.	n_2	\bar{x}	S.E.	P
Glucose concentration								
Ambient	19	21	2.64	0.37	1	1.3	-	0.272
Traffic	33	14	3.22	0.93	5	1.78	0.24	0.893
White	27	9	8.08	3.13	4	1.63	0.37	0.188
Lactic acid concentration								
Ambient	11.5	21	1	0.09	1	0.89	-	0.937
Traffic	31.5	14	0.92	0.06	5	0.92	0.07	0.781
White	8	9	0.97	0.04	4	1.14	0.11	0.148

Table 3.5. Output from standard and log transformed linear regressions showing the relationship between courtship characteristics and haemolymph glucose and lactate concentrations (mmol L⁻¹) in each acoustic condition. Bold P-values indicate a significant result.

	Data	Y	P	r ²	Figure
Courtship duration					
Glucose					
Ambient	log	-0.0004*x+0.458	0.272	0.066	-
Traffic	log	0.0002*x+0.267	0.638	0.019	-
Lactic acid					
Traffic	normal	-0.0003*x+1.041	0.171	0.1499	-
White	log	-0.0003*x+0.053	0.016	0.746	Figure 3.9a
Stridulation rate					
Glucose					
Ambient	log	-0.002*x+0.597	0.169	0.103	-
Traffic	log	-0.0007*x+0.467	0.695	0.014	-
Lactic acid					
Traffic	normal	-0.0008*x+1.045	0.463	0.05	-
White	log	-0.0006*x+0.067	0.01	0.645	Figure 3.9b
Dominant frequency					
Glucose					
Ambient	log	0.09*x+-0.972	0.128	0.124	-
Traffic	log	-0.005*x+0.476	0.966	0.0002	-
Lactic acid					
Traffic	normal	-0.004*x+0.912	0.949	0.0005	-
White	log	0.004*x+-0.08	0.776	0.015	-
Mean inter-tick interval					
Glucose					
Ambient	log	0.002*x+-0.185	0.27	0.064	-
Traffic	log	0.005*x+-0.938	0.088	0.2405	-
Lactic acid					
Traffic	normal	0.008*x+0.457	0.395	0.066	-
White	log	0.001* x + -0.322	0.085	0.4134	-

Table 3.6. Output from generalized linear models with gamma error structure identifying relationship between courtship characteristics and haemolymph glucose and lactate concentrations (mmol L⁻¹) in white and ambient noise conditions. Bold P-values indicate a significant result.

	Df	F	P	Figure
Courtship duration				
Glucose - White	1,6	0.002	0.969	-
Lactic acid - Ambient	1,18	0.084	0.775	-
Stridulation rate				
Glucose - White	1,6	0.117	0.744	-
Lactic acid - Ambient	1,18	0.102	0.754	-
Dominant frequency				
Glucose - White	1,6	6.638	0.042	Figure 3.9c
Lactic acid - Ambient	1,18	1.213	0.285	-
Mean inter-tick interval				
Glucose - White	1,6	0.477	0.516	-
Lactic acid - Ambient	1,18	0.02	0.889	-

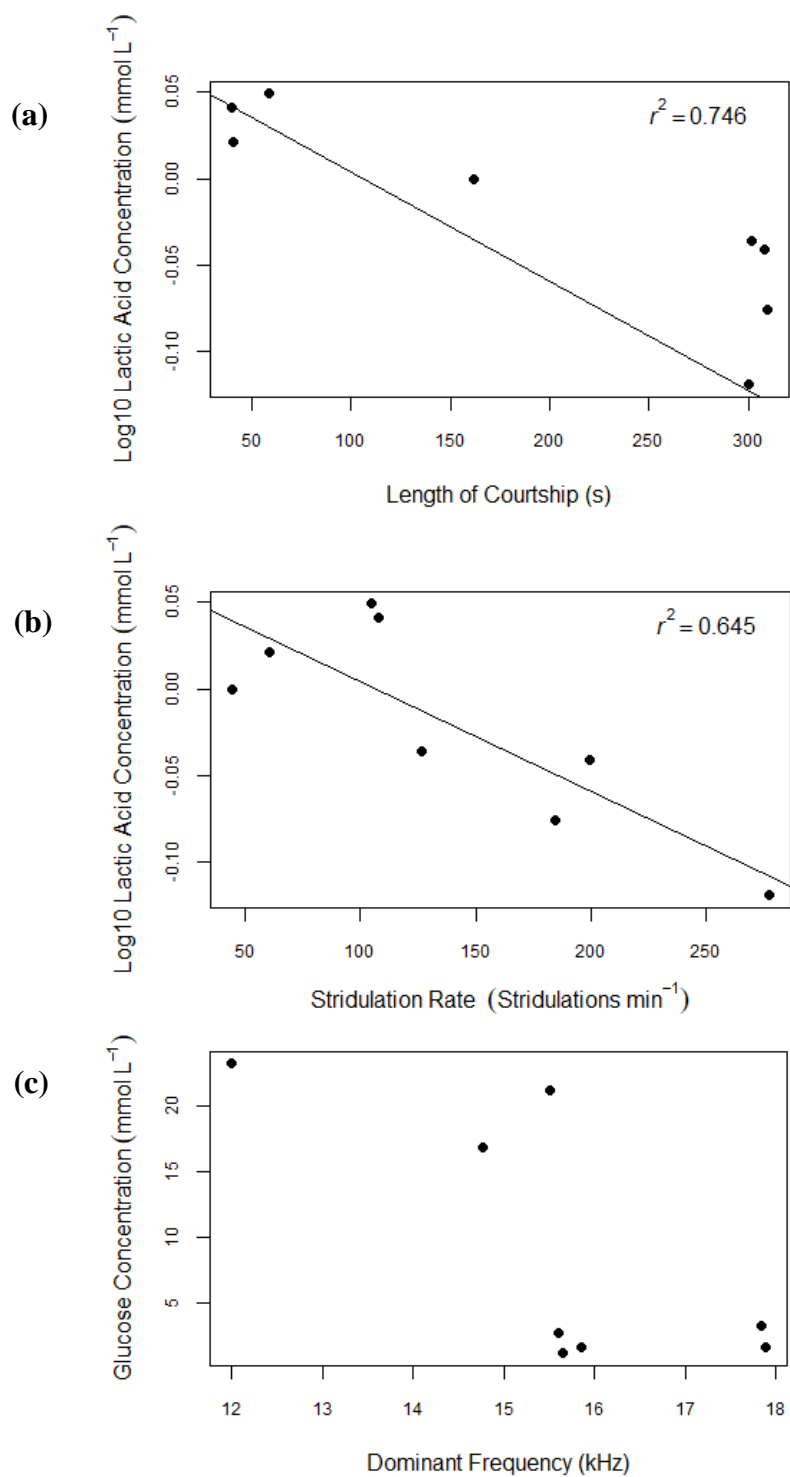


Figure 3.9. Scatter plots showing the significant relationships between courtship song characteristics and haemolymph metabolites in white noise conditions. Relationships shown between (a) courtship length (seconds) and log transformed lactic acid concentrations, (b) stridulation rate (average number of stridulations per minute) and log transformed lactic acid concentrations and (c) dominant frequency (9-20kHz) and glucose concentrations. A line through the data points represented the model outcome of a linear regression.

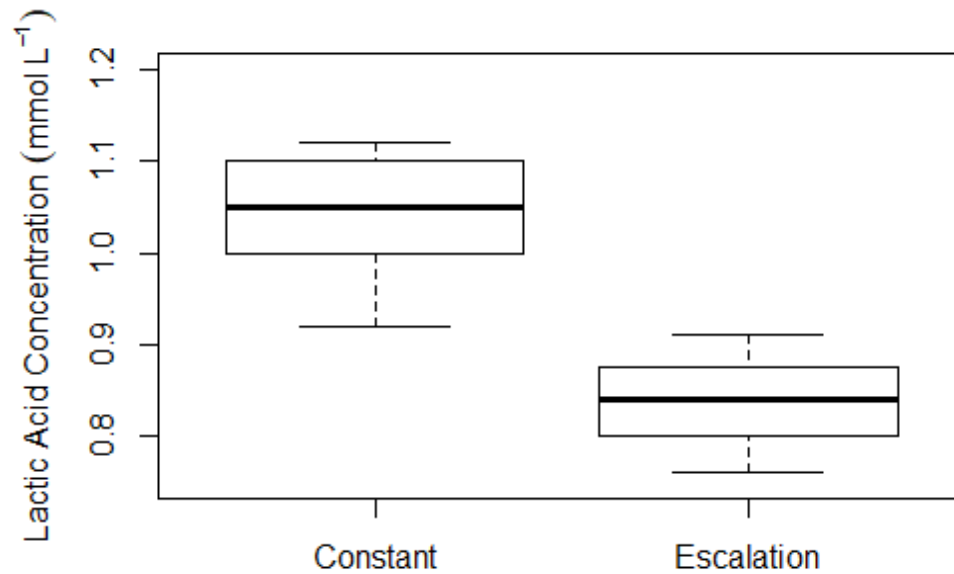


Figure 3.10. Differences in haemolymph lactic acid concentration between individuals that escalated their signal and those that did not, under white noise conditions. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively.

Discussion

The experiments presented in this chapter have shown differences in male signalling behaviour between acoustic conditions, including reduced tendency to court females and increased signalling effort. These results highlight the disruption to courtship behaviours, as well as courtship interaction outcome, under unfavourable acoustic conditions, thus adding to the limited but growing body of literature on how anthropogenic noise affects courtship displays (De Jong et al., 2016; Whalen et al., 2018). Furthermore, as differences were found between certain acoustic condition, but not others, I have potentially identified which acoustic characteristics are necessary to disrupt this sexual signalling system. Tests on haemolymph metabolites in relation to these acoustic conditions and behavioural changes show that physiological trade-offs may be in effect when individuals alter their acoustic signals in response to environmental pressures.

Among acoustic conditions, there was a clear difference in the number of successful courtships interactions (if the female mounted the male). This result seems to be driven by the reduction in males that are choosing to signal, not the reduction in females mounting. As the acoustic courtship signal is necessary for mating to occur in *G. bimaculatus* (Alexander, 1961; Shestakov and Vedenina, 2015), this leads to a reduction in the amount of successful courtship interactions. Here, the primary differences lay between the courtship behaviour of males in ambient noise and white noise conditions, but not between ambient noise and traffic noise conditions. As the two types of noise stimuli (traffic and white noise) were played back at the same average amplitude, we can conclude that this level of noise is powerful enough to disrupt signalling systems, but additional characteristics are also needed. This additional trait is likely a characteristic that differs between the two noise stimuli; namely frequency (Hz) and/or fluctuations in signal characteristics. Unlike the calling song of this species (Chapter 2), the courtship song contains two distinct elements that differ in frequency and temporal characteristics (Libersat, Murray and Hoy, 1994; Rantala and Kortet, 2003). However, the low frequency ‘pulses’ of this signal are thought to hold no useful purpose, in fact making the song more preferable to females when the pulses are absent (Rantala and Kortet, 2003; Shestakov and Vedenina, 2015). The other part of the signal is the ‘ticks’, which are higher in both frequency (12-18 kHz, Figure 1.1b) and amplitude. If a noise stimulus were to mask these ‘ticks’ on a frequency level, the signal would need to have energies at these frequency bands also (Naguib, 2013). This is exactly what we see in white noise, as it is broadband in its frequency (covering many frequency bands), but not in traffic noise, which is relatively low in its dominant frequency.

Alternatively, the courtship signal may be made undetectable by the constant nature of white noise, unlike the fluctuating characteristics seen in most anthropogenic noises, including the traffic noise stimulus used here. The courtship song is a rhythmic and repetitive signal, which may be either to ensure the signal is transmitted in full (along the same lines as the redundant signal hypothesis; Zuk, Ligon and Thornhill, 1992) or because

each aspect of the signal, such as the number of repetitions, details something of the signallers quality (Mowles and Ord, 2012; Mowles, 2014). Theoretically, both of these functions would be disrupted by a continuous noise stimulus (like white noise), but a fluctuating stimulus (such as traffic noise) would only notably disrupt signals that carry non-redundant information. Regardless of how the noise stimulus disrupts the desired signal, males under white noise conditions may have avoided signalling if they did not think their signal would be received by the female. This is further supported as males that did not signal invested more energy in to the juddering (mate guarding) behaviour, potentially to keep females around until more favourable acoustic conditions were present. This behaviour is mainly associated with males preventing females from re-mating or to keep them around until they have a spermatophore ready (Parker and Vahed, 2010), but as spermatophore presence did not differ significantly between the acoustic conditions and behaviour was only recorded prior to mating, this is not likely to be the case here. Furthermore, this is unlikely a shift to another modality (as seen in other species; Partan, 2017) as individuals that did not signal acoustically were never mounted by females. Seemingly, female responsiveness to males that courted did not differ between the acoustic conditions, despite previous work showing a reduced responsiveness to calling songs under anthropogenic noise conditions (Chapter 2; Schmidt, Morrison and Kunc, 2014). However, these results were close to the 0.05 critical significance threshold, sometimes referred to as a non-significant trend, meaning a biologically significant effect may still be present here. Reduced statistical power due to a smaller samples size (less males signalling) may have led to an increase in the likelihood of committing a type 2 statistical error. This potential effect warrants further exploration, so it shall be investigated under greater scrutiny later in this thesis (Chapter 4).

Courting males also altered their behaviour in the presence of a noise stimulus by changing various aspects of their song. Notably, differences were found in stridulation rate, song escalation and the dominant frequency of the high-frequency 'ticks' between noise conditions. Alterations in

signalling behaviour is widely reported throughout the Animal Kingdom in response to anthropogenic noise (Nowacek et al., 2007; Cunnington and Fahrig, 2010; Díaz, Parra and Gallardo, 2011; Melcón et al., 2012; Shieh et al., 2012; Potvin, 2016; Orci, Petróczki and Barta, 2016), often to combat any conflict between signal transmission and noise stimuli. Changes in the pitch (frequency) of the song, is one of the main types of alterations observed (Cunnington and Fahrig, 2010; Lampe et al., 2012; Potvin, Mulder and Parris, 2014; Luther, Phillips and Derryberry, 2016), which is evidence for the frequency of noise being the most important aspect of its disruptive potential. In the present study, males under white noise conditions signalled at a higher frequency than those in either traffic or ambient noise conditions. As previously discussed, of the acoustic conditions used in this experiment, only white noise possessed energy at the frequency band at which courtship song ‘ticks’ are broadcast (12 - 18kHz). By elevating the frequency of their song, males may be attempting to signal at a frequency band that does not have any conflicting noise energy. Unlike the calling song, where frequency is essential for species and signal recognition (Popov, Markovich and Andjan, 1978; Kostarakos, Hartbauer and Römer, 2008), the frequency of the courtship song is much more variable (Gray, 2005), and females are less selective based on the dominant frequency (Shestakov and Vedenina, 2015). However, as white noise has a broadband range of frequencies, it possesses energy at these higher frequency bands also, which would explain why no increase in successful courtships are seen in individuals with higher frequency ticks.

Signal escalation was also shown to differ between acoustic conditions. To my knowledge, no other studies of acoustic signals under conditions of anthropogenic noise have found individuals more likely to escalate their signals (progressively decrease intervals between signals components) under these conditions, as we have seen in the present study. Escalations in sexually selected signals are thought to communicate something of the signaller’s quality (Mowles and Ord, 2012) or alternatively display their level of motivation (Hof and Podos, 2013). Potentially then, the males in this experiment that escalated their signal did so as some aspect

of the noise conflicted with the female's perception of the male's quality and/or motivation. The rate of stridulation (amount of stridulations per minute) was also observed to differ between acoustic conditions, but only in free female trials. As signalling rate is known to reflect aspects of the signaller's quality, shown by a higher preference for signals with a higher rate (Rantala and Kortet, 2003), we might have expected to see an increase in this characteristic under noise conditions where quality perception could be disrupted. However, in the present study we observed that males in ambient and traffic noise conditions were more likely to have a higher stridulation rate than those in white noise conditions. Potentially, this result highlights the energetic trade-off of acoustic alterations.

