Examining the effect of environmental factors on acoustic signalling of a nocturnal ensiferan insect, *Acanthogryllus asiaticus*

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Dedicated to

Papa

"She did not stand alone, but what stood behind her, the most potent moral force in her life, was the love of her father."

-Harper Lee, Go Set a Watchman

Declaration

The work presented in this thesis has been carried out by me under the guidance of Dr. Manjari Jain at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bona fide record of original work done by me and all sources listed within have been detailed in the bibliography.

Richa Singh

Date:
Place:

In my capacity as the supervisor of the candidate's thesis work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Manjari Jain

Date:
Place:

Acknowledgements

Tell me, what is it you plan to do with your one wild and precious life? —Mary Oliver

And I planned to do a PhD. What an incredible journey it was! This was only possible because of wonderful people I met during this time. I think five years of PhD deserve at least five pages of acknowledgements.

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Richa Singh (Survived not only a PhD but a pandemic- CORONA)

Synopsis

Several animals communicate using sound under non-ideal environmental conditions. It is expected that the physical features of the environment, in which an animal lives and communicates, will play an important selective role in the evolution of its signals and signalling behaviour. In addition, the biotic environment constituted by other signalling species, both conspecific and heterospecific, will also exert additional selective pressure on animal signalling. Finally, rapid urbanization, unprecedented growth in human population and increased anthropogenic activities have substantially altered the natural surroundings of animals. The three major environmental factors, light, temperature and noise, have been altered by anthropogenic activities. Together these three environmental features form the core sensory backdrop for many organisms which are likely to be affected by global changes to these factors. Under such altered conditions, the physiology and behaviour of animals may be affected and it may lead to disruption to their ability to communicate efficiently. It is well known that alteration to sexual signals or behaviour of animals may have serious implications on the Darwinian fitness of an organisms. In the last decade or so, many studies world over have focused on understanding the degree of change in these three environmental features, the impact it has on animals and whether animals have alternate strategies to mitigate problems associated with change in these factors.

In my thesis, I have focussed on studying the influence of the above three environmental factors: light, noise and temperature on acoustic signalling of a nocturnal ensiferan insect, a field cricket, *Acanthogryllus asiaticus*. Crickets are nocturnal, ectothermic insects and primarily use sound as a mode of sexual communication. These biological traits together make them a good model system to examine the impact of altered profiles of ambient light, temperature and noise on the signalling behaviour of animals.

My study system, *Acanthogryllus asiaticus*, is native to the Indian subcontinent and to my knowledge, this is the first study on this species despite its wide distribution. Therefore, my first work chapter of the thesis serves as an introduction to the study species. In this chapter, I examined the temporal variation in calling activity of *A. asiaticus* on a diel and seasonal scale. I also compared the acoustic parameters of calls produced in sexual context, namely, long distance mating call (LDMC), courtship call and post copulatory call. Finally, I examined the relationship of sound producing structures with body morphometry and tested whether peak frequency of LDMC was indicative of male body size. I found that the calling activity of *A. asiaticus* varied on both diel and seasonal scale. I also found all three calls to be acoustically distinct from each other. This study provides the first acoustic characterization of post copulatory calls of a field cricket. Morphometric analyses revealed that both inter-tooth distance and teeth width varied along the file length. I also found that peak frequency was significantly negatively correlated with harp area. Harp area was positively correlated with varies proxies of larger body size. This implies that peak frequency can potentially be used an indicator of male body size in this species.

Given that crickets are nocturnal, absence of light is likely to be important for them to have normal activity. From my first work chapter I found that in *A asiaticus*, the peak calling activity is several hours after sunset. Studies on nocturnal animals have shown that the moon phase can potentially alter activity patterns with reduced activity around full moon due to increased ambient light. This has been referred to as 'lunar phobia'. In addition, recent growth in artificial light at night (ALAN) has altered nocturnal landscape worldwide. Studies have reported that ALAN hinders signalling behaviour in birds and frogs. However, till date, there is no study on the effect of ALAN on signalling behaviour of nocturnal insects. Therefore, in my second work chapter, I hypothesized that the presence of ALAN would negatively impact the calling activity of crickets. To address the same, I measured vertical and horizontal variation in light intensities from streetlights in the natural habitat of crickets. I found that artificially-lit areas were significantly brighter than naturally dark areas even under the foliage at the ground level where field crickets are found. I then tested whether

this dim light at night has any impact on the calling behaviour of the species. I also tested the lunar phobia hypothesis which posits that the activity of nocturnal animals reduces around full moon nights. I found that ALAN reduces cricket calling behaviour whereas moonlight does not show any effect on the calling behaviour. All these were done in field conditions in the natural habitat of animals. Given that calling activity is under circadian control and that melatonin plays a role in regulating this rhythm, I also tested, if ALAN affects calling rhythmicity in laboratory conditions and tested the role of melatonin in regulating calling rhythms. I found that ALAN significantly alters circadian rhythm of calling in a laboratory controlled environment and that melatonin (when provided as a supplement) restores the altered calling rhythm.

One of the limitations of acoustically communicating animals is that they need to find ways to communicate in noisy conditions. This noise could be abiotic or biotic. For a female cricket, the call of the male may be rendered too weak or even unrecognizable in presence of high noisy conditions. In fact, environmental noise has long been established as a major obstruction to effective acoustic communication. Signal detection and discrimination both get hampered by acoustic masking interference which occurs when multiple acoustic signallers call at same time and place. This situation is referred to as the 'cocktail party problem'. It refers to the problem of signalling and perceiving relevant signals under high noise conditions. Thus, in my third work chapter, I investigated the problem of conspecific acoustic masking interference in male field crickets and the strategies they use to solve it. First, I examined the potential for acoustic masking for males of *A. asiaticus*. I found that males call from spatially clumped choruses in the field raising the potential for masking. I then estimated the degree or severity of acoustic masking interference in natural choruses of this species. I did this by reconstructing the choruses using inter-male spacing data and estimating the acoustic space overlap for each focal male using signal attenuation profiles to compute masking probabilities. I found that in the spatial aggregations from where males called there was a significant overlap of their broadcast areas from that of neighbouring males. On an average, the number of maskers for a given male was 2 of which only one was audible to the focal

male. This would allow the focal male to actively reset its calling with respect to the audible masker by altering various call parameters. So, I next tested whether such was the case by examining the acoustic interaction of males with their nearest signalling neighbour in the field. Additionally, I also conducted lab playback experiments with simulated neighbouring males to test this. Field and playback experiments showed that males call in alternation with their nearest neighbour, thereby largely escaping masking from the nearest (and most significant) masker. They achieve this by actively resetting various temporal features of their calls. However, they do not make any spectral changes to their calls. I also found that in the presence of either a softer or louder neighbour, focal males modulate their call SPL. However, I found no support for masking avoidance by increasing SPL.

Masking interference can also occur due to abiotic sources of noise such as, wind, water and foliage. While these are sources of noise that would have existed throughout the evolutionary history of animals, anthropogenic noise is more recent. Amongst all sources of anthropogenic noise, road traffic noise is one of the most pervasive pollutant which has been shown to affect acoustic communication of various species inhabiting traffic-prone areas. The bulk of studies examining impact of anthropogenic noise have been on vertebrates while studies on invertebrates are very limited. Moreover, not many studies have dissected whether animals mitigate the problem of masking by traffic noise by exhibiting long-term adaptation or by making short-term adjustments to their calls. Hence, in my fourth work chapter, I examined the effect of exposure to road traffic noise on the acoustic signals of *A. asiaticus* over long and short-term. For this, I examined the signal features and signalling behaviour of crickets with chronic exposure to noise as well as those that were subjected to temporary exposure to traffic noise. Towards this, I carried out noise level measurements in areas prone to traffic noise and those without traffic. To test the effect of chronic exposure to traffic noise on the mating calls of *A. asiaticus*, I also compared the call properties of males living in areas of traffic and those from quiet areas. Finally, in laboratory playback experiments, I examined the response of males to traffic noise to assess the potential of males to

make short-term adjustments to their signalling in presence of traffic noise. My findings suggest that the noise profiles are drastically different between quiet and traffic-prone areas. I also found that the male crickets in noisy areas were significantly louder than those from quiet areas. They also produced high duty cycle calls with shorter chirp periods and higher chirp rates as compared to those from quiet areas. Significantly, the lab playback experiments did not provide any evidence of short-term adjustment to signals or signalling behaviour in these crickets. This study reveals that chronic traffic noise can potentially alter signal characteristics in these nocturnal insects that are unable to make any short-term adjustments in presence of traffic. These changes (louder and faster calls) are likely to be energetically costly, thereby inducing metabolic stress for males from noisier habitats.

Last, but not the least, global increase in temperature in view of climate change has begun to reflect at local scale in the form of increased mean temperatures. Increased ambient temperature is likely to impact the developmental biology and physiology of animals, especially of ectotherms. A plethora of studies have outlined the influence of temperature on development and growth in determining an insect's life history. Besides, studies on acoustically communicating insects have shown that change in ambient temperature also affects acoustic signals. Although the rate of global warming is slower in the tropics than at higher latitudes, several studies have suggested that tropical ectotherms will be negatively affected by global warming. In my fifth work chapter, I studied the effect of temperature on life history traits, such as, hatchability, survival to adulthood, developmental and adult lifespan and body morphometry of *A. asiaticus*. I also examined the seasonal variation in temporal and spectral features of calls and the effect of changing temperature in lab environment on call features. Finally, I examined the effect of developmental temperature on call features of cricket that were reared from egg stage to adulthood in different temperature regimes. I found that, both growth and development rates were faster at higher temperature compared to lower temperature. However, lifespan was shorter in higher temperatures as compared to lower. I also found that various body size parameters differ based on the rearing temperature and

this was true for both males and females. With respect to the differences in call features with temperature, I found both temporal and spectral features of calling song to be different during different seasons. Further, laboratory experiment showed that the calls of *A. asiaticus* are temperature dependent where in, a short exposure to altered temperature regime can significantly alter the call features, even if the rearing conditions were same. Finally, I found significant effect of developmental temperature on the calls of adult males. All the call features were observed to be different between individuals raised at different temperatures. These findings highlight that the critical importance of not just immediate temperature profiles but longer-term temperatures (rearing temperatures) on not only the developmental and growth of these ectotherms but also on their sexual signals.

In summary, my study provides a holistic understanding of how three different environmental features, light, noise and temperature impact different aspects of the biology of an organism, including its behaviour, acoustic communication and life history traits. My study is the first behavioural ecological study of the species, *A. asiaticus.* It provides the first description of the different calls of *A. asiaticus* in intersexual communication. It also provides the first evidence for altered calling behaviour due to ALAN in a nocturnal insect, demonstrated both in field and lab conditions. It also provides the first evidence of population level differences in call features due to traffic noise in an insect. Finally, it is the only other study, to the best of my knowledge, that provides evidence for the impact of rearing temperature on the signals of adult crickets. My study not only helps in understanding the impacts of climate change (acoustic, light and temperature related) on nocturnal, acoustically- active, ectothermic insect but allow a holistic understanding of the ecological and potential evolutionary consequences of increasing anthropogenic disturbances on organisms.

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List of Publications

Publication from thesis

Singh R, Jain M. 2020. Variation in call types, calling activity patterns and relationship between call frequency and body size in a field cricket, *Acanthogryllus asiaticus*. ***Bioacoustics***.1-9.

Singh R, Prathibha P, Jain M. 2020. Effect of temperature on life-history traits and mating calls of a field cricket, *Acanthogryllus asiaticus*. bioRxiv 2020.06.06.137869; doi: 10.1101/2020.06.06.137869.

Manuscript in preparation

Singh R, Jain M. Examining lunar phobia, the impact of artificial light at night and role of melatonin in regulating male calling behaviour in a field cricket, *Acanthogryllus asiaticus*.

Singh R, Bahuleyan J, Jain M. Natural spacing and call alternation reduce acoustic masking in conspecific choruses of crickets.

Singh R, Jain M, Examining the effect of long-term and short-term exposure to road traffic noise on signalling behaviour of field cricket, *Acanthogryllus asiaticus.* Manuscript under preparation.

Other Publication

Sekhar MA*, **Singh R***, Bhat A, Jain M. 2019. Feeding in murky waters: acclimatization and landmarks improve foraging efficiency of zebrafish (*Danio rerio*) in turbid waters. ***Biology letters***.15(7):20190289.

*****equal contribution.

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Chapter 1

General introduction

Picture credit: Nakul Raj

1.1 Animal communication

"The poetry of earth is never dead." - John Keats

The spectacular bright colours of butterflies, the extravagant display of peacock, the alarm calls of vervet monkeys, the emission of pheromones by moths, the electrical signals emitted by some fishes, the claw waving of fiddler crab and numerous other examples exemplify that animal signals are perhaps the most impressive and complex manifestations in nature. Signals serve as the subtle mode of communication between animals (Smith and Harper 2003) and animal communication is one of the most ubiquitous behaviour in the animal kingdom. It involves a sender producing a signal that conveys information and a receiver making a decision on how to respond to that signal (Bradbury and Vehrencamp 1998). Darwin (1872), in his work 'The Expressions of the Emotions in Man and Animals', developed a theory of "expression" and argued that animals, including man, have undergone selection for communication of their emotional state. Animals communicate using diverse sensory modalities including olfaction, electric sensing, acoustics, vision and touch to maintain inter and intra-species interaction and perform various life functions used in different behavioural contexts such as mate attraction, aggressive interactions, parental care and predator-prey interactions (Bradbury and Vehrencamp 1998). For instance, the low-voltage electrical signals are used by fishes to orient, navigate and communicate (Davis and Hopkins 1988), moths release sex pheromones to attract mates (David et al. 1983), vervet monkeys produce alarm calls when detecting predators (Owren and Bernacki 1988) and shrimps snap during aggressive interactions (Hughes 2000). In many species, sexual reproduction relies on signals which are used in the context of mate attraction and play an important role in mate choice (Andersson 1994). Darwin (1871) proposed that these sexual advertisement signals produced by one sex (usually males) of a species in the exclusive context of reproduction, evolve under sexual selection. The display of elaborate tail feathers

in peacock (Darwin 1871), claw waving of male fiddler crab (Latruffe et al. 1999), conspicuous loud advertisement calls produced by male frogs (Ryan 1985) and sex pheromones release by moths (David et al. 1983) are some examples of sexual advertisement signals using different sensory modalities. These ritualized displays in animals that have, in several species, been shown as reliable indicators of the features/quality of the signaller (Andersson 1994). For instance, the extravagant train of a peacock is an honest indicator of male quality (Petrie et al. 1999), the dominant frequency of advertisement calls produced by anurans signify male size (Wagner 1989) and repertoire size of bird song indicates male condition (Lampe and Espmark 1994). Such diversity and intricacies of signalling behaviour reveal that the study of animal communication is an interesting and worthwhile endeavour.

1.1.1 Acoustic communication

Among various sensory modalities, acoustic communication is one of the most prominent modes of communication in many animal taxa, including insects, crustaceans, fishes, anurans, birds, and mammals (Bradbury and Vehrencamp 1998). The ability to carry information over long distances makes acoustic communication suitable for attracting potential mates which are distant or not in immediate view (Bradbury and Vehrencamp 1998). Animals produce sound of varied frequency ranges (Figure 1), for instance, whales use infrasound to communicate with each other across vast distances of the ocean (Wilson et al. 2007), greater horseshoe bats produce ultrasonic social call (Andrews and Andrews 2003), Asian elephants produce infrasonic signals (Payne et al. 1986) whereas songbirds produce songs of audible frequency range (Konishi 1969). A variety of mechanisms are employed by animals for sound production and this varies greatly across different taxa. For instance, birds vocalize using a syrinx while frogs use a larynx for vocalization, even though both use expulsion of air from their lungs to produce sound (Bradbury and

Vehrencamp 1998). On the other hand, Sperm whales produce clicks in the anterior part of the nasal complex involving a specialized structure called spermaceti organ located in the forehead of the animals (Norris and Harvey 1972). While acoustic signalling is widespread among terrestrial vertebrates, in terrestrial invertebrates, acoustic communication is well developed only in insects (Pollack 2017).