Alterations to the signal characteristics discussed here may be energetically costly, like many sexually selected signals are (Kotiaho et al., 1998; Castro et al., 2006; Mowles, 2014; Mowles and Jepson, 2015). If this is the case, males may have a reduced energetic capacity if they have altered their signal in other ways, leading to the onset of fatigue and a reduction in other acoustic traits (i.e. stridulation rate). This would explain the results observed here that males under white noise conditions increased their dominant frequency whilst suffering a decreased stridulation rate. However, like the alteration in frequency, neither higher stridulation rates nor songs that were escalated significantly increased successful encounters. This is perhaps evidence that this species is not accustomed to altering their courtship song in presence of anthropogenic noise conditions, but that they still have some behavioural flexibility to conceivably reduce the consequences of conflicting with other noise sources.

It has long been known that glucose is not the main carbohydrate found in cricket haemolymph, with their physiology being based around the disaccharide trehalose instead (Wyatt and Kalf, 1957; Nowosielski and Patton, 1964), although glucose is still present. However, the equipment used to measure glucose concentrations (Biosen C-line glucose and lactate analyzer; <https://www.ekfdiagnostics.com/biosen-analyzer/>) functions through enzymatic-amperometric (detection of ions from enzyme activity). Thus, only glucose molecules should be able to interact with these enzyme's

active site and produce ions for detection. For these reasons, we believe the glucose measurements to be true and not a measure of overall carbohydrate level. Furthermore, using this technique, we feel that these results should not be interpreted as stores of sugar that may be diminishing under certain conditions, but rather as a signal of sugar mobilisation for respiration (Mowles and Ord, 2012), although this supply can diminish too. With this in mind, the differences in glucose observed could show individuals that are preparing to utilise their energy reserves. In no female trials, this would translate as males in ambient noise conditions mobilising glucose in preparation for something, perhaps a signalling behaviour. In other female conditions, the majority of males would be signalling, so their glucose concentrations would be constantly used up (although no difference was found between female conditions within each noise condition). Glucose concentrations were also found to be linked to the dominant frequency of ticks produced, with higher levels of glucose found in individuals that signalled at a lower frequency. Here, either individuals mobilised the glucose to signal but did not use as much as individuals that signalled at a higher frequency, or they mobilised glucose in anticipation of a future event (such as mating). This result was only observed in individuals under white noise conditions, so is likely a sign of glucose depletion, as these individuals are signalling at a significantly higher frequency than those in other noise conditions.

Lactic acid, a by-product of anaerobic respiration, was also found to be linked to stridulation rate, courtship duration, and escalation, but only under white noise conditions. In line with previous studies (Taigen and Wells, 1985; Matsumasa and Murai, 2005; Mowles, 2014), one would expect to see an increase in lactic acid concentrations when signalling activity or courtship intensity increased. However, in the present study, I observed exactly the opposite. Increases in lactic acid concentrations were here linked to decreasing courtship durations and decreasing stridulation rates. Furthermore, individuals that escalated their signal had lower lactic acid level than those that held a constant signal. This result could be showing that only individuals that have greater anaerobic capabilities can maintain a

highly energetic signal (i.e. escalated), and that individuals with reduced capabilities are reaching their energetic threshold and thus producing a less energetic signal (i.e. constant). Alternatively, acoustic courtship signals may not be entirely produced through anaerobic respiration, as previously thought (e.g. Mowles, 2014). For example, the acoustic components of aggressive encounters in *Acheta domesticus* Linnaeus, a species closely related to *G. bimaculatus*, are the least costly of seven distinct aggressive behaviours (Hack, 1997). Finally, outcomes from these physiological tests should be discussed with caution. Primarily, this is because sample size in these tests were much reduced (under 50% of the individuals tested) due to the difficulty collecting a full 10µl of haemolymph from every individual. I will rectify the issues with this methodology in future experiments (Chapter 4). This reduction is most prominent in samples taken from males that were under white noise conditions, which is interesting as this is the condition where most of the statistically significant differences were found. I conclude that more research into the physiology and metabolic activity of study organisms, as well as developments on testing protocols, are necessary to fully understand the complexity of acoustic signalling and physiology under conditions of anthropogenic noise.

Conclusions

In this chapter, I have shown that acoustic signals performed as part of courtship displays are at risk of disruption from competing acoustic stimuli. Primarily, male perception of the acoustic environment led to a reduction in acoustic courtship behaviour, leading to a complete failure to mate. This reduction in signalling behaviour, despite no reduction in female response, may highlight that other aspects of the courtship signal are being disrupted (e.g. quality indicators; Chapter 4). Acoustic amplitude alone was not the main factor causing this disruption. In *G. bimaculatus*, acoustic signals are relatively high in dominant frequency and thus are not disrupted by common low-frequency anthropogenic noises, although higher frequency sources do exist (Smagowska, 2013). However, many other species are known to signal at a lower-frequency during courtship displays (Sparling, 2007; Smith and van Staaden, 2009), which may be more vulnerable to the low frequencies

of anthropogenic noise. Males were also shown to alter their signal in a number of ways, a common phenomenon in animals under anthropogenic noise conditions, but this did not heighten their courtship success. Finally, coupled with the results of altered acoustic behaviour, analyses of circulating haemolymph metabolites revealed potential energetic trade-offs that may be limiting the amount an individual can alter their signals in response to anthropogenic noise.

Chapter 4

Perception of Mate Quality under Conditions of Anthropogenic Noise

Abstract

By attending to sexual signals produced by conspecifics, individuals can make informed decisions on the best choice of mate, which in turn can offer considerable benefits to their reproductive fitness. However, these communication systems are open to disruption from other stimuli present in the environment. Anthropogenic noise may act as one such unwanted stimulus, leading to disruptions in optimal mate choice decisions, and thus reductions in an animal's fitness. In this study, the courtship behaviours of female *Gryllus bimaculatus* were tested when presented with artificial male acoustic courtship songs of differing 'quality' and under different acoustic conditions. Females maintained their preferences, shown by mounting success and latency, under ambient noise conditions, but this result was not seen in traffic noise or white noise conditions. Additionally, 'high quality' courtship songs had an increased mounting latency in traffic and white noise conditions, when compared to ambient noise conditions. As the effect of noise was seen in both traffic and white noise conditions, this suggests that it is a shared characteristic of the two stimuli, such as average amplitude, that may be causing this disruption. Making non-optimal mating decisions, such as the ones seen here, can lead to deleterious consequences for both the individual and the population as a whole, if an alteration in signalling behaviour does not evolve rapidly.

Introduction

Mate choice is a well-established subset of the theory of sexual selection (Andersson and Simmons, 2006), which regards the decisions individuals make when selecting a conspecific to mate with (Andersson, 1994). This choice of mates is commonly observed in the sex that invests the most into

reproduction, typically the female sex, but mate choice in males is not uncommon (Edward and Chapman, 2011). Through mate choice, choosy individuals can gain considerable direct (resources based) and indirect (genetic based) benefits that lead to an increased reproductive fitness. Mate choice decisions based on resources may involve food items (nuptial gifts) or access to laying sites or shelter, all of which may increase the fecundity of the choosing individuals (Gwynne, 1984; Johnson and Searcy, 1993; but see Vahed, 1998). However, the majority of research regarding mate choice in the Animal Kingdom focuses on the genetic benefits that individuals can gain when mating with high quality conspecifics. Mates with highly desirable sexually selected traits are thought to be of higher genetic quality and thus have ‘good genes’ (although increasingly more work is now being dedicated to the importance of compatible genotype selection; Puurtinen, Ketola and Kotiaho, 2009). When an individual chooses to mate with a conspecific with ‘good genes’, there are two main ways that this can boost their fitness. Zahavi's (1975) handicap theory suggests that ‘good genes’ are implied through exaggerated sexually selected traits, as only individuals with high survivability can handle the costs of bearing these traits. If this survivability is heritable, then mating with these individuals would lead to high offspring survival rate, and thus potential fitness benefits. Alternatively, Fisher (1930) proposed a more cyclical idea, based around individuals preferring traits because that trait is preferred in the population (i.e. latent preferences). If these traits are heritable, then mating with these individuals will result in offspring that also possess the trait and, thus, will be attractive (i.e. ‘sexy sons’). Fitness benefits occur here, not because offspring are more likely to survive, but because they would be more likely to reproduce. Regardless of the source of these mate choice decisions, they offer sizeable benefits to an individual's fitness, and are thus widespread throughout many taxa (Zuk, Ligon and Thornhill, 1992; Censky, 1997; Wagner and Reiser, 2000; Amundsen and Forsgren, 2001; Clutton-Brock and McAuliffe, 2009; Henneken et al., 2015).

An integral component of mate choice theories is the necessity for individuals to make informed decisions. Where the benefit gained from

mating is access to a resource, individuals can directly assess the resource and decide if it is worth mating with the owner (Thornhill, 1976). However, when the benefit of mating is based around a conspecific's genotype, or based on specific phenotypic traits, individuals must utilise signals and/or cues from potential mates to make these informed decisions. Signals attended to by individuals to make mate choice decisions are diverse, not only in the modality used, but also what aspect of the individual's quality they reveal. For example, female Iberian rock lizards, *Lacerta monticola*, decide which male to associate with, and are thus more likely to mate with, based on the pheromonal signals produced by the males. These signals are correlated with fluctuating asymmetry in femoral glands and thus developmental stability (Martín and López, 2000). Alternatively, visual signals based on carotenoid colouration, and the associated selection of mates based on them, is seen throughout many avian species (Svensson and Wong, 2011). These visual signals are known to be affected by parasite load, and thus reflect the individuals immunocompetence (Martínez-Padilla et al., 2007). Additionally, animals may even use multiple signals of different modalities produced by potential mates to make informed mate choice decisions (Candolin, 2003). One of the most conspicuous modalities of sexual signals, however, is acoustic communication, such as vocal, stridulatory or percussion signals. Acoustic signals are utilised in decisions of mate choice throughout different taxa, including mammals (Charlton, Reby and McComb, 2007), birds (Searcy, 1992), amphibians (Gerhardt, 1991), and fish (Amorim et al., 2016), as well as invertebrates such as insects (Brown, 1999), arachnids (Rivero et al., 2000) and crustaceans (Salmon and Atsides, 1968).

Issues may arise from using these signals to make informed mate choice decisions when transmission or perception of the signal is disrupted. The best example of this can be seen in studies where researchers have artificially manipulated certain properties of the sexual signal making the bearer appear more or less attractive in mate choice decisions (Andersson, 1982; Basolo, 1990; Amundsen and Forsgren, 2001; Drăgănoiu, Nagle and Kreutzer, 2002; Charlton, Reby and McComb, 2007; Shestakov and

Vedenina, 2015; Amorim et al., 2016). By altering the transmission of these sexual signal, individuals that are making mate choice decisions would act on unreliable information, which may ultimately lead to them mating with lower quality individuals, or missing mating opportunities with higher quality individuals. Artificial manipulation is a type of human disruption with the intent of understanding sexual signalling systems. However, it is far outnumbered by the amount of other, potentially more damaging, types of anthropogenic disruptions. Anthropogenic noise, for example, is known to heavily disrupt many different communication systems, including sexual signals, across numerous different taxa (Chapter 1, 2 and 3). Potentially, this evolutionarily recent selection pressure may disrupt signals associated with mate choice, and thus lead individuals to make non-preferable mating decisions.

The experiment presented in this chapter aims to observe differences in mate choice based on acoustic sexual signals under conditions of anthropogenic noise, whilst simultaneously aiming to understand the acoustic characteristics necessary to cause this disruption. To accomplish this, I observed the acoustic courtship signals of the Mediterranean field cricket, *Gryllus bimaculatus*. In this species, and commonly throughout the genus *Gryllus*, males produce a number of acoustic sexual signals by means of wing stridulation (Alexander, 1961). This includes a ‘courtship song’ which is used by males to encourage a female to mate with them (Chapter 3), and is formed of a series of high frequency ticks and low frequency pulses (Figure 4.1). This acoustic signal is well suited for this study as it is known to be used by females to make mate choice decisions (Shestakov and Vedenina, 2015), has been shown to correlate with aspects of male quality (Gray and Eckhardt, 2001; Rantala and Kortet, 2003), and is known to be costly to the performer (Mowles, 2014; Zuk and Kolluru, 1998) which is important for maintaining signal reliability (see Chapter 1). These experiments involved manually silencing mature male crickets and playing pre-edited acoustic recordings when they attempted to signal in staged courtship interactions. I observed female mounting choice and latency as a sign of mate preference; a common practice in mate choice experiments

(Shackleton, Jennions and Hunt, 2005; Kostarakos, Hartbauer and Römer, 2008; Shestakov and Vedenina, 2015; Loranger and Bertram, 2016). As anthropogenic noise does not generally affect mounting latency (Chapter 3), any difference in this experiment due to the presence of anthropogenic noise will be as a result of differences in the perception of the male's quality. Additionally, differences in behaviours and latencies between acoustic conditions will also highlight the impact of certain acoustic characteristics causing a disruption in mate choice signalling systems.

Methods

Study Organisms

Individuals used in this study were reared as in Chapter 2, but here both males and females were utilised in the behavioural trials. For this experiment, it was also necessary to mute the males, in order to then play an artificial courtship song when they attempted to signal, which is a common practice in studies using orthopterans (Libersat, Murray and Hoy, 1994; Gray, 2005; Bailey, Gray and Zuk, 2010; Logue et al., 2010; Stoffer and Walker, 2012; Shestakov and Vedenina, 2015). To accomplish this, I placed males at -20°C for 5 minutes to partially anaesthetise them, followed by cutting both their forewings anterior to the stridulatory file. This results in them no longer being able to produce any acoustics, but still allows them to exhibit courtship behaviours. Males were operated on at least 24 hours before they were used in a behavioural trial.

Acoustic Stimuli

The noise conditions I used in this experiment were the same as those used previously (Chapter 2, Figure 2.1) and the recording equipment used to create the artificial courtship song were the same as the those described in that chapter. I made two songs that differed in “quality” based on their tick rate, a feature that females show preference for and is linked to beneficial phenotypic traits (Rantala and Kortet, 2003). The base courtship song which I used to create both the ‘high quality’ and ‘low quality’ courtship songs (Figure 4.1) was recorded from a single sexually active male. I presented the male with a tethered female, so that she could not mount him and courtship

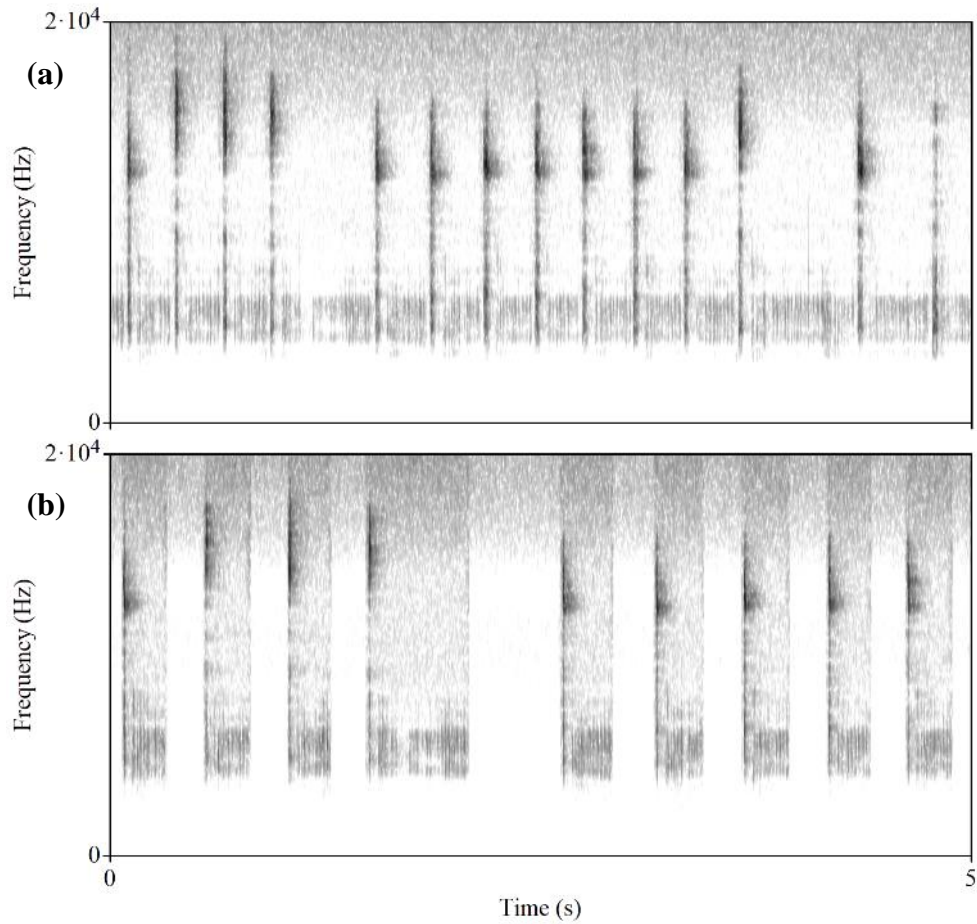


Figure 4.1. Frequency spectrograms showing (a) the ‘high quality’ and (b) ‘low quality’ courtship songs created for this experiment. High quality average TP = 0.341s, average TR = 168 ticks min⁻¹. Low quality average TP = 0.561s, average TR = 108 ticks min⁻¹. Spectrograms were created using Praat with the following properties: window length: 0.005s, time range as shown (0 – 5s); frequency range: 0 – 20000Hz

could continue for a longer period of time (Chapter 3). A 5 minute recording was taken of his courtship song, whilst simultaneously sampling the amplitude of the song (46 ± 2.6 dBA). Additionally, the recording was taken in the same temperature range as the one used in the behavioural trials, as song performance is known to fluctuate due to temperature (Hedrick et al., 2002). From this recording, I took a subset of the most active part of the song and removed extended periods of silence, as well as occurrences of chirps, resulting in a 1 minute sample. This sample was then looped a number of times to create a 15 minute ‘high quality’ courtship song. The