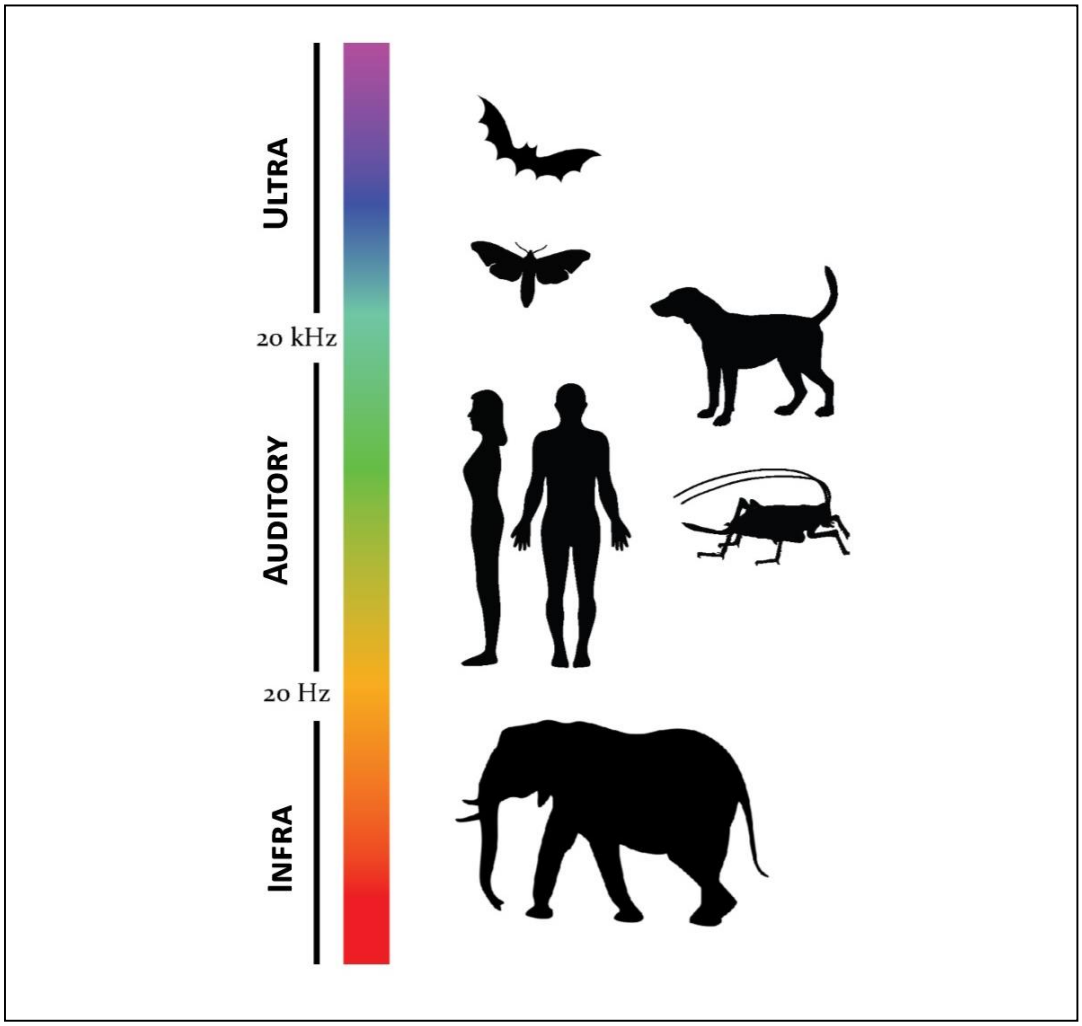

Figure 1.1 The acoustic spectrum. Sound frequencies used by different animals. (Illustration: Atharva)

1.1.2 Acoustic communication in insects

Insects are known to be the first terrestrial animals to use sound signals for long-distance communication (Senter 2008). The sound produced by some of these insects has been shown to be similar to those which were produced during Paleozoic and Mesozoic eras (Senter 2008). Sound producing insect orders are primarily, Orthoptera, Hemiptera, Lepidoptera, Blattodea, Diptera and Hymenoptera. In Orthoptera, sub-orders Caelifera (grasshopper) and Ensifera (crickets and katydids) are mainly known for producing sound of various frequencies using stridulation (rubbing of body parts) and wing mechanisms. Hemiptera which include true bugs, plant lice and cicadas produce sound using tymbals (Strauß and Lakes-Harlan 2014; reviewed in Greenfield 2016). In Dipterans such as true flies, fruit flies and mosquito and in Hymenoptera such as sawflies, wasps, bees, ants, low-frequency sound is produced using wingbeat mechanism. In Lepidoptera, moths and butterflies use tymbals, stridulation and wing mechanisms to produce a sound of typically ultrasound frequency (Strauß and Lakes-Harlan 2014). Blattodea (cockroaches) also produce audible sound by the expulsion of tracheal air (Strauß and Lakes-Harlan 2014).

Insects emit these sound signals in various behavioural contexts including avoiding predators, interacting with competitors or even towards location of hosts (Hoy 1992; Pollack 1998; Zuk and Kolluru 1998). Yet, the majority of sound signals produced by insects are in the context of mate attraction where typically males call to attract conspecific females (Gerhardt and Huber 2002). Various selective forces and constraints, namely, sexual selection, predator-parasites, phylogenetic constraints, morphological constraints, physiological constraints and environmental constraints drive the evolution of acoustic signals (Endler 1992). Among these selective forces, environmental constraints play an important role in shaping acoustic signals and signaling behaviour. Understanding the

relationship between environmental constraints and signal features or signalling behaviour provides an imperative view of signal evolution (Forrest 1994).

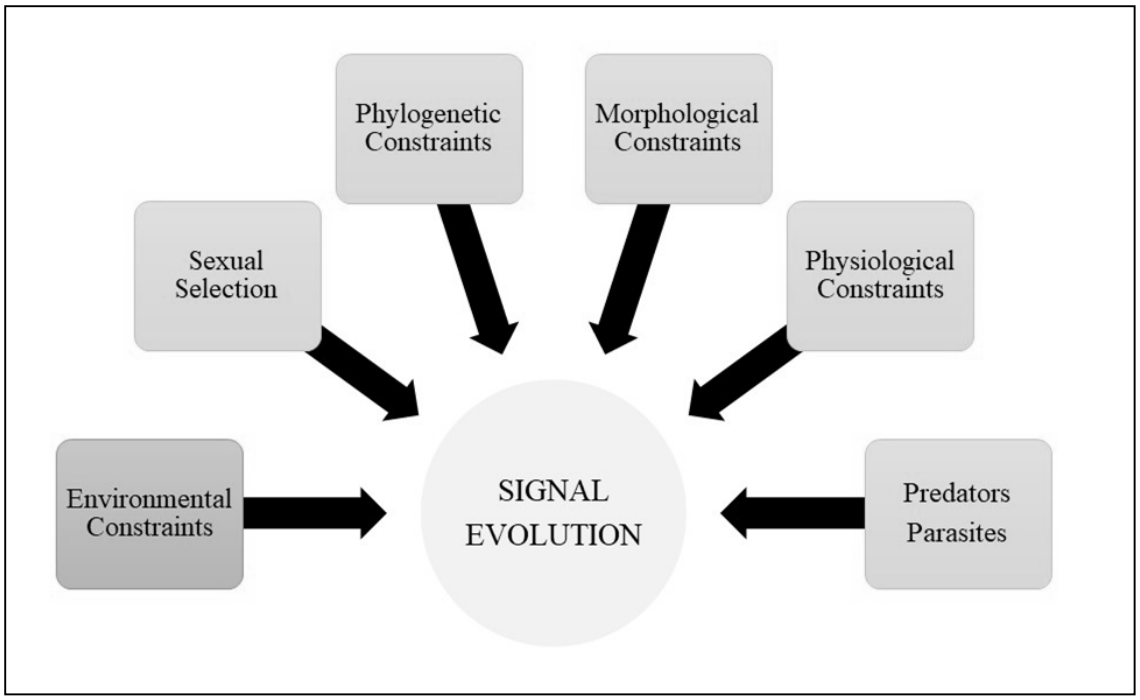

Figure 1.2. Impact of various selective forces on the evolution of signal (adapted from Forrest 1994).

1.2 Environment as a selective force

Communication, like most other behaviours, occurs under non-ideal environmental conditions that occur in the natural habitats of animals. Long range acoustic signals get altered significantly while propagating through the habitat. Various abiotic and biotic environmental factors are known to impact signal features (Otte 1992; Forrest 1994). For instance, wind and temperature gradients, turbulence and vegetation structure can alter the temporal (time-dependent) structure of a transmitted signal through irregular amplitude modulation and reverberation (Wiley and Richards 1978). Whereas, the spectral (frequency-dependent) content of the signal changed by absorption as it causes frequency

dependent loss because of which high frequency sounds do not travel as far as low frequency sounds (Bass 1991). Ambient noise plays an important role in effective communication as it determines the signal-to-noise ratio, which is the difference, in dB, between the level of the background noise and a signal (Forrest 1994). Along with this, the biotic environment involves disturbance to animal signal caused by signalling of same member species (conspecific) or member of other species (heterospecific) (Otte 1992). Signal attenuation, reduction in signal to noise ratio due to high ambient noise, distortion during propagation together determine the transmission range of signals (Jain 2011).

While the natural environment itself impacts communication, anthropogenic changes to the natural environment impose additional constraints. Recent growth in urbanization and human population have severely affected the environment inhabited by organisms. Sih et al. (2011) described that organisms are facing novel environments associated with human-induced rapid environmental change (HIREC) caused by habitat fragmentation, the introduction of exotic species, noise and light pollution and climate change. While environmental change is not a new phenomenon, human-induced changes are occurring on a larger spatial scale and at a faster rate than in the past. The three major environmental factors, light, temperature and noise, have been affected by human-induced changes. Under such altered conditions, the sensory and physiological processes in organisms are likely to be affected. This interferes with the ability of animals to receive and perceive information about their environment (Wong and Candolin 2015). For example, due to anthropogenic activities, increased turbidity level affects foraging behaviour in zebrafish as it impairs their visibility (Sekhar et al. 2019). Animal communication, especially in sexual context also gets affected, which in turn can potentially impact the fitness of an organism (Candolin 2019). Therefore, understanding behaviour due to a change in environmental factors is an exciting and important challenge (Sih et al. 2011). In recent years, a plethora of studies

have focused on behavioural changes in organisms in response to altered environmental condition. In my thesis, I focused on understanding how the behaviour of an organism gets affected by these three environmental factors: light, noise and temperature in natural and altered environmental condition.

1.2.1 Light

Natural light and darkness together, shape the life of the organisms. Various biological phenomena at molecular to ecosystem level are majorly regulated by sunlight (Foster and Kreitzmann 2004). For instance, natural light acts as the most important environmental cue for biological timings (Gaston et al. 2014). These include daily, annual and/or lunar cycles which regulate the lives of most organisms by influencing their circadian rhythm (Gaston et al. 2017). This light-dark cycle synchronises endogenous circadian clocks which allow organisms to adapt to daily variation in environment and thus optimally organise its behaviour, metabolism and physiology (Foster and Kreitzmann 2004; Gaston et al. 2017). During night time, moonlight plays an important role in regulating various behavioural activities of nocturnal organisms. For instance, insects, amphibians and bats are known to reduce their behaviour during full moon conditions to avoid predation (Morrison 1978; Tuttle et al. 1982; Lang et al. 2006). Morrison (1978) coined the term 'lunar phobia' for such changes in the behavioural response to increased moonlight intensity (during full moon). Given that behavioural and physiological traits of animals are synchronized to such daily and seasonal rhythm governed by natural light, changes in patterns of natural light and darkness is likely to impact the biology of the organism in a negative manner. The recent introduction of artificial lighting during night time has increasingly eroded and altered the natural light cycle (Longcore and Rich 2004). Moreover, over 23% of the land surface of the earth has undergone alteration in light levels at night (Falchi et al. 2016). NASA Earth observatory has released a map exhibiting the spread of artificial light all over

the world in the year 2016 (Figure 1.3). Natural light levels from day to night varied from ~100,000 lux to ~0.001lux (clear night) but artificial light during the night may increases lux level from 0.001 lux to magnitude of 3 or more (Gaston et al. 2014). There are a wide variety of light sources, ranging from vehicle headlights to constant night time lighting from streetlights (Gaston et al. 2014). Light qualities for such sources vary from narrow to broad emission spectra and from low to high emission intensities (Gaston et al. 2014). The severity of light pollution is such that it is not just confined to urban cities, but rapidly spreading into pristine areas (Gaston et al. 2017). In fact, it has been shown that artificial light at night impacts an array of behaviours and physiological mechanisms results in fitness reduction, has caused a worrying decline in species abundance and shifts in species interactions and community composition (Longcore and Rich 2004). It affects several taxa, including mammals, birds, reptiles, amphibians, fish, invertebrates and plants, both in terrestrial and aquatic ecosystems (Gaston et al. 2014; Longcore and Rich 2004). It affects diurnal organisms such as songbirds (Jha and Kumar 2017; Taufique and Kumar 2016; Dominoni et al. 2013; Kempenaers et al. 2010; Miller 2006) and nocturnal organisms such bats and insects (Owens and Lewis 2018; Desouhant et al. 2019; Stone et al. 2015).

One of the behaviours which has been substantially altered by night time lighting is sexual signaling. In the recent years, some studies have provided evidence of this in invertebrates, such as impact of night time lighting on visual signalling in fireflies (Owens and Lewis 2018; Desouhant et al. 2019) and reduction in sex pheromone production and alteration in its composition in winter moths (Van Geffen et al. 2015a, 2015b; also see Table 1.1 for more examples). Disruption in the acoustic signalling in vertebrates due to artificial light has also been reported (male green frogs: Baker and Richardson 2006). However, what happens to acoustic signalling in invertebrates due to artificial light at night (ALAN) is still a question.

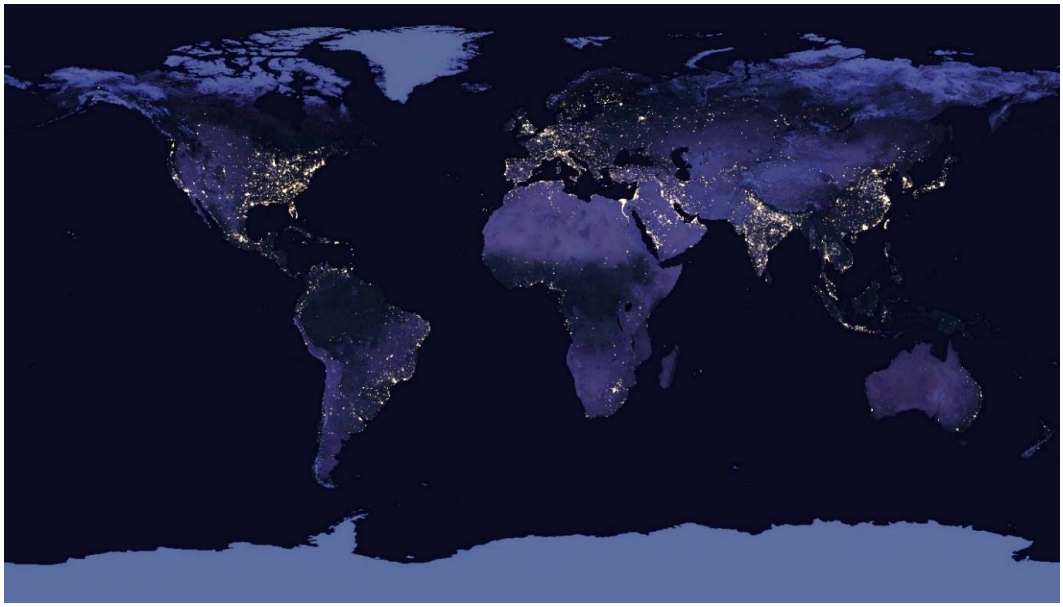

Figure 1.3. A world-view of light pollution in 2016. Source: NASA Earth Observatory images by Joshua Stevens, using Suomi NPP VIIRS data from Miguel Román, NASA's Goddard Space Flight Center.

1.2.2 Noise

"Acoustic communication and hearing in humans and non-human animals did not evolve in sound-proof rooms, but under real-world conditions which are often characterized by a considerable amount of noise." - Schmidt and Romer, 2011

The detection and recognition of signal are dependent not only on the energy in the signal but also on the amount of background noise in the environment. Noise has long been established as a major obstruction to effective acoustic communication. It is defined as any unwanted or irrelevant sound that interferes with the detection or transmission of the signal (Forrest 1994). The source of noise could be abiotic such as wind, running water, foliage or biotic including noise from other signalling organisms, conspecific or heterospecific (Wiley and Richards 1978; Brumm 2004). Increase in ambient noise results in relevant signals getting buried in irrelevant signals, resulting in reduced signal to noise ratio (SNR) (Jain 2011). Given that, signal detection and discrimination are better at high SNR, low

ambient noise is considered to be essential for communication (Romer 1993). Further, when multiple acoustic signallers call at the same time and place, acoustic masking interference occurs which hampers signal detection and discrimination (Otte 1992). This lead to a situation of 'cocktail party problem' which creates difficulty for receivers to perceive relevant signals under noisy conditions. Such noisy environments can have detrimental effects on the fitness of both senders and receivers, especially when it impairs mating related communication (Bee and Micheyl 2008). Masking has been reported in form of jamming in bats emerging in large numbers (Hase et al. 2018), dawn choruses in songbirds (Langemann and Klump 2001) flocking and colonial birds (Aubin and Jouventin 2002) and frog and insect choruses (Gerhardt and Huber 2002; Also see Table 1.1 for more studies on invertebrates). The problem can be addressed at the level of the sender and/or the receiver by showing alteration of signal structure over evolutionary time, signaller behaviour and/or alteration to receiver behaviour or physiology (Römer 2013). Senders can employ several strategies to deal with high levels of masking noise for effective communication including increase signal amplitude to actively avoid noise, partition signal timing at seasonal, diel or fine-temporal scale (Jain et al. 2014)

Another form of environmental noise which impacts acoustic communication systems is abiotic noise. This could be generated by the habitat such as the rustling of leaves or the noise of flowing water. On the other hand, it also includes anthropogenic noise, the one that emerges from human activities. This includes noise from industries, construction sites, road traffic, mining etc. Given the global nature of anthropogenic noise, which is increasing rapidly and changing the ambient environment, Slabbekoorn (2019) gave the new term "acoustic climate change". It affects a wide range of organisms in both marine (Slabbekoorn et al. 2010) and terrestrial (Barber et al. 2010) environments. In the terrestrial environment, road traffic noise is one of the most pervasive forms of human-generated

noise which affect various organism severely (Brumm and Slabbekoorn 2005; Rabin et al. 2003; Raboin and Elias 2019). It can significantly reduce reproductive success or survival of the exposed organism (Barber et al. 2010). It alters behavioural patterns, causes population declines, imposes physiological stress such as hearing loss and results in elevated stress hormone levels and hypertension (reviewed in Barber et al. 2010). This form of noise has the potential to obstruct the acoustic communication of organisms near the roadside by overlapping or masking animal signals that occur in a similar frequency range (Halfwerk et al. 2011). Various studies have investigated the impact of traffic noise on a widening range of taxa and demonstrated that animals can change their signalling behaviour to avoid masking (Halfwerk et al. 2011). While most of the studies have examined the effect of traffic noise on acoustic communication in vertebrates, studies on terrestrial invertebrates that communicate acoustically are very few (Raboin and Elias 2019; also see Table 1.1 for more studies on invertebrates).

1.2.3 Temperature

Global warming has led to an unprecedented rise in temperature and an increase in the frequency and severity of heatwaves (Stone et al. 2010). IPCC suggests that since 1880, the earth's average surface temperature has increased by 1.62°F (0.9 °C) which increased carbon dioxide level (from 280 ppm to 412 ppm) and other human-made emissions into the atmosphere (Figure 1.4). Most of the warming has occurred in the past three decades. Such alteration in the ambient environment varies across the globe and have biological impacts as temperature influences all levels of biological organization (Abram et al 2017). This increase in temperature has attributed to shifts in geographical ranges, seasonal phenology, community interactions, genetics and extinctions. Given that temperature influences all levels of biological organization including metabolism, development, growth, movement and reproduction (Abram et al. 2017), such rapid increase in temperature is likely to impact

at organismal, community and ecosystem levels (Coumou and Rahmstorf 2012). Most terrestrial animals are ectotherms which do not maintain constant body temperature (Angilletta 2009). Although the rate of global warming is slower in the tropics than at higher latitudes, several studies have suggested that tropical ectotherms will be negatively affected by global warming (Sunday et al. 2014; Deutsch et al. 2008). This is because tropical insects are sensitive to temperature change and are currently living very close to their optimal temperature whereas temperate species have wider thermal tolerance and warming may even increase their fitness (Deutsch et al. 2008). Increased ambient temperature increases metabolic rate which impacts the developmental biology and physiology of animals, especially of ectotherms (Angilletta 2009). For instance, an increase in environmental temperature of 10°C results in a two to sixteen-fold increase in growth rate in molluscs, arthropods, and fish (Angilletta 2009). Temperature also influences signalling behaviour in a wide range of taxa constituting terrestrial, aquatic and marine animals through various chemical and physiological regulation, neuronal activity and muscular contraction (Sueur 2019). For instance, the effect of temperature on the structure and rate of production of the sound signal has been studied in anurans (Gerhardt 1978; Llusia et al. 2013), and fish (Connaughton et al. 2000). Because insects are ectotherms, the increasing temperature may affect their developmental biology and behaviour (see Table 1.1 for examples). Given that acoustic signals are controlled by the neuromuscular system, change in environmental temperature can affect the signalling behaviour of ectotherms. Therefore, effect of temperature on the acoustic properties of the signal must be taken into account when comparing calls across individuals, populations and species (Ragge and Reynolds 1998).

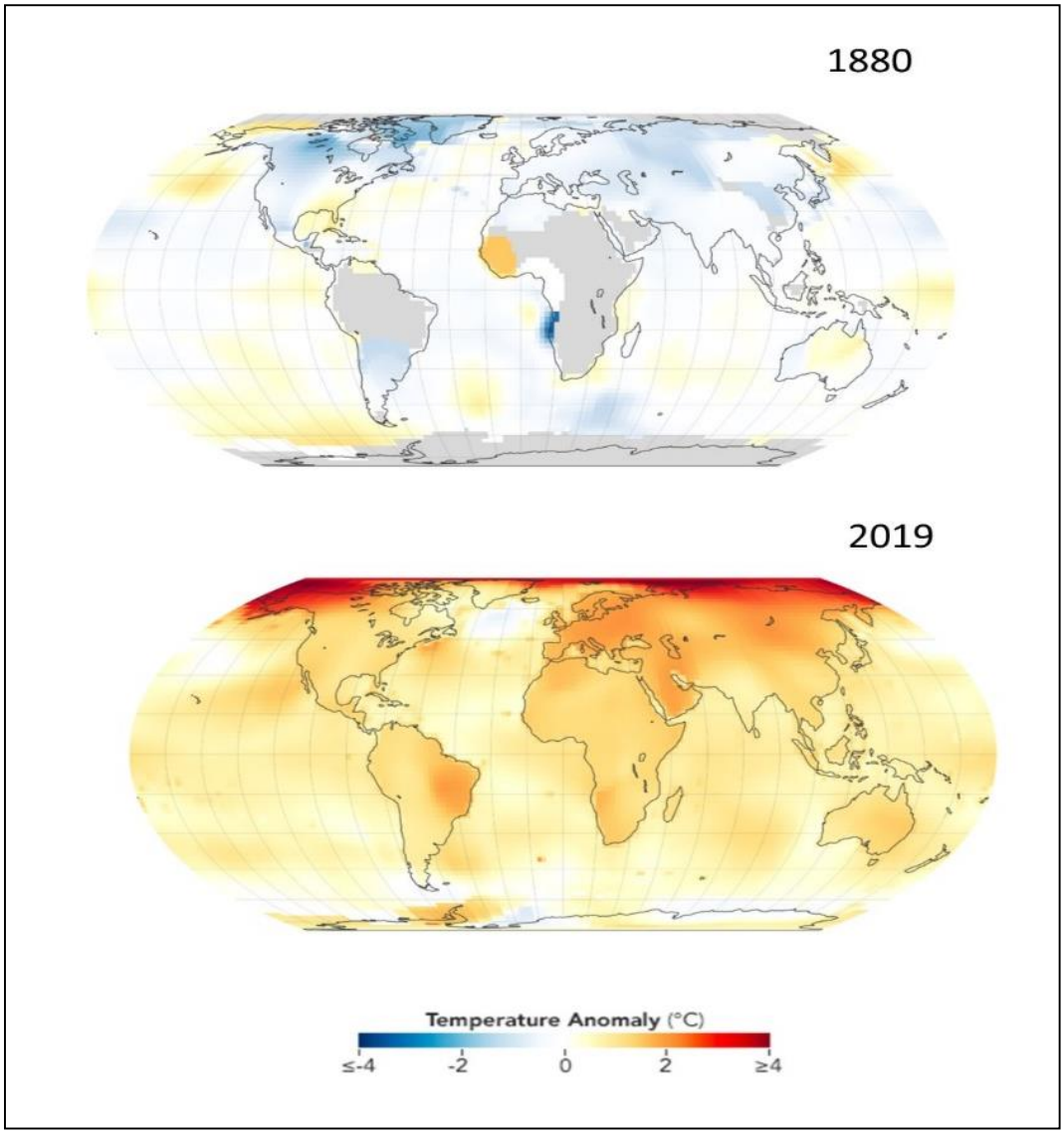

Figure 1.4. Increase in temperature variation between 1880 to 2019. Source: NASA/ GISS

Table 1.1. Literature survey of the effect of environmental factors (light, noise and temperature) on invertebrates.

Environmental Factors	Invertebrate model system	Findings	References
Light (Natural)	Glowworm: *Lampyris noctiluca L*	The luminescent display of larva were higher during late summer when the nights are darker.	Dreisig 1974
	Scorpion: Buthus occitanus	On bright moonlit nights, active adults reduced their locomotion, foraged mainly under bushes.	Skutelsky 1996
	Wasp: *Apoica flavissima*	The rates of foraging flights were higher during the full moon and last quarter moon phases.	Nascimentro and Tannure-Nascimento 2005
	Katydids	During full moon the background noise level dropped because of the reduced calling activity of katydids.	Lang et al. 2006
	Field crickets: *Teloegyrllus oceanicus*	The calling activity was low during the full moon phases compared to other nights.	Loher and Orsak 1985
Light (Artificial)	Moth: Mamestra brassicae	Artificial night lighting reduced sex pheromone production and altered its chemical composition.	Van Geffen et al. 2015
	Firefly: *Photuris versicolor P. pyralis*	Artificial light treatments reduced the flashing rate in *Photuris versicolor* and courtship behaviour in *P. pyralis.*	Firebaugh and Haynes 2016
	Leafhopper: *Empoasca onukii*	Under continuous illumination activities of locomotion, cleaning, and searching were significantly suppressed during the night.	Shi et al. 2017
	Field cricket: *Teleogryllus commodus*	Under artificial lights in laboratory conditions, the females were slower to initiate movement towards playback speaker, but the movement pattern was unaffected.	Thompson et al. 2019
	Field cricket: *Teleogryllus commodus*	Chronic exposure to different light intensities showed no effect on the number of courtship calls or their signal structure.	Botha et al. 2019
Noise (Natural)	Cricket and Katydid assemblege	Masking avoidance in heterospecies chorus by increasing call intensity and frequency tuning.	Jain et al. 2014
	Crab: *Panopeus spp.*	The proportion of clams consumed decreased in presence to the predator catfish (*Ariopsis felis*) acoustic cues.	Hughes et al. 2014
	Tarbush Grasshoppers: *Ligurotettix planum*	The signalling males actively compete with each other and adjusts their own signal to avoid overlap with the signals of other males.	Minckley et al.1995
	Cricket: *Plebeiogryllus guttiventris*	Males increased the length of the chirp or chirp rate to produce energetically expensive signal from their neighbour.	Mhatre and Balakrishnan 2006
	Cricket: *Mecopoda elongata*	Signallers aggregate together and call synchronously to increase the amplitude and the broadcaster area.	Hatbauer et al. 2014

Noise (Anthropogenic)	Cuttlefish: *Sepia officinalis*	The frequency of color change in the visual display of cuttlefish increased during the playback of anthropogenic noise.	Kunc et al. 2014
	Shore Crabs: *Carnicus maenas*	Crabs exposed to the playback of ship noise were more likely to suspend feeding.	Wale et al. 2013
	Tree cricket: *Oecanthus pellucens*	In response to fluctuating traffic noise the male shorten their chirp duration and were highly probable to pause singing in increased noise conditions.	Orci et al. 2016
	Field cricket: *Gryllus bimaculatus*	Chirp rate of male calls decreased in response to noise from passing cars.	Gallego-Abenza et al. 2019
	Grasshopper *Chorthippus biguttulus*	In a noisy area the males produce courtship songs with higher frequency.	Lampe et al. 2012
Temperature	*Beetle species: Callosobruchus maculatus, C. chinensis*	The duration of death feigning (an anti-predatory response) is negatively correlated with ambient temperature.	Miyatake et al. 2008
	Aphid: *Sitobion avenae*	The total number of aphid defences produced per hour increased with temperature.	Le lann et al. 2014
	Parasitic wasp: *Aphidius rhopalosiphi*	Females reared at high temperature on the foraging patch than those at developed at lower temperature.	Le Lann et al. 2011
	Ant: *Tapinoma nigerrimum*	At higher temperatures, the foraging activity reduced, and degradation of pheromone increased.	Oudenhove et al. 2011
	Field cricket: *Gryllus bimaculatus*	Individuals reared at higher temperature were more explorative.	Niemelä et al. 2019
	Field cricket *Acheta domesticus*	The running speed and jumping speed increased with increased testing temperature. Also cold acclimated crickets showed better jumping performance in colder testing conditions than warmer.	Lachenicht et al. 2010
	Firefly: *Photinus greeni*	The inter-pulse intervals of bioluminescent flashes of courtship display decreased with increase in ambient temperature.	Michaelidis et al. 2006
	Spider: *Habronattus clypeatus*	The courtship display rate increased with temperature to a point, and then decreased.	Brandt et al. 2018
	Field cricket: *Teleogryllus oceanicus*	The chirp rate of both long and short chirp of male calls increased with temperature.	Walker and Cade 2003
	Field cricket: *Gryllus integer*	The increase of temperature from 18-30°C results in a 400 Hz increase in peak frequency.	Martin et al. 2000
	Field cricket: *Gryllus integer*	Males showed a preference for warmer cracks and their calls from warmers cracks had shortened syllable period and chirp pauses.	Hedrick et al. 2002

1.3 Cricket as a model system

Light pollution, noise pollution and increasing temperature are pervasive global problems the world is facing. These may impact the behaviour and biology of several organisms including invertebrates. Recent studies have shown steep declines in insect diversity and biomass globally, across Germany, the Netherlands, Sweden, the British Isles, Puerto Rico and Costa Rica (Owens et al. 2020). Given that, insects play a significant role in terrestrial and freshwater food webs (van Veen et al. 2006) and contribute to various ecosystem services (Schowalter et al. 2018), their decline could pose a serious threat to life on the earth. Earlier, factors for such decline were predicted as habitat loss, use of pesticides, invasive species and climate change. However, recently, insect decline has become a global phenomenon and terms like 'Ecological Armageddon' or 'Insect Apocalypse' have been coined predicting that various anthropogenic stressors are responsible for such decline (Leather 2018). Studying the effect of the three most important environmental factors: light, noise and temperature on nocturnal insects through the lens of signalling behaviour, can contribute towards understanding the problem of insect apocalypse. Such a holistic study on any nocturnal organism examining the impact of these factors is completely lacking. Given that, crickets are nocturnal, ectothermic insects and primarily use sound as a mode of communication, they can be a good model system to examine the impact of altered profiles of ambient light, noise and temperature on the signalling behaviour of insect (Figure 1.5). It is expected that light during night time might alter their nocturnal landscape while increase in ambient noise might disturb their signalling behaviour. Change in temperature is likely to impact their communication given that according to Dolbear's law there is a relationship between the sound emitted by crickets and the air temperature (Dolbear 1897; see Chapter 6).

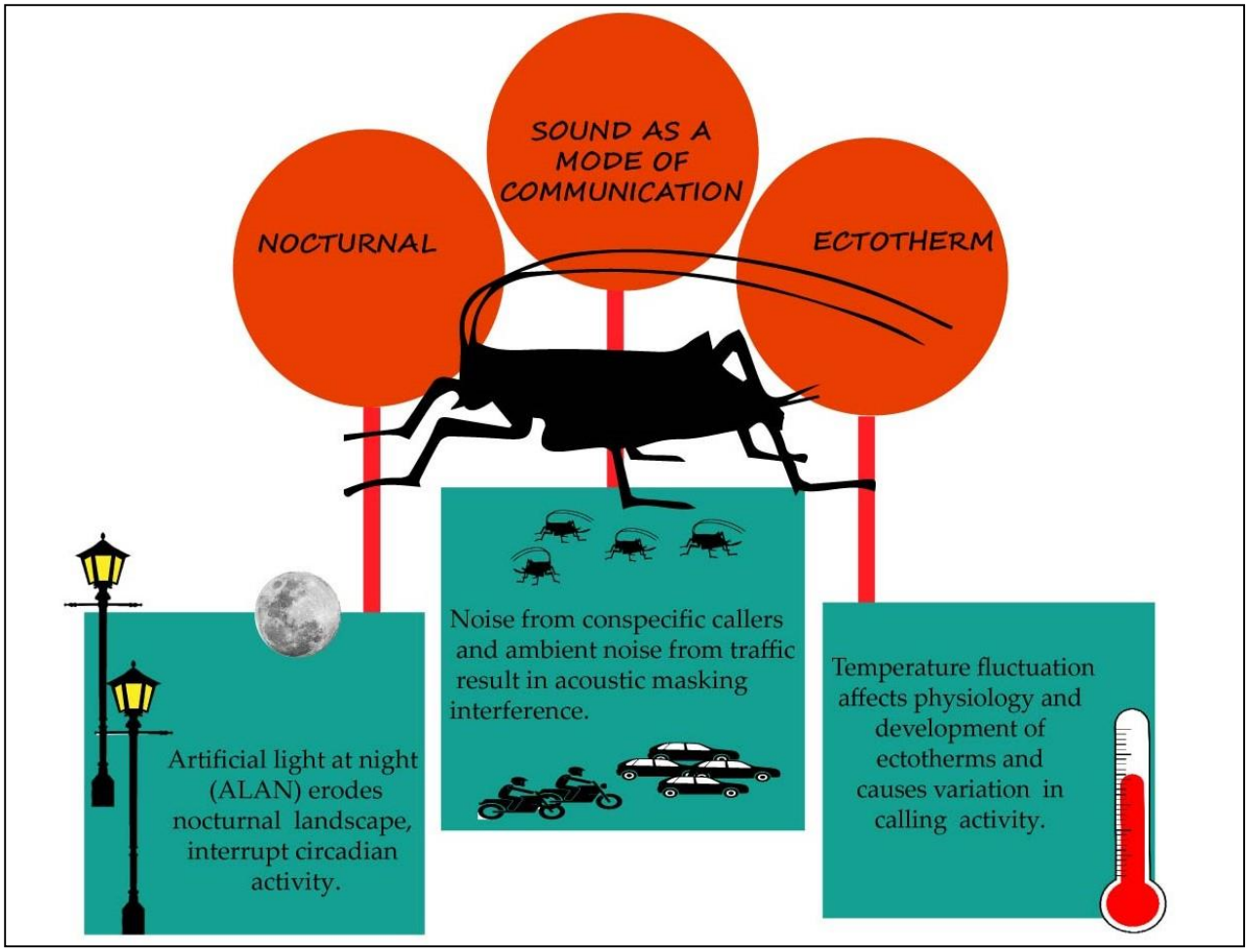

Figure 1.5. Graphical representation of the major focus of the thesis to examine the effect of environmental factors: light, noise and temperature on cricket calling behaviour.

1.3.1 Introduction to crickets

"True crickets were among the first musicians on the planet and were part of the nocturnal circumambience for some 150 million years before there were human ears to hear them."

- Otte 1992

Crickets are known to be distinct singers since ages and have evolved highly specialized sound-producing structures (Alexander 1967). Males produce signals typically in the context of long-distance mate communication to attract females that are mute. Acoustically oriented females detect and recognize conspecific signals and track down calling male in the dark (Alexander 1967). Sound is produced by stridulation of wings wherein the male raises it forewings and closes the right wing over the left, the plectrum (a highly sclerotized edge of the wing) of the left wing strikes teeth on the file on the underside of the right wing (see Chapter 2). Each wing closure results in one syllable which is a basic unit of sound (Bennet-Clark 2003). The song of each species is highly conspicuous and has a unique set of temporal and spectral features as well as amplitude profiles (Otte 1992), which is used by females to distinguish conspecific from heterospecific males (reviewed in Gerhardt and Huber 2002).

1.3.2 Terminology used for call components

Each wing closure results into one syllable which is a basic unit of sound (Bennet-Clark 2003). A collection of many syllables forms a chirp, wherein, two consecutive syllables are separated by a silent interval indicative of the period of disengagement of wings. Typically, the call of a field cricket consists of numerous chirps occurring at a certain interval of time. Temporal (time-domain) and spectral (frequency-domain) features provide a structural characterization of the call (Figure 1.6). These features are as follows:

Temporal features

Temporal features of the call represent how energy in the call is distributed over time and are typically depicted using an oscillogram (Figure 1.6).

i. Chirp duration: onset of one chirp to its offset.
ii. Chirp period: onset of one chirp to the onset of the subsequent chirp.
iii. Chirp rate: number of chirps occurring in a given time period.
iv. Syllable duration: onset of one syllable to its offset.
v. Syllable period: onset of one syllable to the onset of the subsequent syllable.
vi. Number of syllable per chirp

Spectral features

Spectral features of the call represent how energy in the call is distributed over different frequencies and are typically depicted using a power spectrum (Figure 1.6).

Peak frequency: the frequency produced with maximum amplitude.