‘low quality’ courtship song was created from the same 1 minute sample, but additional periods of silence were added after each tick period, approximately the same duration as the low amplitude pulses. This was then looped a number of times to create a 15 minute ‘low quality’ courtship song. In creating the two song types in this manner, the songs only differed in their tick period (TP, the time between the start of one tick and the start of the next) and consequently tick rate (TR, the number of ticks per minute), whilst maintaining frequency, amplitude and other temporal characteristics (High quality song: average TP = 341ms, TR = 168 ticks min⁻¹; Low quality song: average TP = 561ms, TR = 108 ticks min⁻¹). Both the ‘high quality’ and ‘low quality’ courtship song were played back to ensure that the average tick amplitude was the same as the original recording.

Courtship Interactions

I staged behavioural interactions in transparent plastic terraria (15 x 8 x 10cm) with similar protocols to previously conducted experiments (Chapter 3, Figure 3.1). Each terrarium was equipped with a layer of sand to act as substrate and an opaque middle partition to separate the individuals prior to the interaction. Two Veho®360° capsule speakers were positioned 20cm above each end of the arena for acoustic condition playback. An additional speaker was also placed 15cm above the arena for playback of the courtship song. All interactions were staged during the day phase of the day:night cycle (specifically between 09:00 - 11:00 local time) and between 25 - 29°C . However, unlike the previous experiment, only females that were free to move around and mount males when they wanted were used in this experiment. Additionally, females were only used for one experiment, but males were used for up to four (at least 24 hours between each and randomly spread between acoustic and quality conditions) to reduce the number of males that needed muting.

Prior to the start of the interaction, a muted male was placed into one half the behavioural arena to acclimate overnight (16 hours minimum). Following this period, a female was placed into the other half of the arena, and left to acclimatise for 20 minutes. After this, the interaction was started by simultaneously removing the opaque partition and broadcasting the

acoustic condition (ambient, traffic or white noise). These interactions continued for a period of 15 minutes, or until the female mounted the male. When the males attempted to court the female, which was noticeable from the movement of the wing stubs, either an artificial courtship song was broadcast ('high quality' or 'low quality') or nothing was broadcast ('no song' trials). As male courtship performance is known to be affected by acoustic condition (Chapter 3), and female response was important to observe, I repeated trials until there were 20 occurrences of male courtship in each set of conditions (9 in total). Following behavioural interactions, females were placed into the breeding stock, whereas males may have been kept separately for reuse in another trial. All interactions were recorded visually using as SONY HDR-CX625 HANDYCAM® positioned directly above the arena (creating "bird's eye view" footage).

Behavioural Analysis

Footage of the behavioural interactions were event coded using the software B.O.R.I.S. (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). Courtship interactions were scored on multiple events, including counts of successful signalling attempts (if the male attempted to sing) and courtships (if the female mounted), as well as latencies and lengths of courtship behaviours (e.g. latency to start courting, latency of female to mount male).

Statistical Analyses

I carried out all statistical analyses in the statistical program R studio (Rstudio Team, 2016; R Development Core Team, 2017), with the packages 'dunn.test' (Dinno, 2015), 'multcomp' (Hothorn, Bretz and Westfall, 2008), and 'hmisc' (Harrell Jr., 2006). All graphs and plots were created using base R and with the package 'ggplot2' (Wickham, 2016) and Presented effect sizes (Log odds ratio \pm SE) were acquired from the model summary. Where I used GLZMs, I also visually assessed plots of residuals vs. fitted values to ensure that models fit the data well, and that the data did not violate the model assumptions. I tested scale data for normality where necessary, using a Shapiro-Wilk test, and used non-parametric tests where appropriate. I used

a generalized linear model (GLZM) using a binary logistic function to test for differences in courtship occurrence between interactions presented here (males with cut wings) and those of a previous chapter (males without cut wings; Chapter 3). I also used binary GLZMs to test for differences in the occurrence of courtship behaviour between quality conditions and acoustic conditions. In trials where males courted, I used binary GLZMs to test for differences in female mount choice between quality conditions, but within acoustic conditions, and female mount choice between acoustic conditions, using only “high quality” trials (as this is the closest trial to normal behaviour). I also used Kruskal-Wallis tests to analyse differences in movement latency, courtship latency (from conspecific contact), and female mounting latency between both acoustic and quality conditions. When a statistically significant ($P < 0.05$) result was found, I used suitable post-hoc pairwise tests (TukeyHSD multiple comparison analysis for binary GLZMs, Dunn’s tests with bonferroni corrections for Kruskal-Wallis tests).

Results

Courtship and Mounting Occurrence

There was no difference in courtship occurrence between ambient no song conditions and the ambient free female conditions used in Chapter 3 (GLZM(b): Wald $X^2_2 = 1.589$, $N = 44$, $P = 0.208$). Additionally, no difference was found in courtship occurrence between quality conditions within each acoustic condition (Ambient GLZM(b): Wald $X^2_2 = 2.729$, $N = 70$, $P = 0.256$; Traffic GLZM(b): Wald $X^2_2 = 0.956$, $N = 66$, $P = 0.62$; White GLZM(b): Wald $X^2_2 = 0.056$, $N = 85$, $P = 0.973$). However, males showed a reduction in courtship performance in white noise conditions (-9.163 ± 0.416) when compared to other acoustic conditions (GLZM(b): Wald $X^2_2 = 11.397$, $N = 221$, $P = 0.003$, Figure 4.2). Individuals in white noise conditions were less likely to signal than those in traffic noise conditions ($N = 151$, $P = 0.01$), but no effect was found between ambient and white noise conditions ($N = 155$, $P = 0.07$), or ambient and traffic noise conditions ($N = 136$, $P = 0.616$).

In ambient noise conditions, there was a reduction in the occurrence of mountings in no song conditions (-2.197 ± 0.869) when compared to other quality conditions (GLZM(b): Wald $X^2_2 = 8.845$, $N = 60$, $P = 0.012$, Figure 4.3). Females were less likely to mount males in no song trials than those in high quality trials ($N = 40$, $P = 0.03$), but no effect was found between high quality and low quality trials ($N = 40$, $P = 0.656$) or low quality and no song trials ($N = 40$, $P = 0.127$). A similar difference was not found in traffic noise conditions (GLZM(b): Wald $X^2_2 = 1.18$, $N = 60$, $P = 0.554$) or white noise conditions (GLZM(b): Wald $X^2_2 = 0.959$, $N = 60$, $P = 0.619$). Additionally, the occurrence of mountings did not differ in high quality trials between acoustic conditions (GLZM(b): Wald $X^2_2 = 4.145$, $N = 60$, $P = 0.126$).

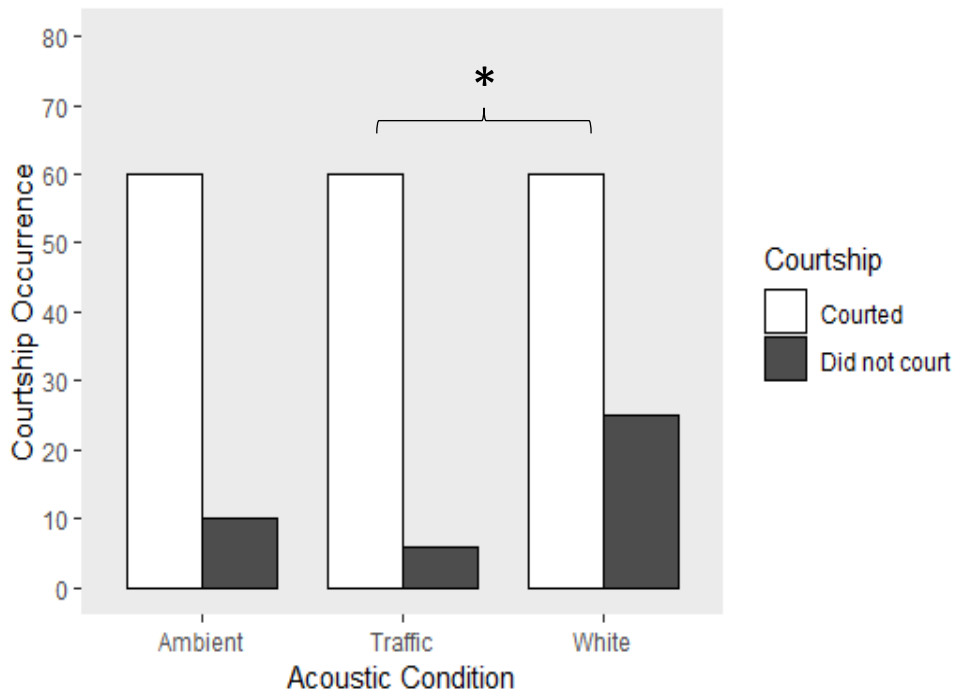


Figure 4.2. The number of males who courted or did not in courtship interactions between ambient ($n = 70$), traffic ($n = 66$) and white noise ($n = 85$) conditions. Brackets with an asterisk show a significant result from pairwise analysis.

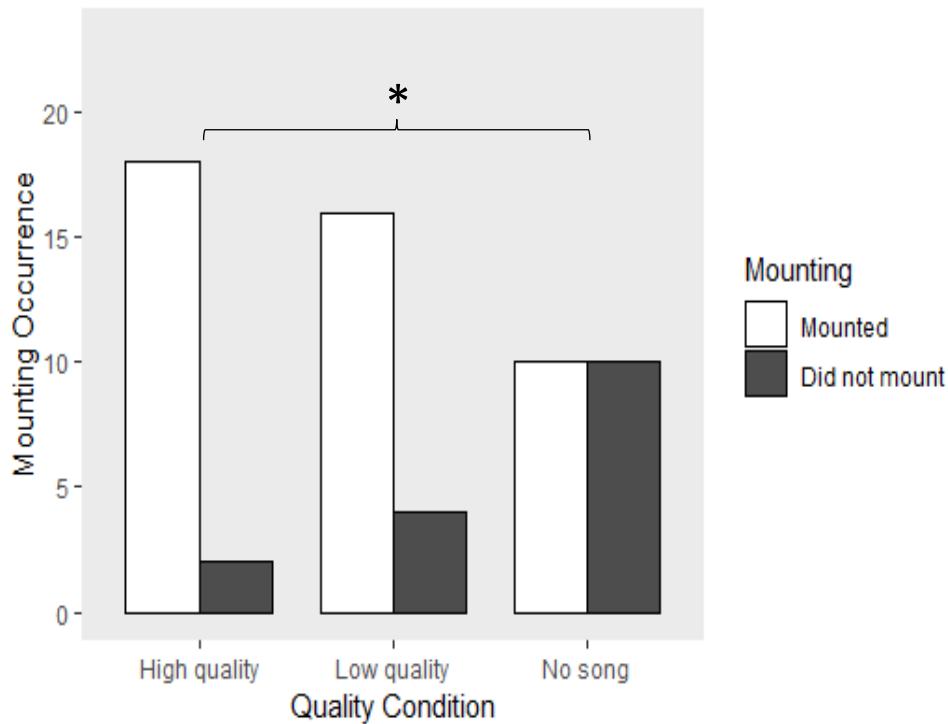


Figure 4.3. The number of females who mounted or did not mount males in ambient noise courtship interactions between quality conditions. Brackets with an asterisk show a significant result from pairwise analysis.

Behavioural Latencies

Neither male nor female movement latency was affected by quality conditions or acoustic conditions (Table 4.1). Additionally, males did not take longer to signal, following contact with the female, between quality conditions or acoustic conditions (Table 4.1). Mounting latency differed between quality conditions in ambient noise trials (Table 4.2, Figure 4.4). Females were quicker to mount in high quality trials than they were in low quality or no song trials, but there was no difference between low quality and no song trials. This effect was not found under traffic noise or white noise conditions (Table 4.2). Additionally, latency to mount in high quality trials was found to differ between acoustic conditions (Table 4.2, Figure 4.4), where females were quicker to mount in ambient noise trials when compared to white noise trials, but showed no difference between ambient and traffic noise conditions or traffic and white noise conditions. No similar

difference in mounting latency was found when comparing low quality trials or no song trials between acoustic conditions (Table 4.2).

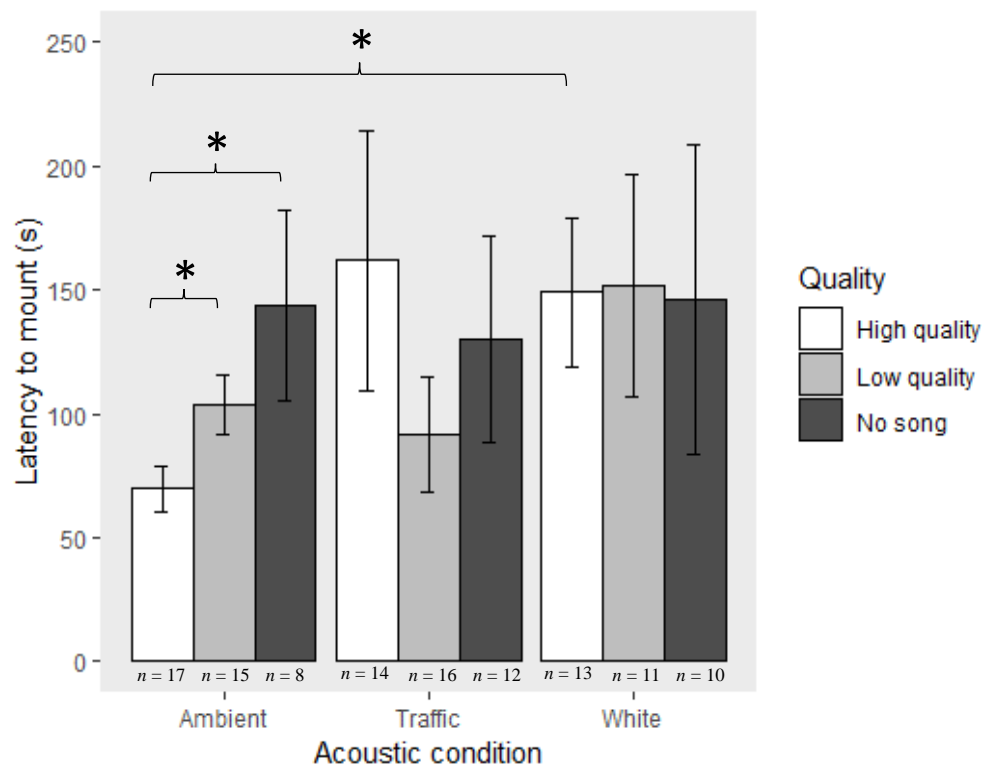


Figure 4.4. Latency of females to mount ($\bar{x} \pm \text{SE}$) between acoustic and quality conditions. Brackets with an asterisk show a significant result from Dunn's post-hoc tests, where an overall significant affect was first found.