Harmonics: direct multiples of the peak frequency.

Bandwidth: range of frequencies containing most of the signal energy. Narrow frequency band call is tuned while broadband call has wide range of frequency.

Q factor: a measure of the sharpness of tuning (Q) is obtained by dividing the peak frequency by the bandwidth 3 dB or 10 dB down.

SPL (Sound pressure level)

It is the loudness of the call measured in dB. Sound pressure level is defined by SPL = 20 log P/Pr where P is the pressure and Pr the pressure reference.

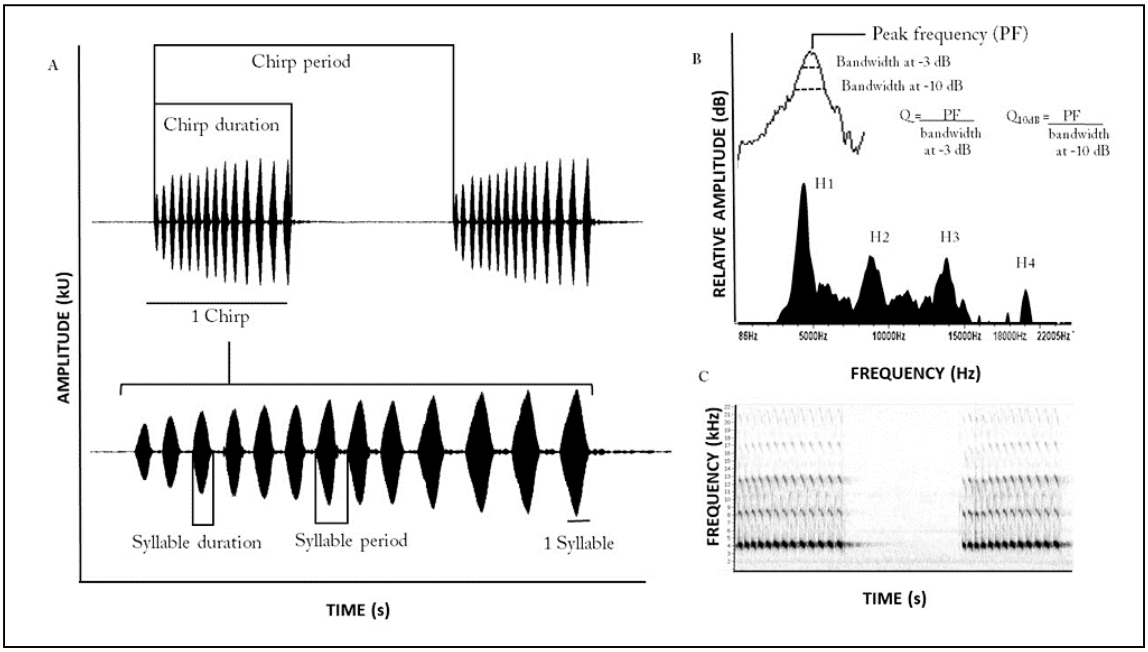

Figure 1.6. Characteristics of a cricket long-distance mating call: Terminology used. A. Oscillogram showing temporal features. B & C. Power spectrum and spectrogram respectively showing spectral features: peak frequency, bandwidth, Q and harmonics (H1, H2, H3, H4).

1.4 Study species

To examine the effect of these environmental factors on calling behaviour of field cricket, I selected *Acanthogryllus asiaticus* as the model species (Figure 1.7). *Acanthogryllus asiaticus* belongs to Gryllidae family and is native to the Indian subcontinent (Gorochov 1990). Males of *A. asiaticus* call from naturally occurring cracks, temporally shelters such as leaf litters or in open (field observation; Figure 1.8). Gorochov (1990) described of the habitus of *A. asiaticus* as follows:

"*Body size small for genus. Head large, red along entire length and angularly bent clypeal suture, apex of angle approximately at level or lower margins of antenna] pits. Color of head dark brown, with 6 distinct short longitudinal pale lines on posterior part of vertex. Pronotum dark brown, with pale spots in posterolateral angles of disk. Elytra with rather transverse stridulatory ridge, more or less rounded speculum, and distinctly bent diagonal vein, area between diagonal vein and oblique veins relatively wide. Color of elytra pale brown, with dark brown stripe along upper margin of lateral area. Legs, abdomen, and cerci brownish, more or less unicolorous. Genitalia without process in middle part of posterior margin of epiphallus, with very short epiphallic apodemes, and with middle processes extending from distal half or ectoparamere and only slightly extending beyond anterior ends of ectoparamere*s."

To my knowledge, this is the first study on this species despite their wide distribution.

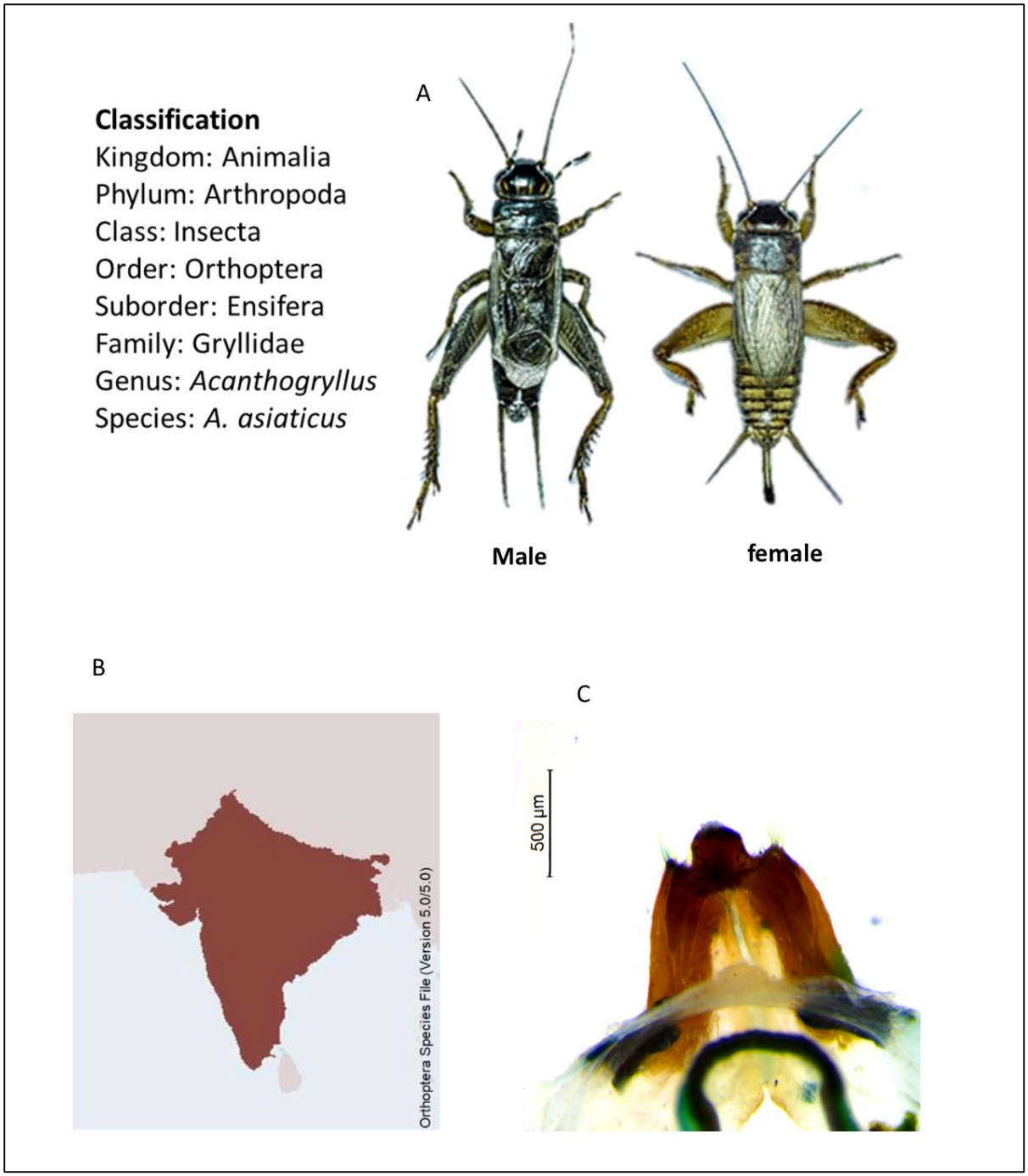

Figure 1.7. A. Habitus of *Acanthogryllus asiaticus* (picture credit: Nakul Raj) B. Distribution of *A. asiaticus* in Indian subcontinent C. Male genitilia of *A. asiaticus* (picture credit: Dr Ranjana Jaiswara).

Figure 1.8 Male of *Acanthogryllus asiaticus* in naturally occurring cracks

1.5 Thesis Objectives and Outline

To address the gap in knowledge in relation to the impact of three environmental factors on the behaviour, biology and acoustic signal characteristics of a nocturnal ectothermic insect, I outlined 4 major objectives. Each broad objective and the underlying sub-objectives are detailed below.

Objective 1. To study the natural history of the *Acanthogryllus asiaticus* with references to its calling behaviour, seasonality, diel cycle, calling apparatus and body size.

i. To examine the temporal variation of calling activity on a diel and seasonal scale.

ii. To determine the acoustic features of the call types produced in the context of reproduction: long-distance mating call (LDMC), courtship call and post-copulatory call.

iii. To investigate the relationships between sound-producing structures, body morphometry and peak frequency of LDMC.

Objective 2. To examine if lunar phobia exhibited by *A. asiaticus* and does artificial light affect their calling behaviour.

i. To examine the vertical and horizontal variation in light intensities of streetlights in the habitat of the species in order to assess the severity of ALAN.

ii. To examine whether light levels are different between full and new moon night and whether that impacts the calling behaviour of males. In other words, do male crickets exhibit 'lunar phobia'?

iii. To examine if areas illuminated by artificial light are brighter than those that are not and whether ALAN impacts calling behaviour of *A. asiaticus*.

iv. To examine if constant illumination affects calling rhythmicity and the role of melatonin in controlling the calling rhythm.

Objective 3. To investigate if acoustic masking interference problem present in *A. asiaticus*, if yes, how do they solve it.

i. To examine male spacing in the field to know if males aggregate in choruses during signalling.

ii. To determine the potential of acoustic masking interference by examining the degree of overlap of signal broadcast areas of signalling males in a given habitat.

iii. To examine the nature of acoustic interactions (if any) of a male with its nearest conspecific neighbour in field and lab environment.

iv. To investigate if males alter call features when calling in presence of a conspecific neighbouring signaller.

v. To test for evidence of Lombard effect in the species as a strategy to avoid masking interference.

Objective 4. To study the effect of traffic noise on the calling behaviour of *A. asiaticus.*

i. To acoustically characterize profiles of ambient noise in regions of the very low and high incidence of traffic in areas where animals are present.

ii. To examine population-level differences in signal characteristics s of males inhabiting 'noisy habitats' with chronic traffic noise and those from 'quiet habitats' without traffic noise.

iii. To investigate whether naïve males make any immediate adjustments to their calls or behaviour in response to short-term exposure to traffic noise.

Objective 5. To examine the effect of temperature on development and calling behaviour of *A. asiaticus*

i. To investigate the impact of temperature on life-history traits, such as hatchability, survival to adulthood, developmental lifespan, adult lifespan, total lifespan and body morphometry.

ii. To examine seasonal variation in temporal and spectral features of the calls of the species.

iii. To examine the influence of change in immediate ambient temperature on the call features.

iv. To determine the impact of developmental temperature on the calling song.

Chapter 2 of my thesis deals with Objective 1 and also serves as an introduction to *A. asiaticus*. Objective 2 on examining the effect of natural and artificial light on the calling behaviour of *A. asiaticus* is discussed in **Chapter 3**. In **Chapter 4**, I present the findings of Objective 3 on examining the effect of natural noise on the calling behaviour of *A. asiaticus* and the findings of Objective 4 which deals with the effect of anthropogenic noise on the calling behaviour of *A. asiaticus* is discussed in **Chapter 5**. Findings of Objective 5 which deals with examining the effect of temperature on life-history traits and the calling behaviour of *A. asiaticus* are presented and discussed in **Chapter 6**. The **Chapter 7** of the thesis is the conclusion which gives the complete picture of the findings from all the objectives and I suggest the future lines of investigations stemming from the insights gained from this study. I believe that the body of work described in this thesis will further our understanding on how insects solve various problems related to signaling and cope with this rapidly changing world. I also hope that the work described in this thesis will also offer insights on the severity of anthropogenic changes and empower policy decisions towards mitigating some of these pervasive alterations to our natural environment.

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Chapter 2

Calling activity patterns, intersexual call types and call producing structures in a field cricket, *Acanthogryllus asiaticus*

Male of *A. asiaticus* courting a conspecific female and producing a courtship call

Singh R, Jain M. 2020. Variation in call types, calling activity patterns and relationship between call frequency and body size in a field cricket, *Acanthogryllus asiaticus*. Bioacoustics.1-9.

2.1 Introduction

The diversity of signals used in animal communication is intriguing. Among the various sensory modalities, acoustic signals are considered to be the principal mode of long-distance communication used by organisms (Bradbury and Vehrencamp 1998). This type of communication is widely used across taxa, ranging from invertebrates like orthopteran insects to large mammals such as whales (Bradbury and Vehrencamp 1998). In insects, crickets are well known for sound production as it modulates some of their most conspicuous behaviours, especially reproduction (Gerhardt and Huber 2002). Their acoustic behaviour depicts temporal organization on the seasonal and diel scale (Gerhardt and Huber 2002). Various ecological, physiological and environmental factors may drive such temporal organization of behaviour. For instance, resource availability, population abundances, parasitism and inability to face extreme environments are known to drive seasonality in insects (reviewed in Wolda 1988). One of the environmental factors which is likely to influence behaviour in crickets, given that they are ectotherms, is temperature (Walker 1975). Emergence, growth, development, reproduction and life cycle stages of crickets can be estimated by understanding the seasonal pattern of calling activity (Masaki and Walker 1987). Diel partitioning of calling activity can be driven by optimal atmospheric conditions for sound transmission, energetic constraints of both signallers and receivers (Gerhardt and Huber 2002), activity time of parasites (Cade 1975; Bertram 2002; Velez and Brockmann 2006), intrasexual aggression (Cade 1979) and the availability of receptive females (Walker 1983; Sakaluk 1987). Moreover, diel partitioning of calling activity also occurs to avoid masking interference from other species which signal at the same time and space (Greenfield 1988). Studies investigating seasonality and diel activity patterns in ensifera have been carried out both at the species level (*Gryllus veletis, G. pennsylvanicus, and G. integer*: French and Cade 1987; *G. rubens*: Velez and Brockmann

2006; *G. texensis*: Bertram 2002; *Cyphoderris strepitans*: Sakaluk and Snedden 1990; *Gryllodes supplicans*: Sakaluk 1987) and the assemblage level (ensiferan assemblage of a tropical rainforest: Diwakar and Balakrishnan 2007; Jain et al. 2014). Investigating such temporal organization of behaviour can provide substantial information about how ecological and environmental factors shape the biology of an organism.

In crickets, acoustic signalling functions only among adults. Generally, males broadcast species-specific calls for the acquisition of a conspecific mate (Alexander 1967). Depending on the location of the females, male crickets tend to produce long-distance as well as short-distance signals, which are directly or indirectly associated with reproduction (Alexander 1967). Three call types in the context of intersexual interaction have been characterized in crickets: a) calling song; b) courtship song; c) post-copulatory song (Alexander 1967). Calling song is a long-distance mating call (LDMC) which is produced as a public signal by a male to attract a sexually responsive female over a long distance (1967). These are condition dependent calls (Wagner and Hoback 1999; Scheuber et al. 2003; Holzer et al. 2003; Hedrick 2005; Judge et al. 2008) and the call components signal male body size, quality, age and attractiveness (Brown et al.1996; Ryder 2000; Deb et al. 2012). When a female is in close proximity, courtship call (CC) is produced by the male to initiate physical contact with the female (Alexander 1967). This call elicits a mounting response in the female which is required for successful mating (*Teleogryllus oceanicus*: Balakrishnan and Pollack 1996; *Acheta domesticus*: Nelson and Nolen 1997). This call is found to be nutrition independent (Wagner and Reiser, 2000). On the execution of mating, after unmounting of female, male produces post-copulatory calls (PCC) (Alexander 1967). The exact function of post copulation calls is unknown and they could be required either for subsequent copulation or during mate guarding, to prevent the female from removing the male's spermatophore before insemination is complete (Alexander 1967). While there

is a multitude of studies on the description and function of LDMC and CC, PCC completely lacks a detailed study on its call structure and precise function.

Male crickets are capable of producing calls because they have evolved very complicated and well-designed sound-producing and resonating structures, namely, the file, plectrum, harp and mirror. During stridulation, an upper side wing with a file of a row of teeth is stroked by plectrum on the lower side wing producing a series of impacts with the file teeth and releasing energy which gets transmitted to the surrounding resonating sound apparatus; mirror and harp (Bennet-Clark 2003). The harp is the significant component of the resonating structure, thereby impacting the frequency of the sound produced (Bennet-Clark 2003). Investigating the relationship between body morphometry, sound-producing structures and call parameters provides information about whether acoustic signals can be reliable indicators of male traits such as body size which is preferred by females during mate choice (Simmons 1986; Brown et al. 1996; Gray 1997; Deb et al. 2012)

This study aims to comprehend the acoustic behaviour in a field cricket, *Acanthogryllus asiaticus,* by focusing on the three primary objectives:

1. Examining the temporal variation of calling activity on a diel and seasonal scale.

2. Determining acoustic features of the call types produced in the context of reproduction: long-distance mating call, courtship call and post-copulatory call.

3. Investigating the relationships between sound-producing structures, body morphometry and peak frequency of LDMC.

Acanthogryllus asiaticus is native to the Indian subcontinent (Gorochov 1990) and to our knowledge, this is the first study on this species despite their wide distribution.