Table 4.1. Output from Kruskal-Wallis tests on measures of male movement latency, female movement latency and courtship latency (from contact), between high quality (H *n*), low quality (L *n*) and no song (N *n*) conditions, and pooled analysis between acoustic conditions. Means and standard error of the mean are shown for populations that were tested (quality conditions or acoustic conditions).

	X^2_2	N <i>n</i>	\bar{x}	S.E.	H <i>n</i>	\bar{x}	S.E.	L <i>n</i>	\bar{x}	S.E.	P
Male movement latency (s)											
Ambient	2.251	24	77.276	18.853	21	89.537	17.625	25	88.191	12.526	0.324
Traffic	0.631	21	107.086	23.376	22	113.398	32.908	23	80.197	16.733	0.73
White	1.53	28	61.319	8.928	29	73.565	13.376	28	81.253	13.023	0.465
Between noise conditions	2.505	70	85.397	9.3724	66	99.664	13.421	85	71.9	6.820	0.286
Female movement latency (s)											
Ambient	0.4	24	81.663	19.445	21	57.421	8.828	25	61.622	12.118	0.819
Traffic	1.452	21	76.425	14.685	22	65.484	8.094	23	73.247	17.813	0.484
White	4.567	28	44.792	7.715	29	51.678	7.463	28	67.002	10.333	0.102
Between noise conditions	1.788	70	66.134	7.803	66	70.549	7.775	85	54.409	5.017	0.41
Courtship latency (s)											
Ambient	0.78	20	20.481	5.167	20	56.588	34.762	20	31.323	9.011	0.677
Traffic	0.209	20	26.012	5.862	20	18.486	4.04	20	33.191	10.228	0.901
White	0.857	20	35.599	19.949	20	47.423	17.58	20	58.711	23.143	0.652
Between noise conditions	0.371	60	36.131	12.049	60	26.072	4.029	60	47.245	11.615	0.83

Table 4.2. Output from Kruskal-Wallis tests on mounting latency (s) between high quality (H *n*), low quality (L *n*) and no song (N *n*) conditions, and between ambient (A *n*), traffic (T *n*) and white noise (W *n*) conditions. Means and standard error of the mean are shown for populations that were tested (quality conditions or acoustic conditions). Dunn's test pairwise results are shown for statistically significant outcomes. Bold P-values indicate a significant ($P > 0.05$) result.

	X^2_2		\bar{x}	S.E.		\bar{x}	S.E.		\bar{x}	S.E.	P
Quality trial differences		N <i>n</i>			H <i>n</i>			L <i>n</i>			
Ambient	8.847	8	143.914	38.596	17	69.094	9.24	15	103.72	12.341	0.012
High - Low					17			15			0.026
Low - No Song		8						15			0.825
No Song - High		8			17						0.015
Traffic	2.062	12	130.119	41.827	14	161.828	52.68	16	91.823	23.013	0.357
White	0.789	10	146.261	62.694	13	148.974	30.356	11	151.971	44.806	0.674
Acoustic trial differences		A <i>n</i>			T <i>n</i>			W <i>n</i>			
High quality	6.688	17	69.094	9.24	15	161.828	52.68	13	148.974	30.356	0.035
Ambient - Traffic		17			15						0.079
Traffic - White					15			13			0.957
White- Ambient		17						13			0.025
Low quality	3.382	15	103.72	12.341	16	91.823	23.013	11	151.971	44.806	0.184
No Song	1.892	8	143.914	38.596	13	130.119	41.827	10	130.119	41.827	0.388

Discussion

The differences in courtship behaviour between acoustic and quality conditions described here highlight the disruptive influence of anthropogenic noises, and the consequences when conflicting with acoustic behaviours that are utilised to make mate choice decisions. I observed no significant difference in female mounting latency and mounting success between males of different levels of perceived quality in traffic and white noise trials. Comparatively, a significant difference was observed in ambient noise conditions, which provides further evidence that adds to the growing body of literature on anthropogenic noise disrupting acoustic signalling systems. Furthermore, this experiment has further supported findings in the previous chapter, as the same reduction in signalling and no reduction in female response was observed here also. My results help to distinguish the important acoustic characteristics of disruptive noise when conflicting with acoustic signalling systems. Additionally, by observing courtship interactions of *G. bimaculatus*, this experiment has aided in defining attractiveness and quality perception in courtship signals, which is understudied when in comparison to the calling song of the species (Shestakov and Vedenina, 2015).

The observed differences in courtship success in ambient noise conditions work as a foundation on which to compare the differences, or lack thereof, seen in other acoustic conditions. In ambient noise conditions, high quality and low quality trials maintained a 90% and 80% success rate (female mounted) respectively, but in no song trials, success rate significantly dropped to 50%. This is not an unusual result, as, in this situation, both a high quality and low quality signal should yield a successful encounter, either through a reduction in a female's selection threshold (variable threshold strategy; Janetos, 2015) or by cumulative effects of the repetitive display (Mowles and Ord, 2012). On the other hand, a complete removal of a sexual signal would significantly reduce any behaviour that is dependent upon it (Gray et al., 2014; Shestakov and Vedenina, 2015), although the higher than expected success rate in the no song conditions suggest an element of multimodal signalling maybe in

effect (Candolin, 2003; Stoffer and Walker, 2012). However, the more interesting results are found in the success rates in traffic noise and white noise conditions, where no significant difference was found between high quality, low quality and no song trials. As I have shown here, and in past experiments (Chapter 3), females are not less likely to mate under traffic or white noise conditions, so we can conclude that this difference is likely due to the perception of male quality. This result suggests that mate choice in this species has been disrupted by the presence of noise as females in these conditions show no differentiation between mating with individuals accompanied by high quality songs, low quality song or no song.

When we consider the differences, or lack thereof, seen in latency to mount a signalling male, this further supports the evidence that anthropogenic noise is disrupting the selection pressures associated with mate choice. Again, an expected response is seen in ambient noise conditions, where males are mounted sooner when accompanied with a high quality courtship song, than with a low quality song or no song at all. In most no-choice tests, such as the one presented in this chapter, the assumption is that females take different amounts of time to mate with males of differing qualities as they need to exceed a threshold (through falling expectations or additive quality perception) before they are deemed viable mates (Shackleton, Jennions and Hunt, 2005; Shestakov and Vedenina, 2015; Backwell and Passmore, 2016). Additionally, the effect seen in most no-choice experiments would be boosted if a choice experimental design was utilised (Dougherty and Shuker, 2015), which potentially reflects a more accurate natural choice (i.e. when presented with more than one male). Thus, we can conclude that, in ambient noise trials, females preferred mating with males with a high tick rate (TR) and shorter tick period (TP), than those with a low TR and a longer TP, or no song at all. However, this difference is again not observed in either traffic noise or white noise conditions, where there was no difference in mounting latency. In previous chapters, I have shown that females do not differ in their mounting latency between acoustic conditions generally (Chapter 3), leading me to conclude that the difference observed here is due to a

disruption in mate quality perception. Additionally, the finding that individuals which were presented with the high quality song mounted quicker in ambient noise conditions, but the lack of differences in low quality and no song trials between acoustic conditions, suggests that high quality males appear less attractive under noisy conditions but low quality males do not appear more attractive.

Anthropogenic disturbances have been shown to disrupt mate choice systems previously (Candolin, Salesto and Evers, 2007), and unfavourable natural noise conditions have also been shown to reduce female preference in mate choice (Wollerman and Wiley, 2002). This study adds to the, currently small, body of evidence that a combination of these factors (i.e. anthropogenic noise) can reduce female perception of mate quality based on acoustic signals (Huet des Aunay et al., 2013). Potential implications of such a disruption of sexual signals may be far reaching and lead to a decrease in population viability. From a female's perspective, they risk mating with a less than preferable male, or avoid mating with a highly preferable male, when they are unable to detect differences in mate quality. This may lead to a reduction or complete loss of offspring viability (Funk and Tallamy, 2000). If female preference behaviour is not altered, through changes in signal modality (Partan, 2017) or other evolutionary responses, then populations may risk extinction (Tanaka, 1996). Alternatively, males that are producing these high quality, and probably costly, signals are receiving no benefit over individuals that may be investing less into their signals. As a result, males may have to increase the costs they are investing to overcome the signal disruptions (Chapter 3; Díaz, Parra and Gallardo, 2011), in order to gain mating advantages, which would disrupt the distribution of resources leading to a potential reduction in survivability (Hunt et al., 2004). However, signalling in noisy conditions may indeed reduce the costs related to sexual signals, in that it could reduce the likelihood of predation, which is a current cost faced by field crickets (Zuk and Kolluru, 1998). In any of these cases, the presence of anthropogenic noise when acoustic mate choice signals are being used can lead to highly deleterious consequences.

The inclusion of both a traffic noise stimulus and a white noise stimulus in the present study allows for discussion on the required acoustic characteristics that can lead to the effects discussed previously. As there appears to be a disruption of mate preference in both traffic and white noise conditions, when compared to ambient noise conditions, this suggests that it is a shared characteristic of the two noise stimuli that is responsible for this disruption. The main shared characteristic between the two stimuli is their average amplitude, for which they are matched, suggesting that this is potentially the main requirement of a disruptive stimulus. Neither the difference in frequency or signal fluctuations led to a noticeable or significant difference. This is an interesting result as I have previously concluded that amplitude alone is not enough to disrupt the signalling system in question (Chapter 2, Chapter 3). In those chapters, my discussion was based around the likelihood that the effect was observed in those experiments was due to frequency masking, based on Naguib's (2013) work. As masking is mostly based on shared frequency bands, we can conclude that this is not the effect seen here, as traffic noise does not contain frequencies similar to the 'ticks' in *G. bimaculatus* courtship song. Instead, this result may be caused by distractions from other signals or a lack of attention to the signal. Naguib (2013) notes that attention based issues with signal reception "would indicate that extracting, storing and recalling more subtle information may well be affected, even in situations where signal detection is less affected by noise". I have shown here, and in previous chapters (Chapter 3,) that signal detection is not reduced in these noise conditions as females still mount males that court. Additionally, as courtship song preference in *G. bimaculatus* is known to be based on the subtle fine scale timing of pulses in the signal (Rantala and Kortet, 2003; Shestakov and Vedenina, 2015), an attention deficit may indeed be the effect seen here. In other species, lower-quality males may exploit this effect of reduced attention by signalling in leks, where females have reduced attentional capabilities and thus altered signal discrimination (e.g. Wollerman and Wiley, 2002).

Finally, my study also adds to the understanding of mate choices based on courtship songs in *G. bimaculatus*. Calling song attractiveness and preference has been studied extensively in field crickets (Simmons and Ritchie, 1996; Wagner Jr and Hoback, 1999; Simmons, Zuk and Rotenberry, 2005; Meckenhäuser, Hennig and Nawrot, 2011; Trobe, Schuster and Römer, 2011; Verburt, Ferreira and Ferguson, 2011; Hirtenlehner et al., 2013; Samuel et al., 2013; Zhemchuzhnikov and Knyazev, 2015; Hedwig and Sarmiento-Ponce, 2017; Zhemchuzhnikov et al., 2017), but by comparison courtship song preference has remained largely understudied (Rantala and Kortet, 2003; Shestakov and Vedenina, 2015). This is despite suggestions that calling songs in *Gryllus* species allows for species identification, whilst courtship songs are more likely to contain information relating to a male's quality (Fitzpatrick and Gray, 2001; Gray, 2005). More research is necessary to understand the evolution behind courtship songs in field crickets, which will in turn better reveal the function of the song and what information females gain from attending to the signal.

Conclusions

The experiment presented here has revealed that anthropogenic noise can lead to alterations in the perception of mate quality, when that quality is advertised through acoustic signals. In this case, both traffic noise and white noise reduced the selection preference seen in females in ambient noise conditions, both in terms of mounting success and latency. This effect does not seem to be due to the dominant frequency of the song, as I suggested in previous chapters, but rather due to the amplitude of the presented stimuli, which has led to potential reductions in attentiveness from the females attending to the courtship signal. This result not only helps to further the work on the consequences of anthropogenic noise, but also highlights the importance of courtship song selectivity in *Gryllus bimaculatus*, a currently understudied topic. However, additional work is required to fully document the consequences of anthropogenic noise when conflicting with sexually selected acoustic signals (i.e. intraspecific aggressive signals; Chapter 5).

Chapter 5

Aggressive Interactions under Conditions of Anthropogenic Noise

Abstract

Many species display intra-specific aggressive behaviours as an adaptation to acquire necessary resources for survival and reproduction. In these aggressive interactions, individuals may perform acoustic signals that detail some aspect of their quality or aggressive motivation, and are important to reduce injurious combat. With global increases of anthropogenic noise, these acoustic signals may be disrupted, resulting in increased aggressive behaviours, or alteration to agonistic encounters and outcomes. In the present study, I investigated the consequences of anthropogenic noise conflicting with acoustic agonistic signals by observing the aggressive interactions of the Mediterranean field cricket, *Gryllus bimaculatus*. Through the lack of behavioural differences observed between acoustic conditions, this chapter has shown the stability of this particular acoustic signalling system under the noise conditions used in the study. This result could potentially detail some fundamental aspects of the aggressive signal used in this species that allow it to be reliable under anthropogenic noise conditions, such as signal function, multi-modal signalling or basic signal-to-noise ratios. A distinct lack of differences in haemolymph metabolites was also found, particularly linked to the occurrence or intensity of aggressive behaviours, the implications of which are discussed.

Introduction

In nature, the demand for resources often exceeds supply. It is this imbalance that causes the diversity of competitive behaviours that we see in the natural world. Animals from all taxonomic groups are known to compete for various kinds of resources, including for food (Fraser, 1976), water (Valeix et al., 2008), shelter or space (Koenig, 1981; Figler,

Cheverton and Blank, 1999), and mates (Alexander, 1961). Successful acquisition of these resources, or even just access to them, can significantly boost the survivability and fitness prospects of the successful individual, whilst potentially being a fatal outcome for individuals which are not successful. Interestingly, successful acquisition of certain resources often leads to successfully acquiring mates too, through the use of food/water as a nuptial gift (Vahed, 1998) or using shelters as a breeding ground to attract mates (Christy, 1982) for example, which further boosts the potential fitness benefits of successful competition. As a result of the benefits of resource acquisition, combative or agonistic behaviours and morphological structures, such as weapons, have evolved to aid in acquiring these resources and thus increase access to mates (Hardy and Briffa, 2013).

Examples of behaviours and weapons utilized for contests are widespread throughout animals in different taxonomic groups. For example, male Japanese horned beetles, *Allomyrina dichotoma*, use their exaggerated horns to displace other males from favourable areas (Siva-jothy, 1987), whilst Scottish red deer, *Cervus elaphus scoticus*, will lock antlers in an attempt to defend or acquire a harem (Clutton-Brock et al., 1979), both of which can lead to significant harm to the individuals involved. However, these behaviours are often part of a larger repertoire of aggressive signals that the receiver uses to gather information on their potential competitor (which is not dissimilar to the purpose of signals used to attract potential mates; Moller and Pomiankowski, 1993). In the case of the red deer example, males precede physical combat with loud roars between the potential combatants, and they do not always proceed to lock antlers as one individual usually withdraws before then (Clutton-Brock et al., 1979). These agonistic displays are also observed throughout different taxonomic groups and in different modalities, for example, electric signalling in fishes (Kramer and Bauer, 1976), visual signals in birds (Pryke, Lawes and Andersson, 2001), vibratory signals in amphibians (Caldwell et al., 2010), and acoustic signals in lizards (Marcellini, 1974).