2.2 Materials and methods

2.2.1 Study location and rearing of animals

The study was carried out from July 2015 to July 2017 at Indian Institute of Science Education and Research campus in Mohali (30°39'N, 76°43'E). Mohali has a humid sub-tropical climate and falls under the 'Cwa' category of Köppen-Geiger climate classification (Kottek et al. 2006). There are three seasons, summer, monsoon and winter with a transitional post-monsoon season. The seasonal and diel cycle was examined in three selected natural habitats of about 225 m^2 each, devoid of any artificial light sources. The vegetation was predominantly grassy with intermittent canopy of trees such as *Populous deltiodes*, *Ficus religiosa* and *F. glomerata*.

Calls analyses was carried-out on wild-caught and laboratory reared crickets while morphometry was performed on lab-bred crickets. Individuals were placed in plastic containers (diameter-12 cm and height-6 cm) covered with cloth mesh. *Ad libitum* food and water were provided and all animals were maintained at 24°C, 40 - 70 % humidity,12:12h light:dark condition in a climatic chamber (Memmert GmbH+Co.KG, Germany). The food provided was Pedigree dry dog food which included 24% of crude protein, 10% of crude fat and 5% of crude fibre. Each adult individual was fed with 2 pellet of the dog food after every 3 days while nymphs were provided with 50 mg powder of crushed pellets after every 3 days.

2.2.2 Temporal variation in calling activity patterns

Calling activity of *A. asiaticus* was monitored by conducting a census of calling males in 3 sampling habitats (Figure A2.1) using psychoacoustic sampling. It is a reliable and non-invasive method to monitor orthopteran species diversity in the given area by a trained

observer who listens and counts the number of calling individuals in given time slots (Diwakar et al. 2007). Census was done from 1800 to 0600 h following a systematic staggered sampling protocol (Figure A2.1). A total of 6 hours of sampling was done every night in two slots of 3 hours each with a 3 hours' rest period in between. Each sampling hour was divided into three sampling slots of 10 minutes. Each sampling slot alternated with a 'rest slot' lasting 10 minutes. During each sampling slot, a designated sampling location was visited and all calling males were psychoacoustically localized within a minute. Census was only done from walking paths along the periphery of plots and not by walking across the plots to avoid disturbing calling animals. Weather parameters such as humidity and temperature were measured just above the ground for every sampling hour using a pocket weather meter (Kestrel 4000, Nielsen-Kellerman, Chester, U.S.A.). Diel calling pattern assessment was carried out for 12 hours between 1800 to 0600h for 270 nights over a period of 12 months from August 2015 to July 2016. Seasonality of calling activity was recorded by conducting census using the same protocol as described above. However, in this case, the census was limited between 1900 to 0200h and the work was done for 543 nights across 24 months between August 2015 to July 2017. Observations were carried out by sampling one natural habitat in a night followed by subsequent sampling in other habitats. Average number of calling males per habitat per minute was calculated on basis of number of callers counted on all replicates in all the habitats in a given time slot.

2.2.3 Call types

LDMC were recorded for wild caught and laboratory-reared virgin adult males (N=25; 2-4 weeks old). In order to record courtship calls (CC) and post copulatory calls (PCC), mating trials (N = 20) were conducted in plastic containers (diameter = 15 cm and height = 18 cm)

wherein a receptive virgin female was introduced to a focal male. All audio recordings were made as 16 bit WAV files at sampling rate of 44.1 kHz using Tascam, Linear PCM Recorder (DR-07 Mk II, TEAC Professional, USA) and the sound pressure level (SPL) dB (LAF in 1/3-octave bands) was measured at a distance of 50 cm from the calling male using a Brüel & Kjær ½″ microphone, Type 4189 (20 Hz to 20 kHz) attached to a Sound Level Meter, Type 2270 (Brüel & Kjær, Naerum, Denmark). For PCC analysis, only calls that were produced after spermatophore transfer were taken into consideration. All the recordings were done in dark silent room (ambient noise at 15 dB at 5 kHz) maintained at 24°C. 10 acoustic parameters, namely, chirp duration, chirp period, syllable duration, syllable period, number of syllables per chirp, syllable repetition rate, peak frequency, harmonics, bandwidth and Q were used for call analysis to characterize the temporal and spectral features of the calls (described in Chapter 1). Q was calculated both at -3dB and -10dB (Bennet-Clark 1999). Raven Pro 1.4 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY) was used for temporal and spectral analysis of the calls whereas and Audacity 2.1.2 Cross-Platform Sound Editor was used to generate the power spectrum for the calls.

2.2.4 Body morphometry and sound-producing structures

Live laboratory bred males were weighed using an analytical balance (Sartorius BSA224S-W, Sartorius AG, Goettingen, Germany) and their LDMC were recorded. After dying naturally, animals were wet preserved in 70% ethanol and the right forewing of males was dissected and used for all morphological measurements. Wing length and width, pronotum length and width, harp and mirror area and right hind leg tibia for 20 individuals were measured using a digital camera (Leica MC120HD, Leica Microsystems GmbH, Wetzlar, Germany) connected with Leica Stereo Zoom Microscope (M 205C, Leica Microsystems

GmbH, Wetzlar, Germany). File length, inter-tooth distance and teeth width (N = 8) were studied using scanning electron microscope (SEM) (JEOL JSM-840 SEMTech Solutions, North Billerica, MA). SEM images were used to count the number of teeth using ImageJ (version 1.50i, National Institute of Health, USA). The file length was separated into three regions: apical, middle and basal (Rakshpal 1960; Montealegre-Z and Mason 2005).

2.2.5 Statistical analyses

Statistical tests were performed using Statistica 64 (Dell Inc.2015, Version 12) and R version 3.3.1. (R Core Team, 2016). Shapiro-Wilk test for normality revealed that data of calling activity (both diel and seasonal pattern) and calls were not normally distributed. A non-parametric Kruskal – Wallis test was done to examine the effect of time of the night and year on calling activity. The same test was carried out to examine overall differences between the three calls. Co-efficient of variation was measured for the call parameters of all the calls. This was followed by pairwise comparison using Mann-Whitney U test (with Bonferroni correction adjustment) on both data sets. Polar plots of diel calling across different months were generated using R packages "ggplot2". To examine the relationships between sound producing structures, morphometry and call (peak frequency), Pearson correlation test was done (as the data followed normal distribution). For Q_{3dB} and tooth width comparison, one-way ANOVA was done followed by Tukey's HSD test.

2.3 Results

2.3.1 Temporal variation in calling activity patterns

Seasonal variation in calling pattern was recorded for 24 months and significant difference in calling activity was found across the seasons (Kruskal-Wallis: $\chi^2 = 169.32$, df = 3, $P < 0.01$; Figure 2.1; Table A1). Peak calling activity was observed during Summer (Mar –

May) while least activity was recorded during winter (Dec – Feb). Moreover, calling activity found to be affected by change in humidity and temperature across the seasons (Figure 2.1 & A2.2).

Calling activity varied on a diel scale for all the months of the year as the peak calling period was different for different months (Figure A2.3). Since the peak calling activity was recorded between Mar – May, it was most relevant to examine the diel pattern of calling in these months. There was a significant difference in diel calling activity of crickets during this season over 12-hour period and 2100-0000 was found to be the peak calling time (Kruskal-Wallis: $\chi 2 = 182.34$, df = 3, $P < 0.01$, Figure 2.2).

2.3.2 Call types and comparison

LDMC were found to be highly stereotypic with all chirps having the same features whereas CC and PCC were composed of two different kinds of chirps each: short chirps (CCS and PCS respectively) and long chirps (CCL and PCL respectively) (Figure 2.3). A chirp of LDMC was composed of 13-14 syllables with chirp period of 0.9 ± 0.1s (Mean ± SD) (Table 2.1) and within a chirp, syllable duration as well as syllable period found to be increased with syllable number (Figure A2.4). While chirp of CCS and PCS were found to be composed of 3 syllables, chirps of CCL and PCL were found to be composed of 9 and 12 syllables respectively (Table 2.1). We compared LDMC, CC and PC considering CCS, PCS, CCL and PCL separately. All calls were found to be significantly different from each other due to difference in one or more acoustic parameters except CCS and PCS, which were not found to be different by any parameter (Kruskal-Wallis Test, Figure 2.4, Table 2.2). These calls varied from each other more by temporal features than spectral features. The power spectrum of these calls showed the presence of at least 5 harmonics considering the fundamental frequency as the 1st harmonic. While $bandwidth_{3dB}$ and Q_{3dB} also varied

across the calls, all three call types of *A. asiaticus* were narrowband with a maximum bandwidth$_{3dB}$ of 320 Hz and bandwidth$_{10dB}$ of 599 Hz found in CCL (Table 2.1 & 2.2). LDMC is narrowband with a Q_{3dB} of 16.04 ± 3 (Mean ± SD) which is typical of field crickets (Bennet-Clark 2003).

2.3.3 Body morphometry and sound-producing structures

The average number of teeth observed on male right forewing was found to be 121.86 ± 9.5 (Mean ± SD). The inter-tooth distance increased until middle region and then declined in basal region (Figure 2.5A & B). Tooth width in all the three regions were found to be significantly different from each other with highest width in middle and lowest in the apical region (one-way ANOVA test, $P < 0.0001$, Figure 2.5C, Table A2.2). We found no correlation for number of teeth with file length, wing length or pronotum width (Figure 2.5D & Figure A2.5, Table 2.3), whereas file length was found to be highly correlated with wing length, width and pronotum width (Figure 2.5E, Table 2.3). Harp and mirror area were found to be significantly correlated with each other (Figure A2.6, Table 2.4). Harp area was also found to be significantly correlated with pronotum width, wing length and wing width but not with hind leg tibia length. (Figure 2.6B & Figure A2.6, Table 2.4). Peak frequency of LDMC was found to be significantly negatively correlated with harp area (Figure 2.6C) but no significant correlation was found with pronotum width or other body size parameters (Figure 2.6D & Figure A2.6D).

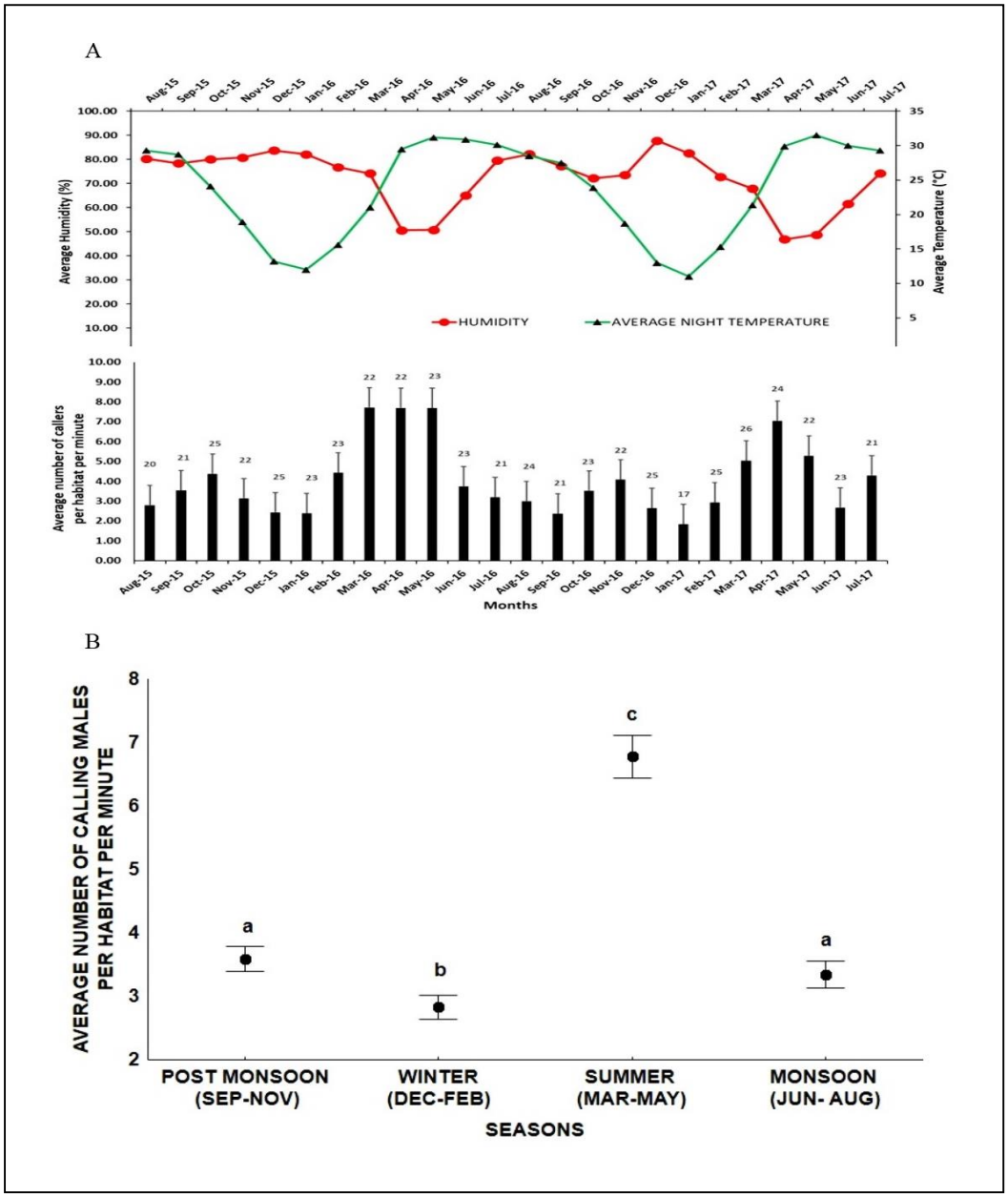

Figure 2.1. Seasonal variation in the calling activity of *A. asiaticus*. A. Variation in calling activity (represented as Mean ± SD), average temperature and average humidity across 24 months (2015-2017). Values on the top of each bar represent number of nights sampled for the respective month. B. Calling activity across the four seasons represented as Mean ± CI. Different letters a, b and c indicate significant differences.

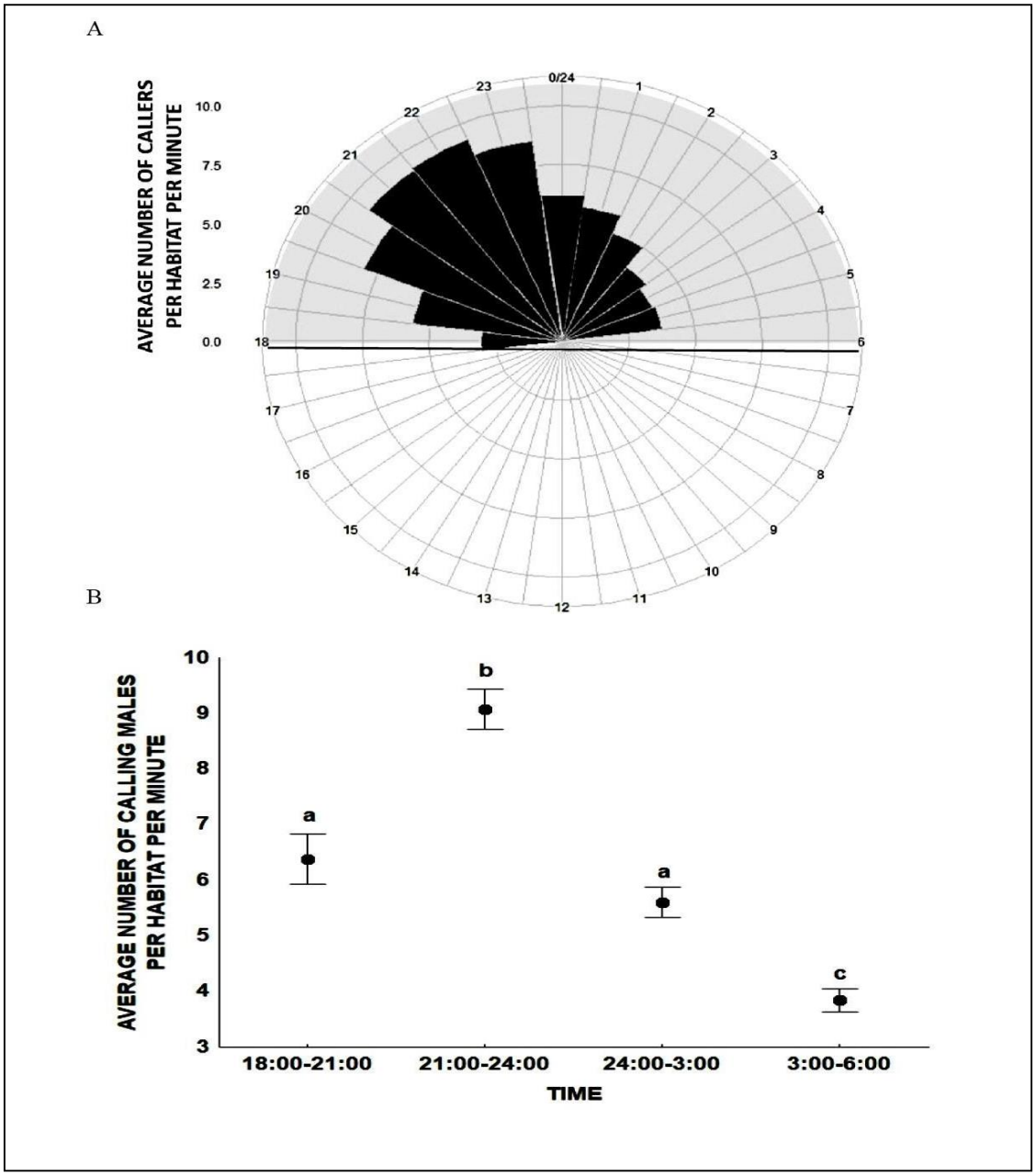

Figure 2.2. Diel variation in the calling activity of *A. asiaticus*. A. Polar plots showing the diel calling activity for 12 hours (1800-0600 hrs). Radius of the polar plot shows frequency of calling males and circumference shows 24 hours-time period. B. Calling activity across 12 hours between 1800-0600 hrs represented by Mean ± CI. Different letters a, b and c indicate significant differences.