Most commonly, these signals are performed prior to any injurious behaviours, as a way to avoid the potentially costly outcomes of a physical

contest. Various models, such as the energetic war of attrition model (E-WOA; Payne and Pagel, 1996), the sequential assessment model (SAM ;Enquist et al., 1990), and the cumulative assessment model (CAM; Payne, 1998), explain these signals or repertoires as indices of an individual's resource holding potential (RHP) or willingness/desire to acquire the benefits of competition, either informing the signalling individual or a potential competitor. By utilizing these signals and repertoires, competitors can predict the outcome of aggressive interactions and ultimately avoid any unnecessary injurious combat. Alternatively, these signals can occur after potentially injurious physical combat has already occurred, and are often referred to as victory displays, but can be performed by either the dominant or subordinate individual, or both. These signals do not follow the assumptions and predictions of the previously mentioned models, but potential alternative functions, although comparatively understudied, have been suggested for these displays, such as submission, browbeating, or advertisement (Mesterton-Gibbons and Sherratt, 2006; Lippold et al., 2008; Rook, Fitzsimmons and Bertram, 2010). Submission displays are produced by losers and function to prevent further aggression from a dominate individual or any other nearby individuals (Lippold et al., 2008). Alternatively, if a display is produced by the winner, it is thought to be either for browbeating (reducing the likelihood of a subordinate individual initiating a contest in the future) or advertisement (communicating contest status to potential competitors and mates; Mesterton-Gibbons and Sherratt, 2006). Regardless of the function, these signals and repertoires are an integral part of many biological systems and are widespread throughout the Animal Kingdom.

Acoustic signals are a common modality for behaviours that are part of an aggressive repertoire, and are observed in mammals (Clutton-Brock et al., 1979), reptiles (Marcellini, 1974), birds (Searcy, Anderson and Nowicki, 2006), and invertebrate taxa, such as insects (Alexander, 1961). As with all signal modalities, these acoustic signals have evolved to overcome certain environmental conditions that would otherwise disrupt signal transmission, such as dense vegetation or heterospecific signals. However, these signals

can also be affected by increasing global anthropogenic noise levels, an evolutionarily recent and disruptive selection pressure that is known to conflict with animal acoustics (see Chapter 1, 2, 3 and 4). If anthropogenic noise conflicts with the aforementioned agonistic acoustics, this could lead to deleterious consequences for interacting individuals. For example, in agonistic encounters suggested by the SAM (Enquist et al., 1990), signal disruptions could lead to further repetitions of the behaviour, which could be costly, or unnecessary injurious fighting due to inaccurate assessment of the opponents RHP. Similarly, disruptions to post-conflict submission (Lippold et al., 2008) or browbeating displays (Mesterton-Gibbons and Sherratt, 2006) could also lead to unnecessary further conflict, which may be costly to both contestants. Current evidence shows that just the presence of anthropogenic noise may alter aggressive encounters (Bruitjes and Radford, 2013), but there is no evidence of this being due to issues with signal transmission. Thus, documenting the affect anthropogenic noise has when conflicting with acoustic agonistic signals, if any, is a necessary endeavour.

This chapter aims to identify the behavioural differences and energetic consequences of agonistic interactions with acoustic components when they are affected by anthropogenic noise. Furthermore, this chapter also aims to identify integral characteristics of a noise stimulus that lead to this initial disruption, where such disruption is evident. I investigated this necessary topic by using the acoustic agonistic interactions of male Mediterranean field crickets, *Gryllus bimaculatus*, as a model system. Male crickets of the genus *Gryllus* are well known for their stridulation based songs and conspicuous sexual behaviours (Alexander, 1961). This includes a repertoire of behaviours which both males and females use on same-sex conspecifics when in competition for resources. The male agonistic repertoire follows a stereotypical pattern which culminates in a victory song produced by the dominant individual (Figure 1.1c). As this acoustic signal follows potentially injurious combat (grappling), it is assumed to have an advertisement or browbeating function (Rook, Fitzsimmons and Bertram, 2010). If anthropogenic noise does conflict with this signal, individuals may

remain in combat for longer when under anthropogenic noise conditions, or alter some aspect of their behaviour to compensate for the loss of signal transmission or reliability. Furthermore, any differences observed in behaviours between different acoustic conditions will reveal the necessary characteristics a noise stimulus needs to possess in order to cause such as disruption.

Methods

Study Organisms and Acoustic Stimuli

The individuals I used in this study were reared as in Chapter 2, but here only male crickets were used. Additionally, I separated males into single individual containers as crickets in high density groups are less likely to display aggressive behaviours (Alexander, 1961), and removing them from other mature individuals avoids past interactions affecting aggressive signalling (i.e. confidence; Simmons, 1986). Furthermore the noise stimuli I used in this experiment were the same as those used previously (Chapter 2, Figure 2.1).

Aggressive Contests

I staged all aggressive interactions in a medium sized (23 x 13 x 18 cm) transparent plastic arena (Figure 5.1), allowing space for individuals to display natural behaviours. The capsule speakers were positioned 24cm up at both sides of the arena, pointing towards the centre. These trials took place between 10:00 and 13:00 local time, during the ‘light’ stage in the light:dark cycle, and at 24 to 28°C. I conducted visual and acoustic recordings of these interactions for later analysis. Video recordings were conducted using a SONY HDR-CX625 HANDYCAM® positioned directly above the arena (creating “bird’s eye view” footage). Acoustic recordings were conducted using a RØDE NTG4+ shotgun microphone (positioned directly above the arena) connected to a TASCAM DR-07MKII Linear PCM recorder (.wav format, 16-bit resolution, 48 kHz sampling rate).

For each interaction, I used males that were within two days of the same age (post-eclosion), to avoid age difference affecting aggressive behaviours, and with a weight difference of no more than 10%, as weight

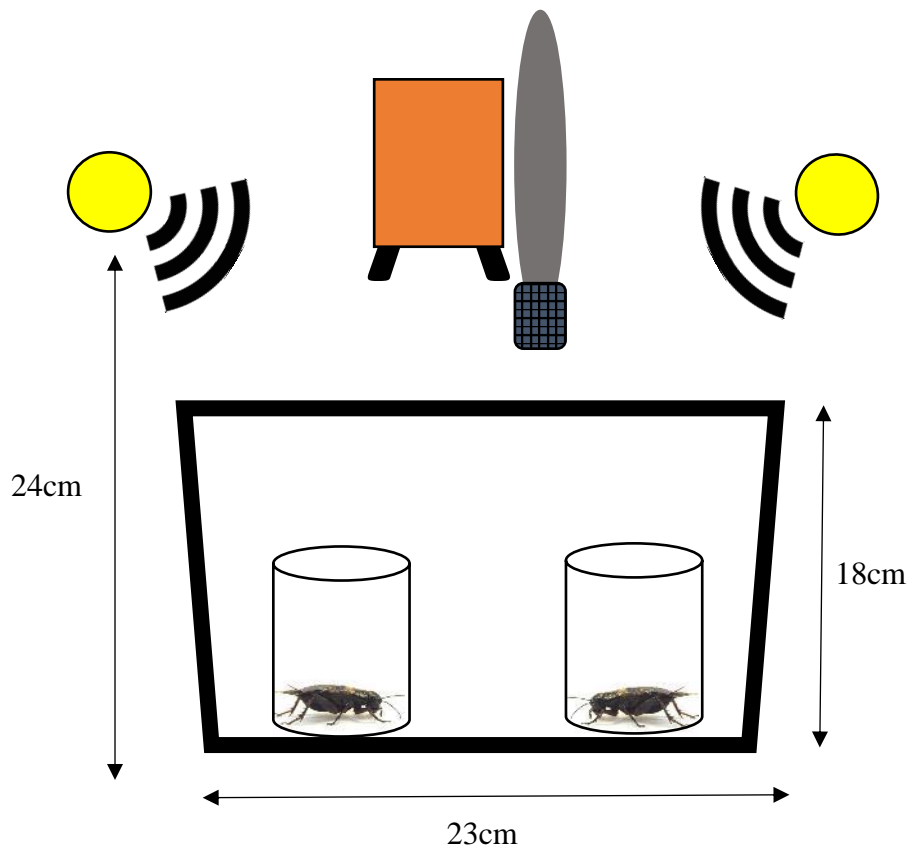


Figure 5.1. Lateral view of arena used to stage all aggressive contests. The large trapezoid represents the behavioural arena with Veho®360° capsule speakers on either side (circles). The camera (small rectangle) and microphone (oval) were positioned above.

difference is known to affect the outcome of aggressive encounters (Alexander, 1961). To distinguish between the two males, I marked both with a different non-toxic coloured insect paint (Natural History Book Service, Devon, U.K.), which was applied to the pronotum. I then placed each male at a different side of the arena, under a transparent 5.5cm diameter plastic cup, and left them to acclimatize for 5 minutes. After acclimatization, I started interactions by releasing the males and initiating acoustic playback simultaneously. No external aggressive prompting was necessary as this species is highly aggressive towards same-sex competitors (Alexander, 1961). I ended the encounter after 15 minutes following the start of the interaction or after a clear dominant individual was established (after the dominate male signalled, and the subordinate male continuously retreated). I also conducted control trials where males were subjected to the

same protocol as in the normal trials, but I did not present them with another male in the arena, thus removing any fighting behaviours. After the individual was released, I allowed the males to explore the arena for 5 minutes, at which point the trial ended. These trials were interspersed between normal trials.

Directly following the end of each trial, I humanely euthanized all males (including both the dominant and subordinate individuals) by placing them into 7.0ml plastic tubes and submersing them in liquid nitrogen. These samples were then stored at -80°C for subsequent analysis of haemolymph glucose and lactic acid concentrations. Prior to starting further behavioural trials, I cleaned the arena with 70% ethanol to remove any potential olfactory cues.

Behavioural and Acoustic Analyses

I conducted behavioural analysis on the recorded video footage through the event coding software BORIS (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). I adapted categories of agonistic behaviours from Adamo and Hoy (1995) and paired them with Sakura and Aonuma's (2013) method for measuring the level of aggression in an encounter (Table 5.1). I assigned each individual an aggression level based on the highest behaviour they exhibited, as well as an interaction outcome (dominant or subordinate), whilst also giving the encounter an aggression level based on the behaviour that both competitors showed (i.e. the highest behaviour level of the subordinate individual). I also coded each individual's aggressive behaviour to include a count of distinct behavioural occurrences and overall duration of these behaviours. Other behaviours, such as non-aggressive signalling and behavioural latencies, were also event logged.

I conducted acoustic analysis on the aggressive songs of the dominant individuals. Using Audacity 2.1.2 (<http://www.audacityteam.org/>, last accessed 7th March 2019), I cut audio files to where the singing occurred and then ran them through the program's noise reduction function twice. I analysed these songs for their temporal characteristic, resulting in

Table 5.1. Aggressive behaviours, description, and their associated level based on behavioural categories by Adamo and Hoy (1995) and aggressive levelling by Sakura and Aonuma (2013).

Name	Description	Aggressive level
Withdraw	Avoidance of opponent/ no aggressive behaviours	0
Antennal fencing	Rapid antennation of opponent's antenna	1
Judder	Short but rapid body rocking	2
Threat posture	Raises itself on its forelegs	3
Aggressive song	Stridulates aggressive song (long chirps)	4
Mandible flare	Hyperextension of mandibles	5
Mandible engagement	Butt heads with opponent/or interlock mandibles	6

a mean number of syllables per chirp, mean chirp duration, mean number of chirps per bout, and mean inter-chirp interval (the time between the end of one chirp and the start of the next within a bout) for each dominant song.

Additionally, I calculated the mean dominant frequency of each aggressive song using the function 'dfreq' (20% threshold) in the R package 'seewave' (Sueur, Aubin and Simonis, 2008; Rstudio Team, 2016; R Development Core Team, 2017).

Physiological Assays

To conduct physiological analysis, I defrosted each sample at room temperature for 20 minutes, after which I removed a single middle leg at the trochanter/thorax joint to extract a small (2 to 10 μ l) sample of haemolymph. I thoroughly mixed this sample with Biosen Glucose/Lactate System Solution at a ratio of 1:50. Within 2 hours of this, I ran the mixed sample through a Biosen C-line glucose and lactate analyzer (EKF Industrie, Elektronik GmbH, Barleben, Germany) to detect glucose and lactic acid concentrations (between 0.5 mmol L⁻¹ and 40 mmol L⁻¹).

Statistical Analyses

I conducted all statistical analyses in the statistical package R studio (Rstudio Team, 2016; R Development Core Team, 2017) with the package 'dunn.test' (Dinno, 2015). Where necessary, I tested data for normality using a Shapiro-Wilk test, and non-parametric tests were used when appropriate. (Harrell Jr., 2006). Where I used GLZMs, I also visually assessed plots of residuals vs. fitted values to ensure that models fit the data well, and that the data did not violate the model assumptions. All graphs and plots were created using base R and with the package 'ggplot2' (Wickham, 2016).

I used a generalized linear model (GLZM) with a binary logistic function and Two-way Chi-squared tests to test for differences in successful encounters (dominance established) between acoustic conditions, and differences in individual and encounter aggressive level between acoustic conditions. I also used these tests to analyse differences between the relative size, weight and age of dominant males between acoustic conditions. I used

Kruskal-Wallis tests to analyse differences in trial duration between acoustic conditions and in movement latency between control acoustic trials.

To test for differences in the occurrence of each level of aggressive behaviour between acoustic conditions, I conducted Binary GLZMs. Furthermore, I used Kruskal-Wallis tests to test for differences in the intensity (total duration) of these behaviours between acoustic conditions. Tests were also extended to look at differences in the occurrence and duration of calling and courtship songs prior to, or during, the encounter between acoustic conditions.

I used Kruskal-Wallis test to test for differences between aggressive song characteristics and acoustic conditions. The characteristics that I tested were song duration, average syllable rate (number of syllables per chirp), average chirp duration, average chirp rate (number of chirps per pulse) average inter-chirp duration (length of time between each chirp) and average dominant frequency.

Where I found no difference in the occurrence or intensity of behaviours between acoustic conditions, I pooled these data to boost statistical power in analyses for the effect of these behaviours on haemolymph glucose and lactic acid concentrations. I tested differences in glucose and lactic acid concentrations based on male weight in control conditions using linear regression analyses if (i) data met parametric criteria or (ii) did so when log transformed. Where data did not meet parametric criteria, I instead conducted GLZMs using a gamma error structure and a log-link function (as data were right-skewed; Fitzsimmons and Bertram, 2013; Mowles and Jepson, 2015). I also used Kruskal-Wallis tests to analyse differences in these metabolite concentrations between acoustic conditions and control acoustic conditions, and conducted Dunn's post-hoc tests when I found a significant result. Additionally, I used Mann-Whitney U tests to analyse the difference in glucose and lactic acid concentrations between individuals that fought and those that did not, and subordinate and dominant individuals. To test for relationships between glucose or lactic acid concentrations and the occurrence or intensity (duration) of each aggressive behaviour, I used GLZMs with gamma error structure and a log-link

function, linear regressions and Mann-Whitney U tests. Finally, I analysed the relationship between acoustic characteristic and glucose or lactic acid concentrations using linear regressions, with normal or log transformed data where necessary.

Results

Encounter Outcome

Acoustic conditions had no effect on whether or not encounters ended with dominance established through the production of an aggressive song (GLZM(b): Wald $X^2_2 = 2.26$, $N = 60$, $P = 0.32$). The acoustic condition also had no effect on the overall encounter's aggression level (two-way Chi-squared: $X^2_{10} = 6.23$, $N = 60$, $P = 0.8$) or on the individual's aggression level (two-way Chi-squared: $X^2_{12} = 14.68$, $N = 120$, $P = 0.26$). The relative size (GLZM(b): Wald $X^2_2 = 0.05$, $N = 52$, $P = 0.97$), weight (two-way Chi-squared: $X^2_4 = 0.67$, $N = 52$, $P = 0.95$) and age of dominant individuals (two-way Chi-squared: $X^2_4 = 5.39$, $N = 52$, $P = 0.25$) did not differ between the acoustic condition. Additionally, the acoustic condition did not affect either movement latency (Kruskal–Wallis: $X^2_2 = 2.43$, $n_1 = 20$, $n_2 = 20$, $n_3 = 20$, $P = 0.3$) or the duration of trials where dominance was established (Kruskal–Wallis: $X^2_2 = 0.7$, $n_1 = 17$, $n_2 = 19$, $n_3 = 16$, $P = 0.71$).