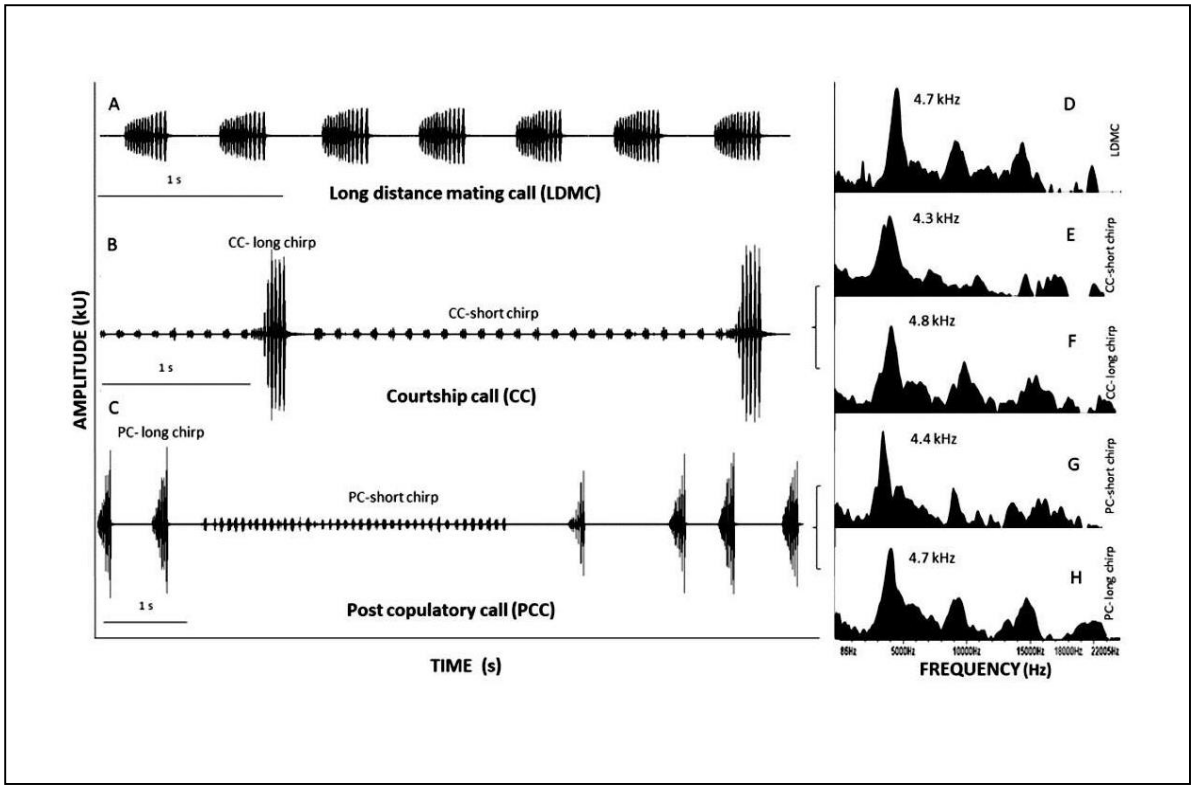

Figure 2.3. Types of calls produced during inter-sexual interaction in *A. asiaticus*. Oscillogram of A. LDMC, B. CC with two components: short chirps & long chirps, C. PC with two components: short chirps & long chirps. Power spectra of D. LDMC, E & F. CC with two components: short chirps & long chirps respectively G & H. PC with two components: short chirps & long chirps respectively.

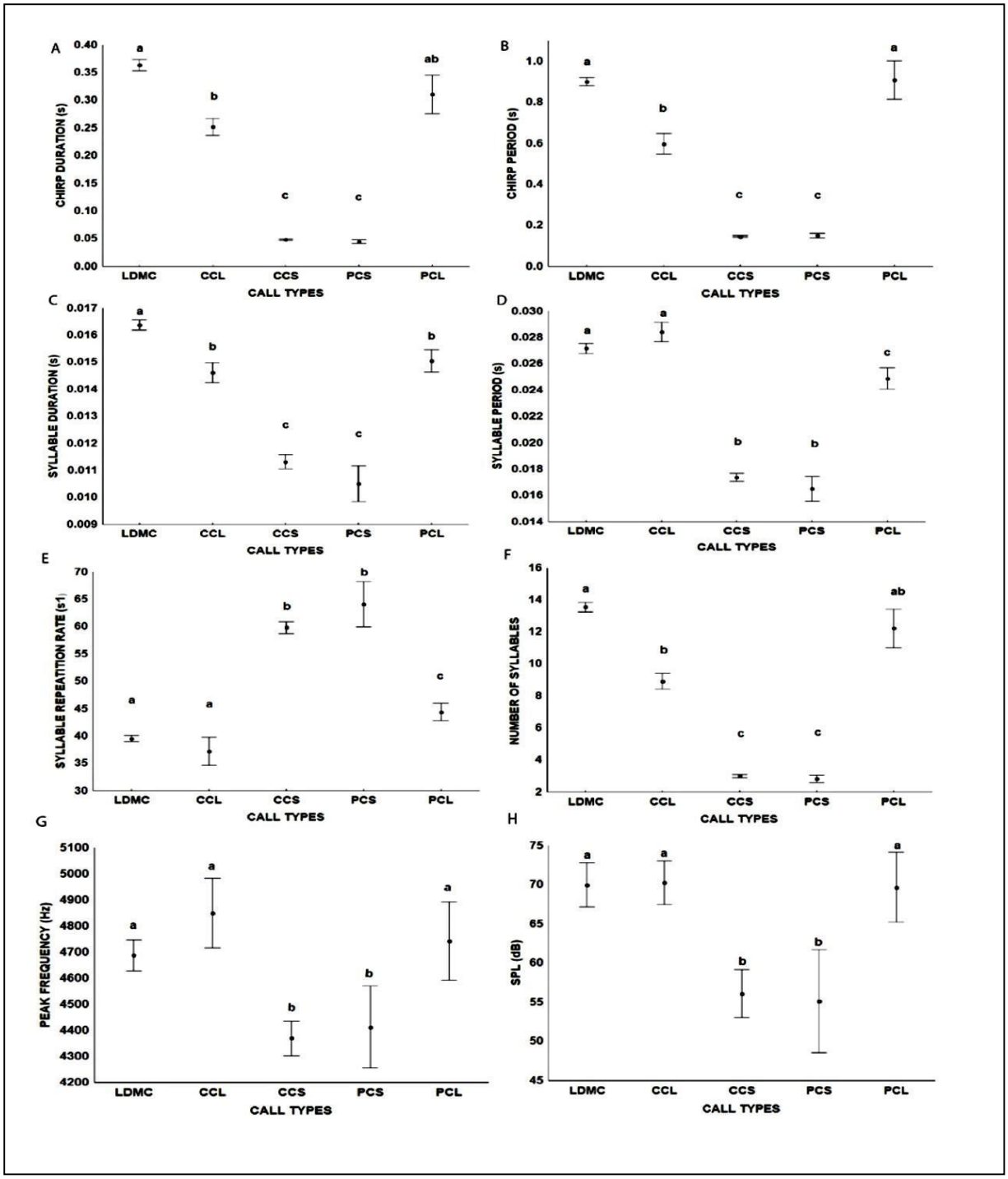

Figure 2.4. Comparison of temporal, spectral components and loudness of three call types LDMC, CC (CCL & CCS), PC (PCL & PCS) in *A. asiaticus*. A. Chirp duration B. Chirp period C. Syllable duration D. Syllable period E. Syllable repetition rate F. Number of syllable G. Peak frequency H. Loudness. Each bar represents Mean ± CI. Different letters a, b and c indicate significant differences.

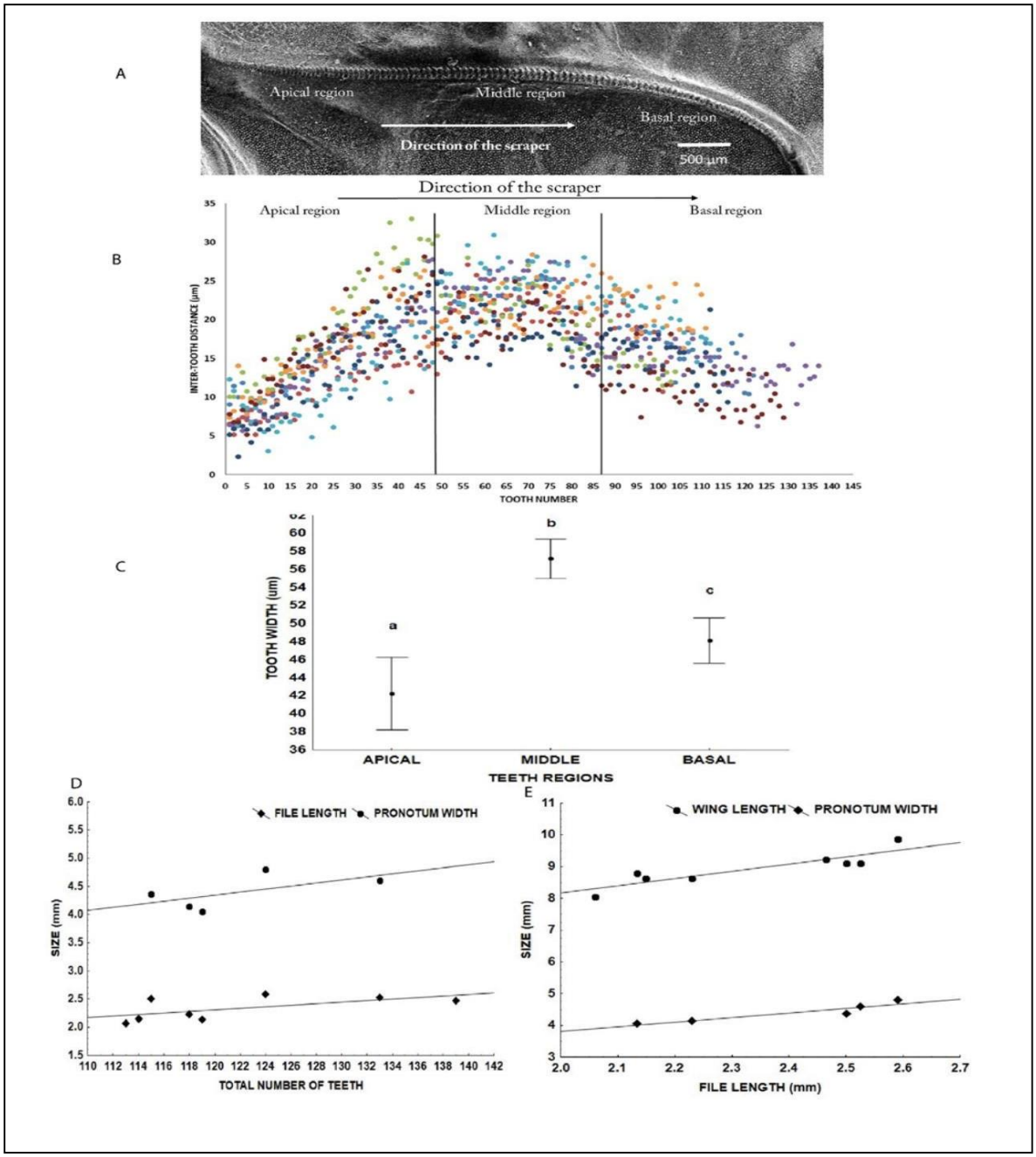

Figure 2.5. Detailed morphology of sound producing structures: file and file teeth of *A. asiaticus* (N = 8). A. Scanning Electron Micrograph of the stridulatory file. B. Inter-tooth distances over the file length. Different colour dots represent individuals. C. Comparison of teeth width in three different regions of stridulatory file. Each bar represents Mean ± CI. Different letters a, b and c indicate significant differences. D. Relationship between total number of teeth with file length and pronotum width. E. Relationship between file length with wing size and pronotum width.

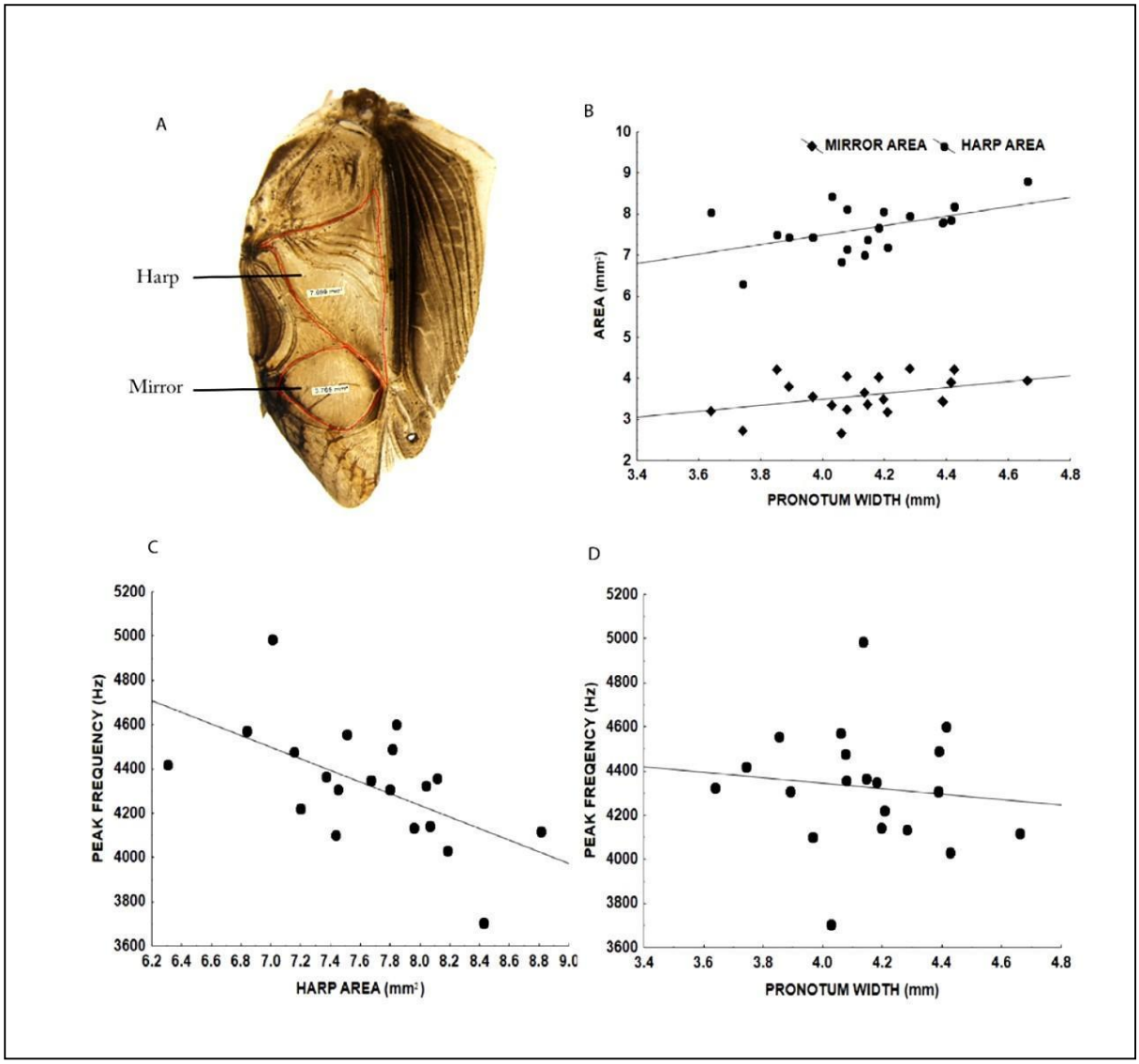

Figure 2.6. Relationship between body morphology, sound producing structure and call characteristics of *A. asiaticus*. A. Right forewing showing harp area and mirror area. B. Relationship between pronotum width and harp area and mirror area. C. Relationship between peak frequency of LDMC (recorded at 24°C) and harp area. D. Relationship between peak frequency of LDMC with pronotum width.

Table 2.1. Descriptive statistics for the call characteristics of LDMC, CC and PCC of *A. asiaticus*. N represents number of chirps or syllables measured (as applicable).

	Long distance mating call			Courtship call						Post-copulatory call					
		LDMC (25 males)			CCL (17 males)			CCS (20 males)			PCL (6 males)			PCS (6 males)	
CALL PARAMETERS	N	MEAN ± STDEV	CV	N	MEAN± STDEV	CV	N	MEAN± STDEV	CV	N	MEAN± STDEV	CV	N	MEAN± STDEV	CV
Chirp duration (s)	250	0.364 ± 0.085	23.36	72	0.253±0.065	26.03	246	0.049± 0.011	23.59	30	0.311±0.09	29.91	30	0.045±0.008	19.67
Chirp period (s)	249	0.902 ± 0.16	17.97	67	0.598±0.201	33.58	233	0.147±0.003	20.42	28	0.909±0.24	26.66	30	0.15±0.03	20.31
Syllable duration (s)	692	0.016 ± 0.003	22.08	616	0.015±0.005	32.49	692	0.011± 0.003	30.00	339	0.015±0.004	25.81	79	0.011±0.003	28.06
Syllable period (s)	692	0.028 ± 0.007	26.01	533	0.028±0.009	31.26	481	0.017± 0.003	19.81	369	0.025±0.008	31.65	67	0.017±0.004	23.47
No. of syllables/chirp	133	13.571 ± 1.78	13.12	72	8.93±2.14	23.94	195	3.010 ± 0.60	19.95	30	12.233±3.25	26.53	29	2.828±0.60	21.28
Syllable repetition rate (s^{-1})	692	38.629 ± 10.36	26.83	88	37.224±1211	32.53	481	59.84 ± 11.91	19.91	369	44.406±15.602	35.14	67	64.14±17.04	26.56
Peak Frequency (Hz)	250	4687.610± 482.08	10.28	73	4849.75±575.42	11.86	246	4369.461± 527.81	12.08	30	4743.047±401.40	8.46	30	4412.24±420.4	9.53
Bandwidth (-3dB)	25	308.6 ± 87.81	28.45	13	320.0769	22.86	19	217.63 ± 63.65	29.25	11	283.36±94.45	33.33	11	207.090±71.11	34.34
Bandwidth (-10dB)	25	522.64 ± 137.3	26.28	13	599.6154	18.67	19	436.263±116.60	26.73	11	523.73±151.12	28.85	11	391±168.97	43.22
Q3dB	25	16.04 ± 3.546	22.11	13	15.489±3.76	24.25	19	21.24 ± 5.34	25.16	11	18.57±5.63	30.31	11	21.949±3.95	18.00
Q10dB	25	9.42±2.075	22.03	13	8.1045±1.45	17.94	19	10.55± 2.56	24.31	11	9.935±3.044	30.64	11	12.2281±3.38	27.69
SPL (dB)	10	70±3.92	5.60	9	70.275±3.63	5.16	10	56.11±4.3	7.67	6	69.67±4.24	6.09	5	55.16±5.33	9.67

Table 2.2 A. Summary of Kruskal-Wallis ANOVA analyses for the comparison of LDMC, CC and PCC on the basis of different call parameters. B. Pairwise comparison of call characteristics of different call types of *A. asiaticus* using Mann-Whitney U test applying Bonferonni correction. ns represents no significant difference.

Call Parameters	χ2	df	N	H	P
Chirp duration (s)	519.42	4	628	491.45	<0.01
Chirp period (s)	533.96	4	607	485.08	<0.01
Syllable duration (s)	591.52	4	3245	746.16	<0.01
Syllable period (s)	576.54	4	2864	814.68	<0.01
No. of syllables/chirp	433.72	4	459	389.02	<0.01
Syllable repetition rate (s^{-1})	601.49	4	2419	791.71	<0.01
Peak Frequency (Hz)	102.14	4	700	86.23	<0.01
Bandwidth (-3dB)	20.35	4	79	26.71	<0.01
Bandwidth (-10dB)	14.11	4	79	19.09	<0.01
Q_{10dB}	14.43	4	79	14.73	<0.01
SPL (dB)	24.71	4	40	27.52	<0.01

CALL PARAM-ETERS	Chirp dura-tion (s)	Chirp period (s)	Syllable dura-tion (s)	Sylla-ble period (s)	No. of sylla-bles/chirp	Syllable repeti-tion rate (s^{-1})	Peak Fre-quency (Hz)	Band-width (-3dB)	Band-width (-10dB)	Q3dB	Q10dB	SPL (dB)
CALL TYPES												
LDMC VS CCL	<0.01	<0.01	<0.01	ns	<0.01	ns	ns	ns	ns	ns	ns	ns
LDMC VS CCS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	ns	<0.01	ns	<0.01
LDMC VS PCL	ns	ns	<0.01	<0.01	ns	<0.01	ns	ns	ns	ns	ns	ns
LDMC VS PCS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	ns	<0.01	ns	<0.01
CCL VS CCS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CCL VS PCL	ns	<0.01	ns	<0.01	ns	<0.01	ns	ns	ns	ns	ns	ns
CCL VS PCS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
CCS VS PCL	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	ns	ns	ns	ns	ns
CCS VS PCS	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
PCL VS PCS	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	ns	ns	ns	ns	ns	ns

Table 2.3. A. Descriptive statistics of wing morphometric parameters. B. Relationship between wing size, file length and total number of teeth in *A. asiaticus* showing P values and R for Pearson correlation coefficient. Significant correlation is indicated in bold.

A. Morphometric parameters	Mean	SD	N
wing length (mm)	8.9213	0.536791	8
wing width (mm)	3.1008	0.153394	8
file length (mm)	2.332	0.209461	8
total number of teeth	121.875	9.508455	8

B.		wing length (mm)	wing width (mm)	file length (mm)	total number of teeth
wing length (mm)	R				
	P				
wing width (mm)	R	**0.73**			
	P	**0.04**			
file length (mm)	R	**0.89**	**0.87**		
	P	**< 0.01**	**< 0.01**		
total number of teeth	R	0.55	0.47	0.62	
	P	0.16	0.23	0.10	
pronotum width	R	**0.90**	0.53	**0.93**	0.62
	P	**0.03**	0.35	**0.02**	0.27

Table 2.4. A. Descriptive statistics for morphometric and song parameters. B. Relationship between body morphology, sound producing structures and peak frequency in *A. asiaticus* showing P values and R for Pearson correlation coefficient. Significant correlation is indicated in bold.

A. Parameters	Mean	SD	N
pronotum width (mm)	4.138	0.2502	20
harp (mm2)	7.650	0.5832	20
mirror (mm2)	3.585	0.4633	20
pronotum length	2.348	0.2007	20
pronotum area	9.751	1.3225	20
wing length	9.100	0.5397	20
wingh width	3.441	0.1708	20
tibiae	4.917	0.2905	20
peak freq (hz)	4328.165	265.4268	20

B.		pronotum width (mm)	harp area (mm^2)	mirror area (mm^2)	pronotum length (mm)	wing length (mm)	wing width (mm)	tibia length (mm)
pronotum width (mm)	R							
	P							
harp area (mm^2)	R	**0.49**						
	P	**0.02**						
mirror area (mm^2)	R	0.38	**0.59**					
	P	0.08	**<0.01**					
pronotum length (mm)	R	**0.772**	0.22	0.39				
	P	**<0.01**	0.33	0.08				
wing length (mm)	R	**0.66**	**0.47**	0.55	**0.54**			
	P	**0.001**	**0.03**	0.12	**0.01**			
wing width (mm)	R	0.43	**0.48**	0.56	**0.49**	**0.54**		
	P	0.05	**<0.01**	0.008	**0.02**	**0.01**		
tibia length (mm)	R	**0.70**	0.35	0.37	**0.57**	**0.64**	0.28	
	P	**<0.01**	0.12	0.102	**<0.01**	**<0.01**	0.22	
peak frequency (hz)	R	-0.118	**-0.57**	-0.113	0.05	-0.12	0.21	-0.255
	P	0.6	**<0.01**	0.6	0.8	0.61	0.36	0.27

2.4 Discussion

2.4.1 Temporal variation in calling activity patterns

Environmental conditions are instrumental in shaping the seasonal and diel activity patterns and play an essential role in the temporal organization of the population and ecosystem functioning. In our study, we found calling activity patterns in *A. asiaticus* to vary with temperature and humidity (Figure A2). On a seasonal scale, peak calling activity was found during summer (Mar – May) while the least calling activity was found during winter (Dec – Feb). Contrary to our finding, peak calling season was found to be during winters for many species of ensifera in the evergreen rainforest of Karnataka (in southern India) (Diwakar and Balakrishnan 2007). This difference in the calling activity is likely due to the variation in the weather conditions for the same months across North and South India. Winter in northern India is extremely cold while in southern India, winter temperatures are moderate even at night. High calling activity during certain seasons indicates the abundance of active adults, appearance of reproductive activity and dispersal. On the other hand, the least calling activity can be due to the avoidance of unfavourable and extreme environmental conditions. A study on *G. bimaculatus* has reported similar results where they found that during winters when temperature decreases below to 15°C, males ceased their calling activity (Van Wyk and Ferguson 1995). In our study, we found that during summer, when the calling activity was at peak, the lowest night-time temperature was > 20°C while during winters, calling activity ceased, as highest temperature for winter was around 17°C.

Diel calling Pattern of *A. asiaticus* reveals the variation in 12 h sampling period (1800-0600h). Peak activity hours were found to be between 2100-2400h during peak seasons (Mar- May). Calling activity declined after midnight though some males were found to be

calling during the morning after sunrise. Decreasing temperatures towards dawn may drive this cessation of calling after midnight. In *Gryllodes supplicans*, calling activity peaked shortly after sunset, decreased gradually throughout most of the night and ended around dawn (Sakaluk1987). Similar results have been found in studies on ensiferan community (Diwakar and Balakrishnan 2007) of tropical forests and three *Gryllus* species (French and Cade 1987) of subtropical climate. Moreover, calling activity on diel scale, between 1800-0600h, also showed variation across the seasons and this is expected due to drastic temperature fluctuations. The extreme cold conditions may cease the calling activity between midnight to morning in winters. Diel pattern of calling activity also reveals the temporal availability of females, which means high activity of females during peak calling hours (Sakaluk 1987).

2.4.2 Call types and comparison

This study provides the first quantitative acoustic analysis of LDMC, CC in *A. asiaticus* and the first description of PCC in a field cricket. These calls were found to be significantly distinct from each other. LDMC found to be loud, narrowband, pure tone calls with long chirps and high chirp rates in which peak frequency lies between 4-5 kHz while CC and PCC are found to be composed of two different chirp types: longer loud chirps and shorter soft chirps of different frequencies. In support of our finding, similar characteristics for these calls have been reported in various field cricket species (Alexander 1967; Balakrishnan and Pollack 1996; Nelson and Nolen 1997; Rebar et al. 2009; Zuk et al. 2008; Harrison et al. 2013). Several studies have focused on the relative importance of different components of LDMC in mate choice decisions by female crickets. Females of several species prefer males whose calls composed of higher chirp rates (Scheuber et al. 2003), longer chirps (Simmons et al. 2001) and those with higher SPL (Walker and Forrest 1989).

Females in turn use CC as a reliable indicator of male attractiveness as shown in *T. oceanicus* females where they choose males based on elements of their courtship call and prefers males with longer courtship songs that have higher duty cycle (Rebar et al. 2009). Analysis of PCC of *A. asiaticus* showed that it is composed of a combination of LDMC-like longer and louder chirps as well as CC-like shorter and softer chirps. We found that PCC was produced rarely (only in 6 out of 20 mating observations). In Oecanthineae, CC and PCC were found to be similar and in *Miyogryllus* where LDMC were found to be similar to PCC, however, no acoustic characterization was reported (reviewed in Alexander 1967). PCC has been referred to as 'pair-maintaining signals', hypothesized to be produced in the context of courting the female for subsequent copulation or to discourage the female from eating the spermatophore by producing high-pitched chirps (Alexander 1967). However, there is no empirical evidence in support of this hypothesis in literature so far. We found that the coefficient of variation (CV) of various temporal parameters of calls to be relatively higher in CC and PCC than in LDMC. Presence of high variability in CC as compared to LDMC has been reported previously and it is proposed that this is because the former gives distinct information about male quality whereas the latter is essential for species recognition (Zuk et al. 2008).

2.4.3 Body morphometry and sound-producing structures

Our study reveals that there exists allometric relationship between sound-producing structures and body morphology. The positive correlation of stridulatory file length with forewing size indicates that larger males with big body size have longer file length. However, number of teeth does not found to be related to body size as well as file length. This is in contrast to a study on bush crickets, where it has been shown that larger files tend to have more number of teeth (Montealegre-Z 2009). The widest teeth found to be in the

middle region of the file, whereas narrower in the basal region as well as in apical region is similar to teeth conditions as described in *Acheta* species (Rakshpal 1960). The tooth spacing found to be gradually increasing (linearly) from the apical to the middle region and thereafter, the spacing decreases. Such a gradual increase in inter-tooth distance in the direction of scraper movement direction have also been noticed in bushcrickets such as *Panacanthus pallicornis* (Montealegre-Z 2005). Various studies have reported that teeth distribution and structure influence stridulatory behaviour in crickets (Robillard and Desutter-Grandcolas 2011) and help to determine the relative velocities of the tegmina at plectrum–tooth impact (Koch et al. 1988; Montealegre-Z and Mason 2005). For example, in *Anaxipha* latipennis and *Oecanthus exclamationis*, carrier frequency and pulse duration were found to be correlated with number and spacing of the file teeth and the distance and speed of movement of the scraper (Walker and Carlysle 1975). The relationship of acoustic parameters with stridulatory teeth spacing is still needed to be investigated in this species.

We also found harp area to be correlated with various body size proxies and peak frequency of LDMC to be significantly negatively correlated with harp area. However, peak frequency was not found to be significantly correlated with pronotum width, wing length and tibia length. Previous studies on the relationship between peak frequency and body size indicate different findings. For instance, no effect of body size on peak frequency was found in *G. bimaculatus*, (Miyashita et al. 2016) and *Plebeiogryllus guttiventris* (Nandi and Balakrishnan 2013). However, in *G. pennsylvanicus*, body size found to be significantly negatively correlated with peak frequency (Harrison et al. 2013). In *G. campestris*, peak frequency was not found to be significantly correlated with a direct measure of body size; however, a negative relationship with an indirect measure of body size was found (Simmons 1995). These previous studies have used different proxies for body size. For instance, body mass (Miyashita et al. 2016), morphometric measures such as head width,

pronotum width, area and height (Harrison et al. 2013) and in other study morphometric features such as pronotum width and tibia length as well as harp area (to examine size-frequency relationship) were used as proxies for body size (Simmons 1995). In our study, we found that peak frequency to be significantly negatively correlated with harp area and the same trend was observed for other proxies of size. This reveals that larger males are likely to have a larger harp area and large harp area in this species implies lower peak frequency. Therefore, peak frequency can be treated as a good indicator of body size in *A. asiaticus*. Similar findings have been reported in *G. bimaculatus, G. rubens, A. domesticus and T. oceanicus* (Moradian and Walker 2008) and *G. campestris* (Simmons 1995). A study on a tree cricket, *Oecanthus henryi* reported that while females do have a preference for larger males, they do not have a preference for lower frequency (Deb et al. 2012). Similarly, in *G. bimaculatus*, females did not show any preference based on carrier frequency (Verburgt and Ferguson 2010). Whether female of this species have any preference for body size and whether they use peak frequency or other call features as a cue for male quality during mate choice, is still to be investigated.

In conclusion, we have described that calling behaviour of *A. asiaticus* shows temporal organization on seasonal and diel scale and can produce three call types in the context of reproduction which are structurally different. This study reveals the natural history of this species and provides a vivid description of the relationship among body morphology, signalling and sound-producing structures.

2.5 References

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2.6 Appendix A

A

B

	NIGHT 1	NIGHT 2	NIGHT 3	NIGHT 4
Time slots	Habitat1	Habitat 2	Habitat 3	Habitat 1
18:00-19:00	*			
19:00-20:00	*	*		
20:00-21:00	*	*	*	
21:00-22:00		*	*	*
22:00-23:00			*	*
23:00-24:00				*
24:00-1:00	*			
1:00-2:00	*	*		
2:00-3:00	*	*	*	
3:00-4:00		*	*	*
4:00-5:00			*	*
5:00-6:00				*

Figure A2.1**.** Habitat of *A. asiaticus*. B. Staggered sampling protocol (shaded cells are the sampling hours). Every habitat was visited thrice in given sampling hour. Observations were carried out by sampling one natural habitat in a night followed by subsequent sampling in other habitats.