Occurrence and Duration of Aggressive Behaviours

The acoustic condition was shown not to affect the occurrence of non-acoustic aggressive behaviours (Table 5.2). Furthermore, I found no difference in the aggressive intensity (total duration) of these behaviours between acoustic conditions (Table 5.3). Additionally, the occurrence of calling songs (GLZM(b): Wald $X^2_2 = 1.46$, $N = 120$, $P = 0.48$) and courtship songs (GLZM(b): Wald $X^2_2 = 1.79$, $N = 120$, $P = 0.41$), as well as their duration (calling song Kruskal–Wallis: $X^2_2 = 0.06$, $n_1 = 5$, $n_2 = 2$, $n_3 = 4$, $P = 0.97$; courtship song Kruskal–Wallis: $X^2_2 = 0.27$, $n_1 = 5$, $n_2 = 3$, $n_3 = 7$, $P = 0.87$), were not affected by the acoustic condition.

Table 5.2. Output from GLZMs with binary logistic function, including test statistic, sample size, and P-value, for differences in the occurrence of aggressive behaviours between acoustic conditions.

	Wald X^2_2	N	P
Antennation	0.39	120	0.82
Juddering	0.41	120	0.81
Threat posture	2.14	120	0.34
Mandible flares	1.58	120	0.45
Mandible engagement	1	120	0.61

Table 5.3. Output for Kruskal-Wallis tests on differences in the total duration (seconds) of each aggressive behaviour between acoustic conditions. A n , T n , and W n show the sample size for each acoustic condition (ambient, traffic and white, respectively) and the population means and standard error of the mean are also shown.

	X^2_2	A n	\bar{x}	S.E.	T n	\bar{x}	S.E.	W n	\bar{x}	S.E.	P
Antennation	1.64	30	3.433	0.483	32	3.275	0.427	32	4.021	0.549	0.44
Threat posture	1.88	24	2.107	0.353	26	1.89	0.299	30	3.408	0.701	0.39
Mandible flares	5.14	24	2.124	0.364	28	2.065	0.318	29	3.816	0.755	0.08
Mandible engagement	1	12	0.388	0.142	14	0.26	0.074	11	0.32	0.085	0.61

Acoustic Differences

Acoustic condition did not affect any of the measured aggressive song characteristics (Table 5.4).

Haemolymph Analyses

Haemolymph glucose and lactic acid concentrations were not correlated with male weight (Table 5.5). Control acoustic conditions affected glucose concentrations (Kruskal–Wallis: $X^2_2 = 7.52$, $n_1 = 10$, $n_2 = 6$, $n_3 = 10$, $P = 0.02$, Figure 5.2). Post hoc tests revealed this significance lay between traffic and white noise conditions ($N = 16$, $P = 0.02$), but not between traffic and ambient noise conditions ($N = 16$, $P = 0.67$) or ambient and white noise conditions ($N = 20$, $P = 0.06$). However, lactic acid concentrations were not affected by control acoustic conditions (Kruskal–Wallis: $X^2_2 = 1.16$, $n_1 = 9$, $n_2 = 7$, $n_3 = 9$, $P = 0.56$). In aggressive trials, males that were not involved in aggressive combat (i.e. neither individual showed any aggressive behaviour) had reduced glucose concentrations, compared to those that were involved in aggressive combat (Table 5.6, Figure 5.3). However, no evidence was found of other relationships between glucose or lactic acid concentrations and the occurrence or aggressive intensity (total duration) of aggressive behaviours (Table 5.6, Table 5.7, Table 5.8, Table 5.9).

Table 5.4. Output from Kruskal-Wallis tests on differences between song characteristics between acoustic conditions. A_n , T_n , and W_n show the sample size for each acoustic condition (ambient, traffic and white noise, respectively) and the population means and standard error of the mean are also shown.

	X^2_2	A_n	\bar{x}	S.E.	T_n	\bar{x}	S.E.	W_n	\bar{x}	S.E.	P
Song duration (s)	4.32	17	5.391	1.669	19	2.91	0.607	16	5.86	1.721	0.12
Syllable rate (syllables per chirp)	3.06	17	3.344	0.577	19	3.661	5.883	16	2.409	0.469	0.22
Chirp duration (s)	0.15	17	0.125	0.022	19	0.118	0.029	16	0.103	0.021	0.93
Chirp rate (chirps per bout)	0.84	17	2.452	0.53	19	2.667	0.567	16	3.603	0.903	0.66
Inter-chirp duration (s)	0.9	17	0.134	0.024	19	0.145	0.027	16	0.127	0.026	0.64
Mean dominant frequency (kHz)	4.378	20	4.921	0.090	18	4.845	0.071	15	5.032	0.045	0.11

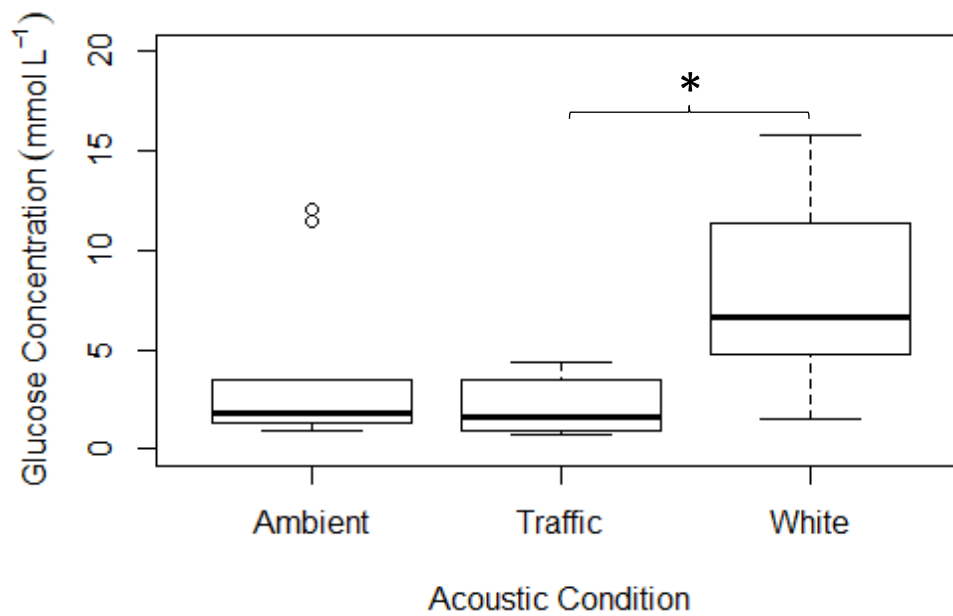


Figure 5.2. Differences in haemolymph glucose concentration between acoustic conditions in control trials. Brackets with an asterisk show a significant result from pairwise analysis. The central line shows the sample median, with box edges and whiskers showing the interquartile and full range (excluding outliers), respectively. Data which were identified as outliers by the function `boxplot()` in R are shown as o.

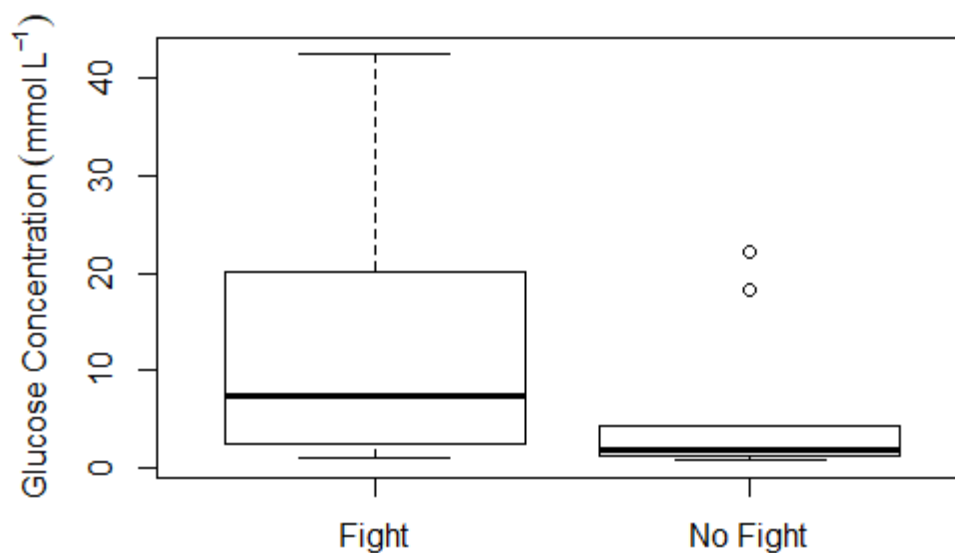


Figure 5.3. Differences in haemolymph glucose concentration in males that were part of aggressive encounters (at least one male showed aggressive behaviours) and those that were not (neither participant showed aggressive behaviours). Data which were identified as outliers by the function `boxplot()` in R are shown as o.

Table 5.5. Output from linear regressions and a gamma GLZM detailing the relationship between male weight and glucose or lactic acid concentrations for each acoustic condition.

	Model	y	df	F	P	r ²
Ambient						
Glucose	Gamma GLZM	-	1,8	2.01	0.19	-
Lactic acid	Log regression	-0.39*weight+0.71	1,7	0.42	0.54	0.06
Traffic						
Glucose	Regression	1.62*weight+0.84	1,4	0.16	0.71	0.04
Lactic acid	Log regression	0.92*weight-0.31	1,5	2.33	0.19	0.32
White						
Glucose	Regression	20.41*weight-7.09	1,8	1.54	0.25	0.16
Lactic acid	Regression	2.86*weight-0.07	1,7	1.99	0.2	0.22

Table 5.6. Output for Mann-Whitney U tests on the relationship between the occurrence of certain aggressive behaviours and haemolymph glucose concentrations (mmol L^{-1}). Means and standard error of the mean are shown for individuals that performed (n_1) and did not perform (n_2) that certain behaviour. Significant results are highlighted in bold.

	U	n_1	\bar{x}	S.E.	n_2	\bar{x}	S.E.	P
Fight Participation	355	48	11.53	1.53	10	5.61	2.49	0.02
Role	283.1	21	11.77	2.5	27	11.34	1.93	1
Antennation	109	43	11.53	1.63	5	11.52	4.87	0.97
Judder	166.5	9	8.97	2.46	39	12.12	1.79	0.82
Threat Posture	178	38	11.1	1.65	10	13.15	3.91	0.77
Mandible Flare	178	38	11.1	1.65	10	13.15	3.91	0.77
Mandible Engagement	232.5	24	10.05	2.22	24	13	2.1	0.26
Aggressive Song	290.5	24	11.29	2.22	24	11.76	2.14	0.97

Table 5.7. Output for Mann-Whitney U tests on the relationship between the occurrence of certain aggressive behaviours and haemolymph lactic acid concentrations (mmol L^{-1}). Means and standard error of the mean are shown for individuals that performed (n_1) and did not perform (n_2) that certain behaviour.

	U	n_1	\bar{x}	S.E.	n_2	\bar{x}	S.E.	P
Fight Participation	194	46	3.26	0.75	10	2.83	0.37	0.45
Role	263.5	19	2.6	0.28	27	3.73	1.27	0.88
Antennation	52	41	3.26	0.84	5	3.31	0.53	0.08
Judder	171	7	2.89	0.44	39	3.33	0.89	0.3
Threat Posture	124	37	2.52	0.2	9	6.32	3.77	0.24
Mandible Flare	124	37	2.52	0.2	9	6.32	3.77	0.24
Mandible Engagement	200.5	24	2.32	0.18	22	4.3	1.55	0.17
Aggressive Song	258.5	22	2.57	0.25	24	3.9	1.43	0.91

Table 5.8. Output from log transformed linear regressions and gamma GLZMs on the relationship between aggressive behaviours and haemolymph glucose or lactic acid concentrations.

	Test	y	df	F	P	r ²
Antennation						
Glucose	Gamma GLZM	-	1,41	0.23	0.64	-
Lactic acid	Gamma GLZM	-	1,39	2.28	0.14	-
Threat posture						
Glucose	Log regression	0.02*Threat duration+0.76	1,35	0.22	0.65	0.01
Lactic acid	Log regression	-0.01*Threat duration+0.42	1,34	0.57	0.46	0.02
Mandible flare						
Glucose	Log regression	0.02*Flare duration+0.75	1,35	0.33	0.57	0.01
Lactic acid	Log regression	-0.01*Flare duration+0.4	1,34	0.23	0.63	0.01
Mandible engagement						
Glucose	Log regression	-0.05*Engagement duration+0.82	1,22	0.21	0.65	0.01
Lactic acid	Log regression	-0.01*Engagement duration+0.35	1,22	0.06	0.81	0.003
Aggressive song						
Glucose	Log regression	-0.002*Song duration+0.83	1,22	0.02	0.88	0.001
Lactic acid	Log regression	0.003*Song duration+0.34	1,20	0.06	0.46	0.03

Table 5.9. Output from log transformed linear regressions on the relationship between aggressive acoustic characteristics and haemolymph glucose or lactic acid concentrations.

	y	df	F	P	r ²
Syllable rate					
Glucose	0.08*Syllable rate+0.42	1,20	2.08	0.16	0.09
Lactic acid	-0.01*Syllable rate+0.46	1,18	0.31	0.58	0.02
Chirp duration					
Glucose	1.7*Chirp duartion+0.5	1,20	1.54	0.23	0.07
Lactic acid	-0.57*Chirp duartion+0.5	1,18	0.31	0.35	0.05
Chirp rate					
Glucose	-0.03*Chirp rate+1.03	1,20	2.04	0.17	0.09
Lactic acid	0.01*Chirp rate+0.33	1,18	0.71	0.41	0.04
Inter-chirp duration					
Glucose	-0.92*Inter-chirp duration +1.1	1,18	0.28	0.6	0.02
Lactic acid	-0.35*Inter-chirp duration+0.47	1,17	0.21	0.65	0.01
Average Frequency					
Glucose	0.09*Average Frequency+0.46	1,18	0.04	0.84	0.05
Lactic acid	-0.08*Average Frequency+0.78	1,16	0.14	0.71	0.05

Discussion

This chapter has highlighted a lack of differences found between individuals that signalled aggressively under different acoustic conditions, both in terms of behavioural differences and differences in haemolymph metabolites.

These results contrast with the differences observed when acoustic condition conflicts with the other acoustic sexual signals in *G. bimaculatus* (Chapter 2, 3, 4). This may result from a number of different factors, including the amplitude of the aggressive signal, its function, and the use of multimodal communication.

Aggressive encounters did not differ significantly in whether or not dominance was established or the level of aggression reached in the encounter, depending on which acoustic condition the encounter was staged under. Furthermore, duration and occurrence of aggressive behaviours, as well as individual level of aggression, were not observed to differ between acoustic conditions either. Differences in the acoustic characteristics of the aggressive song of dominant individuals were also not found between these acoustic conditions. These are interesting results as I have shown in previous chapters that unfavourable acoustic conditions do have the ability to disrupt other acoustic signals in this species (Chapter 2, 3 and 4). Thus, these findings highlight a number of potential factors that may result in an acoustic signalling system that is stable under conditions of anthropogenic noise. Firstly, as aggressive acoustic signals in *G. bimaculatus* are produced at the end of, or sometimes mid-way through, aggressive encounters (Adamo and Hoy, 1995), they are assumed to function as either a browbeating signal or an advertisement signal (Rook, Fitzsimmons and Bertram, 2010). Evidence for the function of this victory display as an advertisement signal can be found throughout Gryllidae. For example, male *G. veletis* and *G. bimaculatus* alter the intensity of their aggressive encounters and rate of victory displays based on the composition of their audience (Tachon et al., 1999; Fitzsimmons and Bertram, 2013). Furthermore, in *Acheta domesticus*, correlations were found between aggressive song and male condition (information which is often used to make mate choice decisions; Bertram et al., 2011), but not between

aggressive song and motivation to fight (Brown et al., 2006). These results, coupled with the result of the present study, may highlight that the aggressive acoustic signals performed in *G. bimaculatus* function more as an advertisement signal to potential mates, and less as a signal to deter further aggression.

Additionally, males may be using other signal modalities to inform their competitor. Unlike other acoustic signals produced by *Gryllus* individuals, aggressive acoustic signals are part of a larger, multimodal repertoire, and these signals may be enough to inform competitive conspecifics. Partan (2017) has shown that many different animal species switch to different signal modalities or pay more attention to other aspects of a multimodal signal when acoustic communication is disrupted by anthropogenic noise sources. Furthermore, individuals that have either a reduced or complete loss of signal have been shown, both here and in *Teleogryllus oceanicus* (Gray et al., 2014), that they do not increase the intensity of their non-acoustic aggressive behaviours, which further suggests that enough information may be acquired from the tactile (non-acoustic) behaviours alone. Female *A. domestica* are known to use tactile signals when selecting a mate (Stoffer and Walker, 2012), and female *G. bimaculatus*, which are unable to stridulate, are capable of engaging and resolving in aggressive interactions (Delago and Aonuma, 2006), confirming that crickets attend to non-acoustic signals from conspecifics. Furthermore, acoustic signalling is known to be the least energetically costly behaviour when compared to other aggressive behaviours (Hack, 1997). This suggests that competitors can determine more about a signaller's energetic reserves or stamina (and thus RHP) from attending to these other aggressive behaviours.

However, a study on *Teleogryllus* individuals that have lost their ability to signal has shown that these individuals reach higher levels of aggression than in encounters where at least one individual can stridulate (Logue et al., 2010). This is evidence against signal function or multimodal signals being the factors that lead to this signalling system remaining robust under unfavourable noise conditions. Alternatively then, the lack of effect

from anthropogenic noise seen in this present study could simply result from a higher signal-to-noise ratio. The aggressive song produced by *G. bimaculatus* is comparable in acoustic structure to the calling song of the species, which is known to be disrupted by anthropogenic noise (Chapter 2), but the aggressive song is produced at a higher amplitude (Alexander, 1962) and performed in close vicinity rather than at long distances. Increasing signal amplitude in order to be heard over disruptive noise, sometimes known as the Lombard effect, is a tactic used by many species (Cynx et al., 1998; Brumm, 2004; Brumm et al., 2004; Holt and Johnston, 2014). Potentially then, the amplitude of the aggressive songs produced in the present study were loud enough to reach the threshold of hearing under anthropogenic noise conditions. In previous chapters (Chapter 2 and 3), I have shown that the amplitude of noise is not enough to disrupt a signalling system alone, but understanding the amplitude of noise necessary to disrupt these aggressive signals is beyond the capabilities of the methods used here.

In the analysis of haemolymph metabolites, I found few differences between acoustic conditions, behavioural occurrences and behavioural intensities. However, a result was detected between acoustic conditions in control trials (where no aggressive behaviour was displayed) suggesting that the acoustic condition may have an effect on physiological processes. Specifically, the concentration of circulating glucose was increased under white noise conditions, suggesting that individuals may be mobilising sugars for use under these conditions (as glucose is not used as a storage molecule in this species; Chapter 3; Wyatt and Kalf, 1957; Nowosielski and Patton, 1964). Interestingly, these results differ from the control trials in a previous chapter (which were staged in a similar manner) where an increase in glucose concentrations was found under ambient noise conditions (Chapter 3). The main difference in protocol between these two control trials was the acclimation time (the amount of time individuals were left in the arena prior to the trial). In the present study, I left individuals for five minutes, whereas in the previous study, I left individuals for a minimum of sixteen hours (overnight). These results indicate that animals may act differently towards acoustic stimuli depending on how acclimated they are

to their environment. The other finding related to haemolymph glucose concentration shows an increase in this metabolite when individuals are involved in aggressive combat (when either individual in an encounter showed an aggressive behaviour). This indicates that individuals that are fighting, or receiving aggressive behaviours, are mobilising sugars to better fight, or evade, the competing individual. Similar differences in metabolism between fighting and non-fighting animals can be seen using oxygen consumption (Hack, 1997; Castro et al., 2006), circulating metal ions (Mowles et al., 2008), and haemolymph glucose concentrations in hermit crabs (Briffa and Elwood, 2004). However, this was the only aggressive behaviour that was found to be linked to either glucose or lactic acid concentrations. Aggressive and acoustic behaviours are known to be energetically costly in crickets (Hack, 1997; Bertram et al., 2011; Mowles, 2014; Houslay et al., 2017), meaning a difference in glucose and/or lactic acid should be detected based on the intensity of the aggressive behaviours. This discrepancy may be plainly due to reductions in sample size. The techniques I used to gather haemolymph lactic acid and glucose concentrations differed to those I used previously (Chapter 3) to increase sample size, and thus measurement reliability, which would enable more powerful statistical analysis. Still, it was unfortunate that I could not collect samples from all individuals reducing the statistical power of metabolite analysis. This may explain some of the discrepancies between this experiment, previous chapters, and other literature.

When acoustic signalling systems are not impacted by the presence of unfavourable acoustic conditions, behavioural responses are unlikely to differ from those normally observed within the species. However, anthropogenic noise may lead to other alterations in the life history of these animals. For example, the acoustic signals of many species are exploited by potential predators to locate them (Zuk and Kolluru, 1998), but their ability to do so may be disrupted by the presence of anthropogenic noises. If this is the case, then the extrinsic (indirect) costs of signalling may be reduced, whilst the signaller suffers no reduction in signal transmission, leading to a potential increase in individual survivability and population viability.

Furthermore, populations that are able to survive and reproduce under anthropogenic noise conditions have a large advantage over those that cannot, due to access to additional resources such as habitat (Cardoso, Hu and Francis, 2018). As levels of anthropogenic noise rise globally (see Chapter 1), additional habitats will be altered due to its presence, leading to further benefits to populations that can still function under these conditions. Alternatively, the presence of anthropogenic noise may have deleterious consequences due to the disruption it causes in (i) other acoustic signals produced in a species (Chapter 2, 3 and 4) or (ii) when perceiving environmental acoustic cues, such as those used for predator detection (Chan et al., 2010). Thus, when detailing the consequences of anthropogenic noise on a given species, the entirety of its life history should be taken into consideration.

Conclusions

I designed the experiment presented here to test for the deleterious consequences of anthropogenic noise when it disrupts the acoustic aggressive signals produced by male field crickets. However, I observed no behavioural or acoustic differences between different acoustic conditions, suggesting that no such disruption occurs. Instead, this chapter has shown the robustness of this particular acoustic signalling system when presented with anthropogenic noise conditions, and discussed potential reasons for how the system remains stable, especially in comparison to the disruptions observed to the calling (Chapter 2) and courtship song of this species (Chapter 3 and 4). Finally, analyses of circulating haemolymph revealed the effect both unfavourable acoustic conditions and agonistic interactions may have on the metabolism of individuals, but small sample sizes mean that the results should be interpreted with caution.

Chapter 6

General Discussions

The experiments presented in this thesis were set up to investigate the effect that anthropogenic noise has when conflicting with sexually selected acoustic signals, and the related behavioural and physiological consequences. Through observations of the signalling systems in *Gryllus bimaculatus*, I have here highlighted such deleterious consequences. The transmission, reception and perception of these signals, produced by male crickets, were shown to be affected by the presence of unfavourable acoustic conditions. Additionally, physiological and behavioural assays revealed potential trade-offs that might occur should males alter their acoustic signals in an attempt to maintain signal efficacy under these conditions. By using specific acoustic conditions in these experiments, I have been able to draw conclusions on the acoustic characteristics necessary to cause such a disruption. Yet, the results presented here also highlight a signalling system that is not affected by the levels of noise that I used in these experiments. I hope that these results can be applied to other animal populations, where they may aid in understanding the changes in behaviours presented or predict potential consequences that occur when animals, across taxa, signal acoustically under the conditions of anthropogenic noise.

Behavioural Differences

The ability of *G. bimaculatus* to produce (Chapter 3 and 4) and receive (Chapter 2 and 4) acoustic sexual signals were shown to be affected by the presence of anthropogenic noises, whilst a significant intra-sexual signalling system (male aggression) appears to remain unaffected (Chapter 5; Table 6.1). The first experimental chapter (Chapter 2) detailed female responsiveness to the calling song of conspecific males when under differing noise conditions. As female *G. bimaculatus* use the calling the song of males to locate them (Alexander, 1961), a reduction in the ability to do this (phonotaxis) could reduce fitness, of both males and females, as a result of impaired mate location. Thus, the finding that females were less

Table 6.1. Information on the acoustic noise stimuli (dark grey table) and sexual behaviours (light grey table) analysed throughout this thesis, with a summary of which noise conditions disrupted each acoustic signal (white table). Information includes approximations of the dominant frequency (kHz), average amplitude (dBA), and general aspects of the temporal structure of each acoustic stimuli.

				Noise Stimuli	Traffic Noise	White Noise
				Frequency	0.5 – 1.5	N/A
				Amplitude	79	79
				Structure	Fluctuating traits	Constant traits
Chapter	Behaviour Observed	Frequency	Amplitude	Structure	Disrupted Behaviour	
Chapter 2	Calling Song - Female reception	4.5 - 5.5	56	Repeated Chirps (made of 3 - 5 pulses)	Yes	No
Chapter 3	Courtship Song - Encounter outcome	13 - 18	46	Repeated singular pulses	No	Yes - Males only
Chapter 4	Courtship Song - Female perception				Yes	Yes
Chapter 5	Aggressive Song - Encounter outcome	4.5 - 5.5	65 - 75	Few long chirps (3 - 20 pulses)	No	No

likely to approach a speaker playing conspecific calling songs whilst under traffic noise conditions, compared to those under ambient noise conditions, shows that such a reduction in individual fitness is highly probable. This result is supported by studies conducted by other researchers (Schmidt, Morrison and Kunc, 2014). Additionally, I also noted a difference in behavioural latency, with individuals under traffic noise conditions making a ‘decision’ more quickly (linked to how soon they stopped attending the signal), compared to those under both ambient and white noise conditions. The connotations of this suggest that females may alter their perception of mate quality if they spend less time attending the calling stimulus; a concept that I further considered in a separate experimental chapter (Chapter 4).

The second and third data chapters presented in this thesis (Chapter 3 and 4) focus on acoustics used during courtship interactions in *G. bimaculatus*, and how this interaction may be disrupted by the presence of anthropogenic noise. As the courtship song in this species is necessary to initiate mounting (and thus required for mating; Adamo and Hoy, 1994), and it also details aspects of the male quality (Rantala and Kortet, 2003; Shestakov and Vedenina, 2015), then a disruption in the transmission, reception or perception of this signal could reduce the fitness of individuals involved in the interaction. The results in Chapter 3 detail the effects anthropogenic noise has on transmission, with males under white noise conditions less likely to signal than those under ambient noise conditions, resulting in a reduction of successful matings. Additionally, those individuals that did not signal displayed greater mate guarding behaviours, whilst the males that did signal under white noise conditions did so at a higher dominant frequency level and had associated reductions in other signal characteristics. The reduction in male signalling has an obvious consequence to individual fitness as the signal is necessary to elicit females to mount, and thus mate, whereas the behavioural and acoustic alterations show a level of flexibility, and associated trade-offs, in courtship interactions within this species. Chapter 4 detailed the affect anthropogenic noise has on signal perception, with females failing to differ in their latency to mount attractive and non-attractive males under both traffic and white

noise conditions. As mounting latency is used widely as a proxy for female mate choice (Shackleton, Jennions and Hunt, 2005; Kostarakos, Hartbauer and Römer, 2008; Shestakov and Vedenina, 2015; Loranger and Bertram, 2016), this results highlights the female's inability to differentiate between males of high or low quality. This could lead to non-optimal mating decisions (i.e. mating with a low quality male, or not mating with a high quality male), and thus reduction in fitness based on this.

The final data chapter (Chapter 5) was concerned with the acoustics used in agonistic encounters between male *G. bimaculatus*, and how these may also be disrupted when anthropogenic noise is present. The function of this signal, which is produced mainly by victorious males, is less clear than it is in the calling and courtship song. Likely it is used to reduce additional agonistic encounters (i.e. browbeating) or to signal fight outcome to nearby conspecifics (i.e. advertisement; Rook, Fitzsimmons and Bertram, 2010). Chapter 5 was concerned with the possibility of increased fighting behaviours under anthropogenic noise conditions due to reduced browbeating signals, either through compensated signals or repeated engagements. However, the results revealed no increase in aggressive behaviours between acoustic conditions. This may suggest that the signal does not function as a browbeating exercise, competitors attend to non-acoustic signals as an alternative, or that the noise conditions used were not sufficient (i.e. loud enough or at the right frequency band) to disrupt the signal. This is an important finding as it demonstrates the variability of signal robustness even within a species, and is further documentation of acoustic aggressive behaviours under anthropogenic noise conditions.

Physiological and Energetic Trade-offs

One of the themes of this thesis was to detail the costs, both intrinsic (direct) and extrinsic (indirect) costs, of anthropogenic noise when it causes disruptions, and thus behavioural alterations, in an acoustic sexual signalling system (Chapter 3 and 5). By measuring circulating metabolites, it is possible to detect metabolic and physiological differences in animals signalling under different conditions, and link this to the behaviours and

behavioural alterations observed. Sampling circulating metabolites is a common experimental practice both in vertebrates and invertebrates, and a variety of different metabolites can be measures, such as glucocorticoids (Blickley et al., 2012), lactic acid (Mowles, 2014), glucose (Matsumasa and Murai, 2005), carbon dioxide (through the sampling of doubly labelled water; Vehrencamp, Bradbury and Gibson, 1989), and even metal ions (Mowles et al., 2008).

In Chapter 3 and 5, I measured the concentrations of haemolymph glucose and lactic acid to analyse energy consumption and rates of anaerobic respiration. Additionally, I linked these results to the behavioural observations to better understand the costs of altering signalling behaviour. The results from Chapter 3 demonstrate this point, with findings that suggest that only individuals that have high metabolic capacities can perform at a higher rate (i.e. courtship duration, signal escalation, stridulation rate). Additionally, individuals that signalled under white noise conditions (the acoustic condition where most of the behavioural differences were observed) had increased the dominant frequency of their song, which was linked to depleted glucose concentrations and decreases in other signal characteristics. This is an indication that there may be a noise induced trade-off occurring here, with males altering their song to best combat the anthropogenic disturbance, but resulting in reduced courtship effort in other aspects. The results from this chapter are an example of the effect anthropogenic noise may have when individuals must alter their acoustic signals to ensure successful transmission or reception, and helps to frame the abundance of literature on acoustic alterations in a new light. In Chapter 5, metabolite analyses highlighted the effect fighting might have on physiology, with individuals that were part of aggressive encounters showing much higher concentrations of glucose than individuals that did not show or receive any aggressive signals. Additionally, differences in glucose concentrations in the control acoustic conditions reveal information on the importance of acclimation time when conducting behaviour trials, especially when compared control conditions in Chapter 3. Unfortunately, in both Chapter 3 and Chapter 5, results on the correlations between circulating

metabolites and behavioural activity were found to be contradictory to previous research (Hack, 1997; Bertram et al., 2011; Mowles, 2014; Houslay et al., 2017). As the protocol for metabolites analyses were newly developed, a discussion into the methods used to collect these data is necessary to identify elements of this particular assay and how they may have affected the outcome of the experiment.

To collect haemolymph samples, I froze individual crickets directly following behavioural observations by submersing them in liquid nitrogen, and then stored them at -80°C. This is common experimental practice in metabolite analyses (Taigen and Wells, 1985; Bertram et al., 2011; Mowles, 2014) as it prevents the samples from degrading until physiological analysis can occur. However, the introduction of ice crystal in the body may rupture the organs of the individual (Lee Jr., 1991), resulting in a diluted haemolymph sample when taken. When haemolymph samples were ready to be taken, I left individual crickets at room temperature to defrost for twenty minutes. I followed this period of time by removing a single middle leg from the defrosted cricket, which resulted in a small droplet of haemolymph forming that I could collect on a glass microscope cover slide and then measure for dilution to be used in the bio-analyser. Collecting haemolymph by making an breach of their internal cavity of the invertebrate is common practice (Harrison, Phillips and Gleeson, 1991; Rantala and Kortet, 2003; Mowles, 2014) as haemolymph is not restricted to blood vessels (like blood in vertebrates) but is freely flowing in the open cavity (Rapp, 1947). However, despite following this protocol strictly, several crickets did not yield enough, or any, haemolymph for analysis. This reduction in sample size leads to a decrease in statistical power, meaning any biological effect that is present may be undetectable. Levels of haemolymph lactic acid and glucose are both used as measures of metabolic activity, looking at anaerobic respiration rate and blood sugar mobilisation respectively (Taigen and Wells, 1985; Harrison, Phillips and Gleeson, 1991; Matsumasa and Murai, 2005; Mowles and Ord, 2012; Mowles, 2014). *G. bimaculatus* are known to utilise anaerobic pathways during signalling bouts (Mowles, 2014), but the presence of glucose may be interpreted both as a level of

activity and level of energy stores (Mowles and Ord, 2012). In most insects, sugars are stored in the form of the disaccharide trehalose (Wyatt and Kalf, 1957). When known concentrations of trehalose were run as a haemolymph proxy (following the same experimental protocol as the haemolymph), no glucose molecules were detected. Thus, absolute haemolymph blood sugar level was not detected, but results could still show depleted reserves or lack of sugar mobilisation.

To analyse haemolymph metabolites, I ran collected haemolymph samples through a Biosen C-line glucose and lactate analyzer (EKF Industrie, Elektronik GmbH, Barleben, Germany). To my knowledge, this is the first time this system has been used to analyse signalling metabolism in non-human animals, but it is widely used to measure circulating metabolites in athletes (Hanon et al., 2010; West et al., 2013; Burden et al., 2015) and in experiments concerned with dietetics (Brands et al., 2013; Lagerpusch et al., 2013; Nowotny et al., 2014). The machine functions using an enzymatic-amperometric method and chip-sensor technology to detect levels of glucose and lactic acid simultaneously. In other words, enzyme activity is measured in the solution by detecting changes in the electrical current due to ions. Thus, only glucose and lactic acid can bind with these specific enzymes, unless inhibitors are present in the sample, so the machine reading should always be correct. I mixed the haemolymph samples with standard system solution (Biosen Glucose/Lactate System Solution) which functions as both a haemolysing and stabilising agent. Thus samples should not differ based on how long it took to analyse them, although no sample was analysed over 3 hours from when it was first taken. Additionally, all blood cells are ruptured (mainly haemocytes, as insect haemolymph does not possess red blood cells; Rapp, 1947), releasing any metabolites into the solution.

In summary, reduced sample sizes were the deficiency in this protocol, with potentially diluted samples also a potential concern. However, the Biosen C-line glucose and lactate analyzer should, in theory, work perfectly to analyse the glucose and lactic acid concentrations of the samples presented, assuming the machine is maintained sufficiently. Thus,

drawing conclusions on the results of metabolite concentrations, whilst not unjustified, should be done with caution.

Disruptive Acoustic Characteristics

As I have shown in this thesis, unfavourable acoustic conditions have the ability to disrupt acoustic sexual signals, leading to potentially deleterious consequences for both signallers and receivers. However, these acoustic conditions can differ in a number of their attributes, such as duration, pressure levels (amplitude), frequency (pitch) and fluctuations in all of these traits (Table 6.1). Throughout the experimental chapters presented here, I have used different acoustic conditions to better understand what characteristics and aspects of the acoustic stimuli are necessary to disrupt the signalling systems I observed. Specifically, I used white noise as an alternative stimulus to the general anthropogenic noise used (road traffic noise) as they shared an average amplitude, but differed in the spread of frequency power and how attributes of the signal fluctuated. Differences, or lack thereof, in the response to these signals, highlighted the potential characteristics necessary for causing disruption.

In Chapter 2 and 3, the results showed a difference in behaviour to one acoustic condition but not the other, suggesting that it was a characteristic that differed between the acoustic stimuli that caused the disruption. Thus, either the frequency aspects of the stimulus or the amount of fluctuations in its characteristics are necessary to cause the behaviour disruptions I observed. Traffic noise, a stimulus that is low in dominant frequency, was shown to conflict with the calling song, a signal with a relatively low dominant frequency. Additionally, white noise, a stimulus with broadband frequency power, was shown to conflict with the courtship song, a signal with power in higher frequency bands. This seems to highlight that the frequency aspects of the acoustic condition seem to be the necessary characteristic for causing a disruption. This is supported with the observation that males would modulate the frequency of their signal (Chapter 3), likely to combat frequency masking. However, as both traffic and white noise share similar acoustic power at the level at which *G.*

bimaculatus individuals are highly tuned (4.5-5.5 kHz; Chapter 2; Popov, Markovich and Andjan, 1978), this suggests that stimuli fluctuations, or lack thereof, is the key characteristic necessary to cause the observed disruption. Alternatively, the differences I observed between traffic noise and white noise responses may be due to differences in male and female perception of the environment. The main behavioural differences in Chapter 2 was reduced female responsiveness, whereas in Chapter 3, the main finding was reduced male signalling behaviour. Additionally, where males show a reduction in signalling behaviour, females did not show a reduced response to any signals (Chapter 3). Males and females may be susceptible to different environmental acoustics, due to how they naturally respond to acoustic stimuli, leading to the differences in observed behaviour in these experiments.

In contrast to the results I presented in Chapter 2 and 3, the results in Chapter 4 show behavioural differences were observed for both presented acoustic conditions, compared to ambient noise conditions, suggesting that it is a shared trait that causes this behavioural difference. Amplitude is the main shared acoustic characteristic between the traffic noise and white noise stimuli I used in these experiments, as both are broadcast at an approximate average of 80dBA. The difference between this finding and those of Chapter 2 and 3 may detail the variety of ways acoustic stimuli can interfere with acoustic signalling systems. For example, frequency masking or disruptions based on fluctuations may reduce signal detection, and thus reduced signalling behaviour or response (Chapter 2 and 3), whereas stimulus amplitude may alter signal perception, leading to alterations to a response rather than a complete reduction (i.e. maintain behavioural success, but alter latency; Chapter 4). Finally, in Chapter 5, I observed no behavioural differences, meaning no conclusions could be drawn on important acoustic aspects. However, as the acoustic stimuli used were broadcast at the same average amplitude, we cannot conclude that amplitude is not important in conflicting with this signal. Rather, we can consider what acoustic alteration would be necessary to elicit a behaviour response. As traffic noise and white noise already vary in their frequency power and acoustic dynamics, it is

more likely that their shared characteristic would need to be altered (i.e. increase stimulus amplitude) to observe a behavioural response. If this did indeed elicit a response, then, again, different responses between acoustic conditions would yield information on what other acoustic aspects are necessary for causing a disruption.

Understanding what aspects of an acoustic stimulus are required to disrupt acoustic signalling systems can highlight the process by which the signal is disrupted, especially when paired with signal function. Naguib (2013) noted multiple different ways that environmental noise may conflict with animal communication systems. These included signal masking, disrupted attentional capabilities, and basic stress/startle responses. As masking works mostly in similar frequency bands (supported by the documented cases of altering signal frequency to increase signalling success; Chapter 3; Cunningham and Fahrig, 2010; Potvin, Mulder and Parris, 2014), this is assumed to be the factor affecting signal detection (Chapter 2), and thus choice to signal (Chapter 3 and 4). However, attentional issues are more likely to be the factor affecting the behavioural differences I observed in Chapter 4, as I found both acoustic conditions show this difference and signal detection was not affected (encounters were still successful). Additionally, as quality perception of acoustic signals often requires sustained attention to the signal (Mowles and Ord, 2012), then attentional issues would disrupt this, leading to reduced responsiveness to otherwise very attractive signals. If anthropogenic noise conflicts with acoustic communication due to multiple conflicting factors, as it does appear to do here, then it only boosts the disruptive power of this noise stimulus.

Dealing with the Problem

This thesis was produced in response to the global increases in anthropogenic noise, which I have here shown may have deleterious consequences when conflicting with sexually selected acoustic communication. Thus, it would be negligent to not discuss the potential strategies for reducing the impact that this human generated selection pressure is having on animal populations around the globe. Most research

regarding this topic is predominately concerned with human health and welfare, but their results can be extended to consider how they might aid in protecting non-human animals as well. Whilst individual choice can greatly impact noise production, responsibility should ultimately fall to governing bodies, to enforce environmental legislation, and companies, to be aware, and make consumers aware, of the noise produced by their products or services.

Remove

As I discussed in Chapter 1, anthropogenic noise is particularly widespread partly due to the sheer number and diversity of its sources. Thus, a logical response would be to attempt to reduce the number of these noise sources. As transport noise is a large contributor to environmental noise, a reduction in the use of certain vehicles could see a significant reduction in environmental noise. For example, private car use could be swapped for public transport or energetic travel, such as cycling or walking, which would reduce the amount of car traffic on our roads, and thus the noise produced by them (Nieuwenhuijsen and Khreis, 2016), although encouraging this change in behaviour is known to be difficult (Tertoolen, Van Kreveld and Verstraten, 1998). Similarly, utilising different strategies for production may lessen the extent to which noise is produced. For example, energy production through deep sea oil extraction processes is known to produce high levels of anthropogenic noise (Greene, 1987). These could be switched to greener methods of energy production, such as solar panels, which likely produce far diminished environmental impacts, including noise production (although noise produced by wind turbines are known to affect animal behaviour; Smith et al., 2016; Whalen et al., 2018). The strategy of removing noise sources from an environment will be, by far, the best way to cut down on anthropogenic noise. However, it would be impossible, and also unethical, to completely remove all source of, for example, transportation noise and production noise. Thus, it is necessary to discuss other potential methods of noise reduction.

Reduce

If removal of a noise source is not possible, then the next alternative precaution should be to reduce the noise that is produced by these sources. This is by far the most researched and discussed category for diminishing the output of anthropogenic noise, and has been topic of discussion for over 60 year (Apps, 1955). For example, advances in the field of road construction include the introduction of “quiet pavements”; specially designed asphalt, or other materials, which reduce the noise produced from pavement-tyre interactions (Praticò and Anfosso-Lédée, 2012). Similarly, technologies in car production now allow for cars that produce less engine noise, such as electric or hybrid engines (Iversen, Marbjerg and Bendtsen, 2013), although safety concerns have led the discussion of re-introducing noise to these vehicles (Sandberg, Goubert and Mioduszewski, 2010). Furthermore, mechanical efficiency should be taken into greater consideration, as these acoustics are often an unwanted by-product of machine operation (Shaw, 1975). Working on system efficiency has a twofold benefit, in that less energy, or fuel, is required for the desired level of response, and unwanted noise produced by the system is decreased. In essence, by altering certain aspects of the noise producers currently present in the urban environment, it is possible to greatly reduce the noise produced by them.

Protect

Finally, if removal or reduction of noise sources is not possible, then the focus should shift to protecting areas from the influence of these unwanted acoustics. Unlike with humans, where hearing protectors can limit the effect of noise at the level of each individual, it would be impossible to control the level of noise exposure for each individual organism. Thus, protection from noise needs to function at a larger scale (i.e. protection for a whole habitat). Examples of this type of strategy include environmental noise barriers (Kotzen and English, 2009) and specialised urban planning (Ariza-Villaverde, Jiménez-Hornero and Gutiérrez De Ravé, 2014), which utilise aspects of architectural design to dampen and redirect noise away from certain areas. While effective, these strategies may lead to other deleterious

consequences such as dividing up habitats, disrupting microclimates and altering run-off and water flow (Arenas, 2008). Additionally, tree belts and areas of vegetation are utilised to reduce environmental noise expanding far from its source (Fang and Ling, 2005; Ow and Ghosh, 2017), but these are small and, likely, unsustainable ecosystems that are even more uninhabitable due to the levels of noise they are subject to. Ultimately, strategies of noise protection, whilst sometimes effective, can have negative effects too, so ideally should only be considered when removal or reduction of the noise source cannot be accomplished.

Future Work

The results presented in this thesis clearly reveal the potential deleterious consequences of anthropogenic noise when interfering with the transmission, reception and perception of sexually selected acoustic signals. Furthermore, I also discussed the acoustic characteristic that were important to cause this disruption. However, I have not directly observed how these unwanted acoustic stimuli are perceived, and thus how this impacts the perception of desired acoustic signal. Through intracellular recordings of identified interneurons, researchers have been able to directly observe the perception of acoustic stimuli in orthopteran species (Popov, Markovich and Andjan, 1978; Stout et al., 1998; Korsunovskaya and Zhantiev, 2007; Kostarakos and Romer, 2015; Schöneich, Kostarakos and Hedwig, 2015), and then compare it to observed behavioural response (Samuel et al., 2013). By utilising these techniques, it would be possible to identify the neuronal responses to different types of noise, as well as neuronal responses to species specific songs when in the presence of anthropogenic noise. Results from such an experiment would help to further explain whether the results presented in this thesis are due to disruptive mechanisms, such as masking or distraction, or due to adaptive behavioural responses (i.e. startle responses).

The experiments I present in this thesis are mostly concerned with the instantaneous effects of anthropogenic noise interfering with acoustic signalling systems. However, developmental experience of these noise conditions is also likely to affect adult sexual behaviour. For example, a

recent study highlighted the impact of anthropogenic noise on developing female field crickets, *Teleogryllus oceanicus*, showing that they had a reduced phonotactic response at maturity when raised under high traffic noise conditions (Gurule-Small and Tinghitella, 2018). Male field crickets are known to sample the acoustic environment to alter plastic phenotypic traits during development, and thus have a behavioural advantage when mature (Bailey, Gray and Zuk, 2010). Thus, it is plausible that the presence of disrupting noise conditions could alter the juvenile male's perception of the acoustic environment, and alter their development negatively. The combination of negative developmental consequences in both males and females may lead to reductions in population viability, if evolutionary adaptations are not first reached. Thus, a longitudinal study design, observing behavioural and developmental differences over more than one generation, would help to improve our understanding of the long term effects of maintaining a signalling system under conditions of anthropogenic noise.

Finally, another subset of individuals that attend these sexual signals, besides juvenile and mature conspecifics, are potential predators. In many species, predators, both micropredators (parasites) and macropredators, utilise sexual signals produced by potential prey animals in order to hunt (locate) them (Zuk and Kolluru, 1998). This is an important aspect of sexual selection as the predator acts as an extrinsic (indirect) cost of the sexual signalling system. If location of prey stimuli through acoustic cues is disrupted due to the presence of anthropogenic noise, similar to that shown in mate searching behaviours (Chapter 2), then predation may be reduced when signalling in unfavourable noise conditions, acting as a benefit to the signalling individual. Thus, studying the behaviour of predators that eavesdrop on acoustic signals under anthropogenic noise conditions will further highlight the costs faced by individuals signalling under these conditions.

Conclusions

The aim of this thesis was to assess the effects, and related consequences, that anthropogenic noise causes when conflicting with sexually selected acoustic communication. The results I presented here detail the deleterious

consequences that can, or not, occur when anthropogenic noises are present during the sexual signalling systems of *G. bimaculatus*. These novel findings highlight the potential fitness costs of disruptive noises, the physiological and behavioural trade-offs related to acoustic behavioural alterations, and begin to understand the key acoustic characteristics that are integral for causing the initial disruptions. Specifically, calling and courtship behaviours were significantly affected in terms of signal transmission, reception and perception, through the reduced signalling behaviours of males and reduced and altered response to these songs by females. Additionally, acoustic alterations shown by the males led to energetic and behavioural trade-offs, shown by the alterations in glucose and lactic acid concentrations linked to increases and decreases in certain behaviours. Finally, the acoustic parameters of the noise stimuli that were necessary to cause such disruptions differed depending on the spectral parameters of the sexual signal and the information that individuals gained from attending to it. The majority of previous literature on this topic has focused on the fitness benefits that are associated with behavioural changes, so my experiments, which were concerned with the consequences related to anthropogenic noise, make a novel contribution to science. Additionally, as *G. bimaculatus* is a widely studied organism in various fields of study, the behavioural findings and discussions in this thesis will aid in future experimentation using this study species. I hope that all of these findings can be applied to other animal populations to better predict or understand the effect anthropogenic noise has when conflicting with their acoustic signalling systems.

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Appendix 1

Manuscript of published chapter: “Anthropogenic noise disrupts mate searching in *Gryllus bimaculatus*”

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