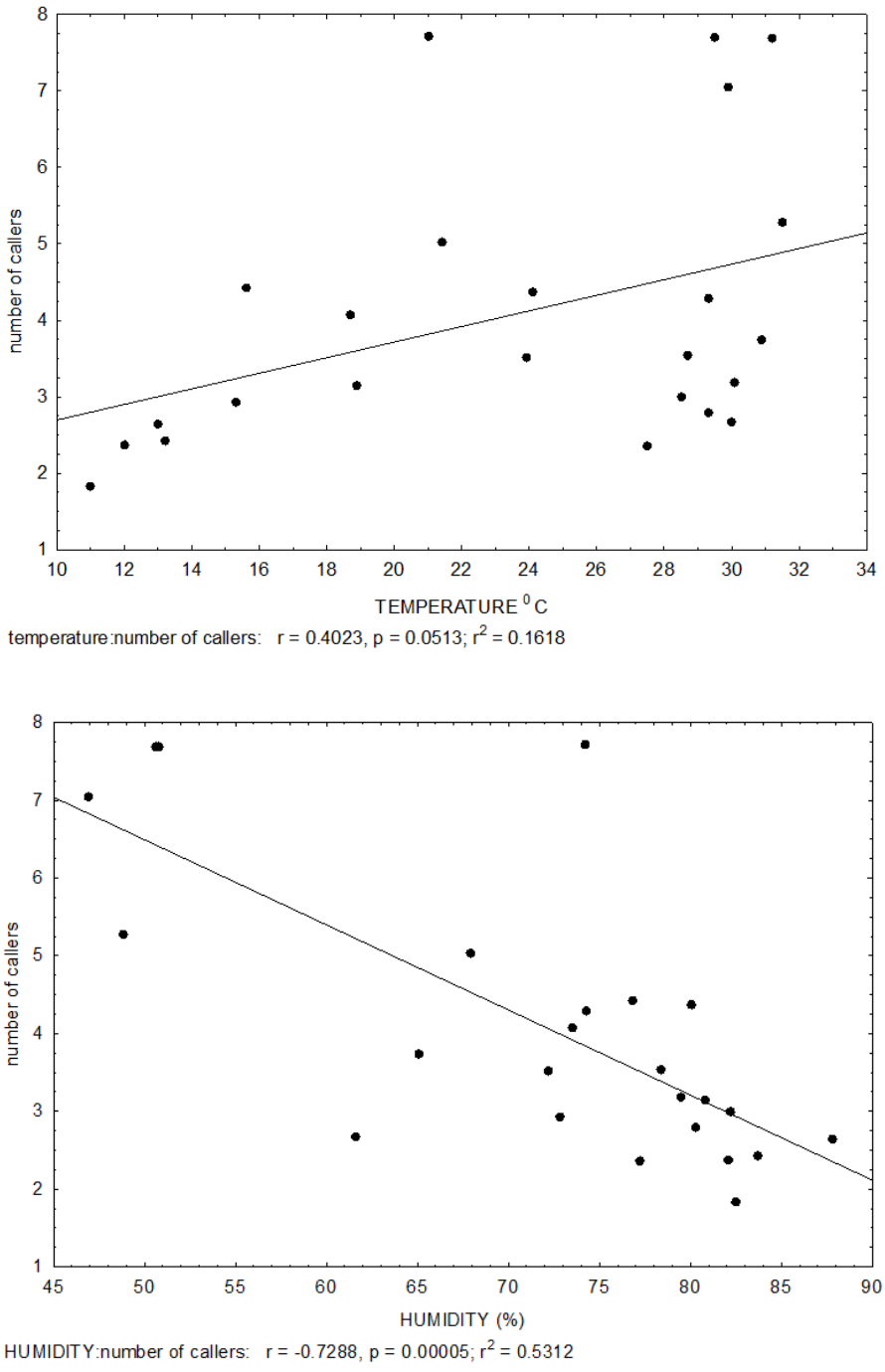


Figure A2.2. Relationship of calling activity with temperature and humidity

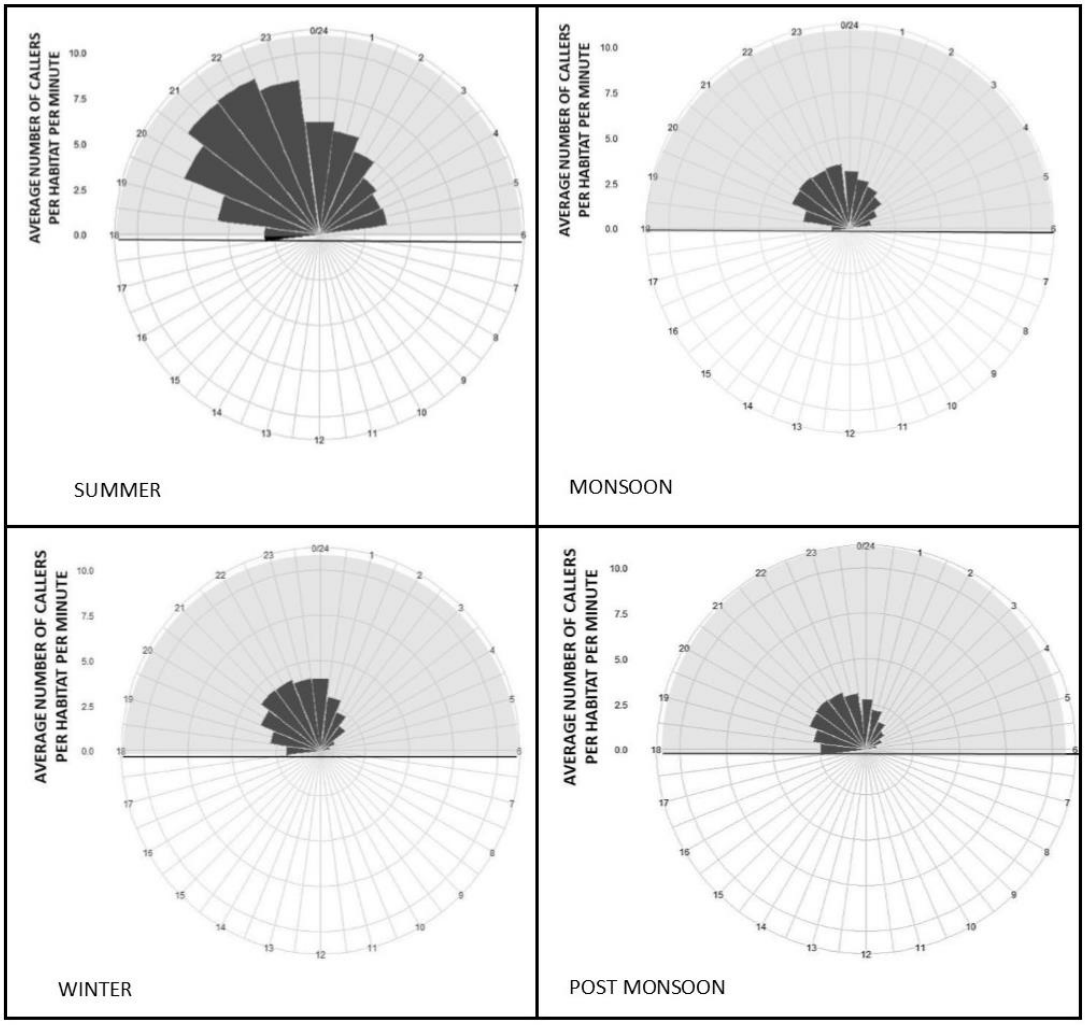


Figure A2.3. Seasonal variation in the diel calling activity of *A. asiaticus*. Polar plots showing diel calling activity for 12 hours (1800-0600 hrs) for the four seasons. Radius of the polar plot shows frequency of calling males and circumference shows 24 hours-time period.

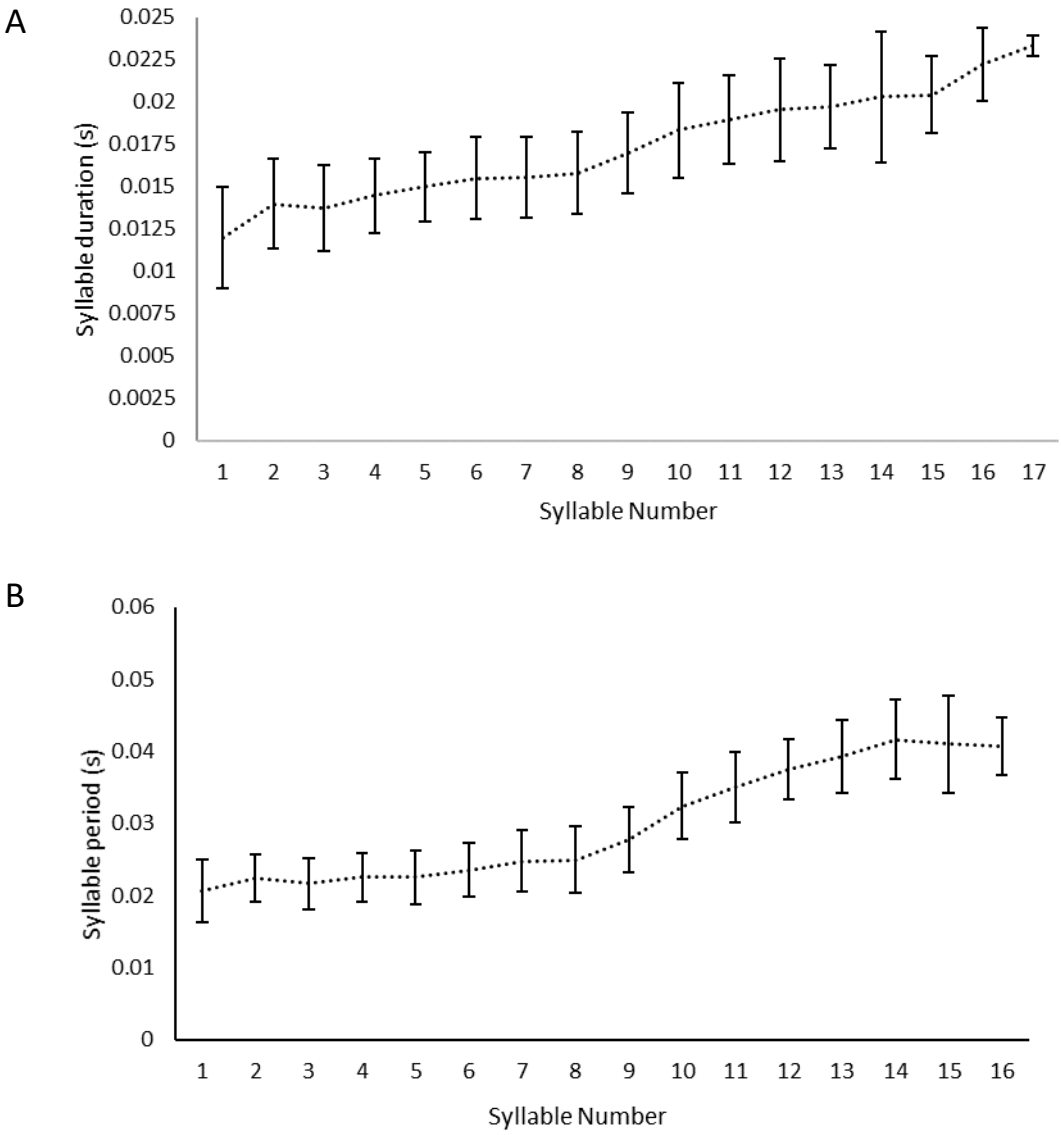


Figure A2.4. Syllable duration and syllable period gradually increases in a chirp of LDMC of *A. asiaticus*. (A) Syllable duration increased from the first syllable to the last. (B) Syllable period increased from the first syllable to the last. Each bar for every syllable position represents Mean±SD.

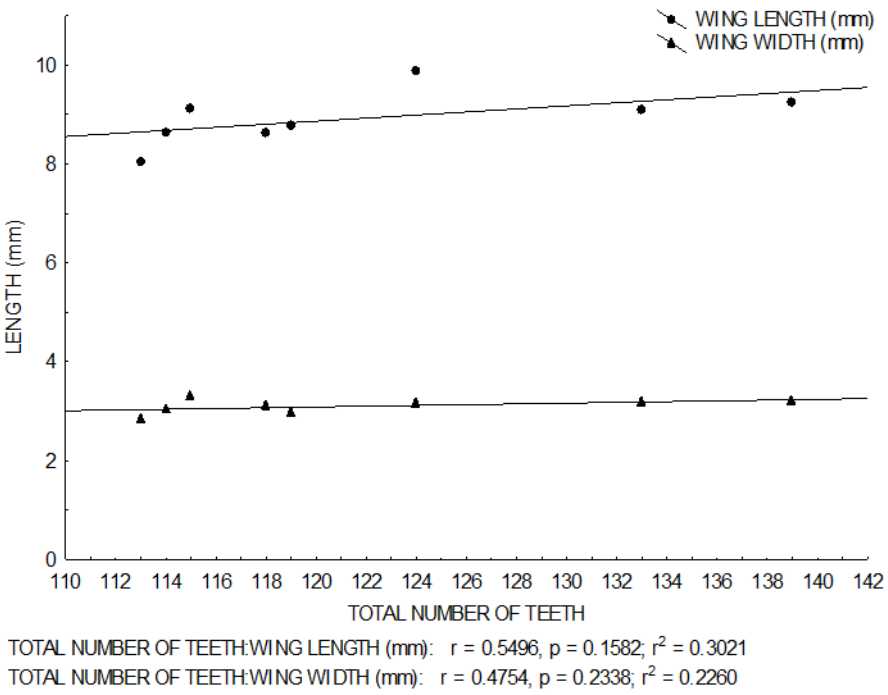
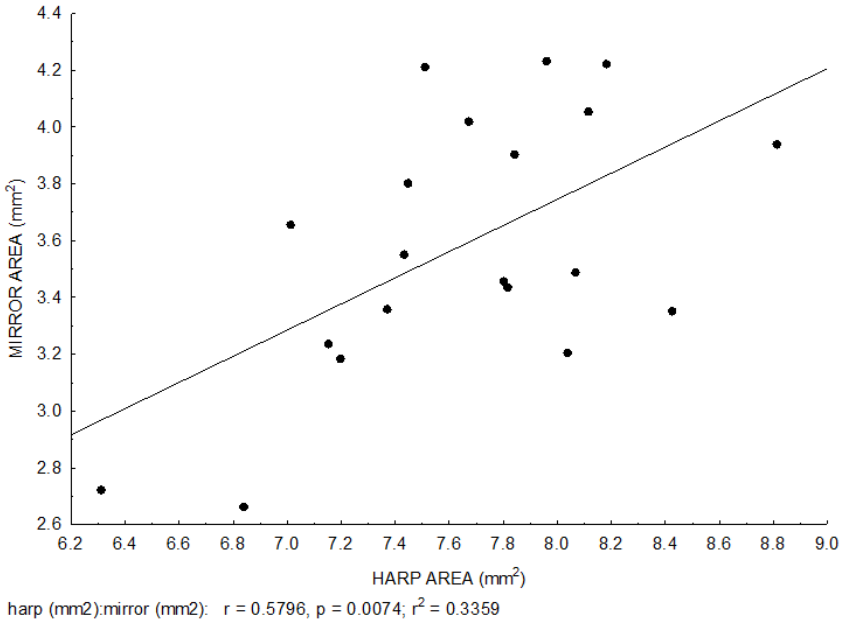
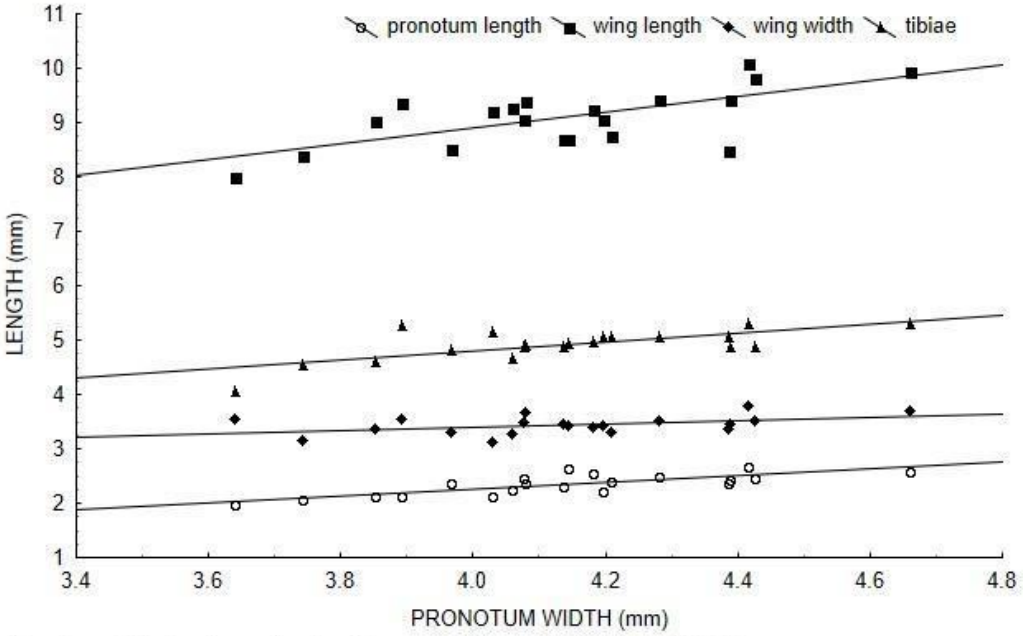


Figure A2.5. Relationship of wing morphology with number of teeth in stridulatory file.

A

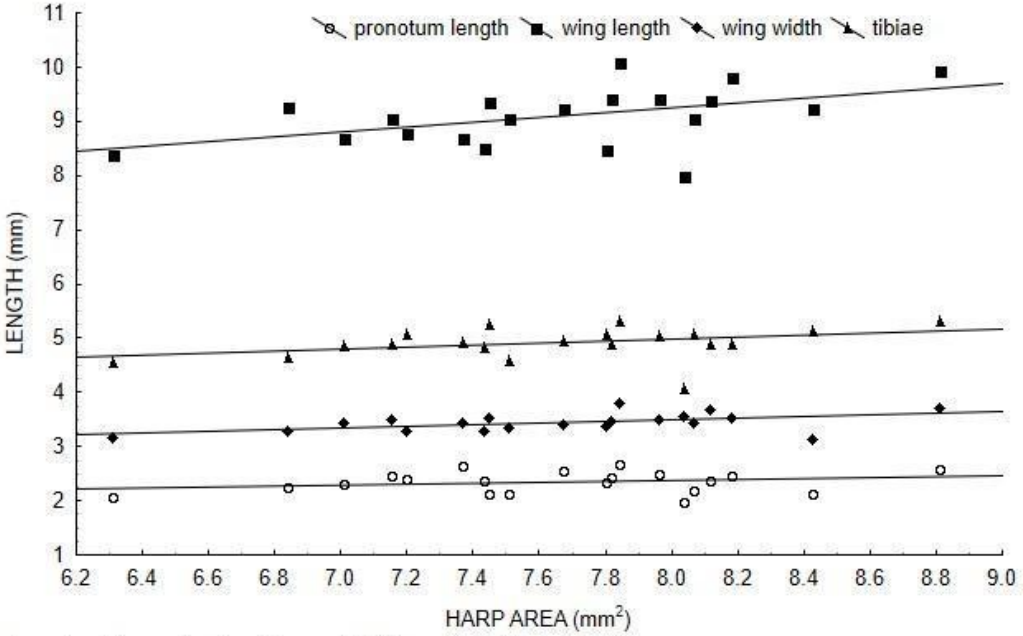


B



Pronotum width (mm):pronotum length: r = 0.7722, p = 0.00007; r^2 = 0.5962
Pronotum width (mm):wing length: r = 0.6684, p = 0.0013; r^2 = 0.4468
Pronotum width (mm):wing width: r = 0.4386, p = 0.0530; r^2 = 0.1924
Pronotum width (mm):tibiae: r = 0.7002, p = 0.0006; r^2 = 0.4903

C



harp (mm2):pronotum length: r = 0.2275, p = 0.3347; r^2 = 0.0518
harp (mm2):wing length: r = 0.4735, p = 0.0350; r^2 = 0.2242
harp (mm2):wing width: r = 0.4849, p = 0.0302; r^2 = 0.2351
harp (mm2):tibiae: r = 0.3552, p = 0.1244; r^2 = 0.1262

D

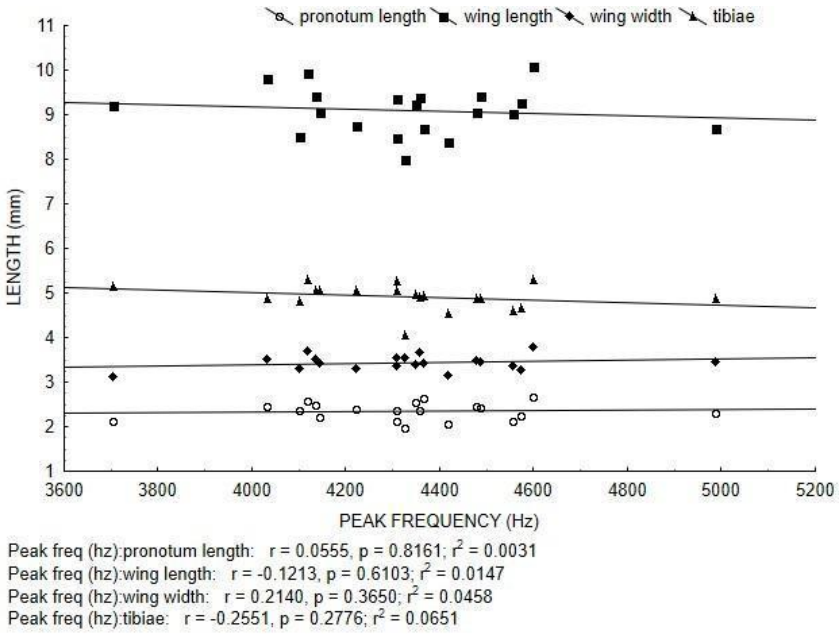


Figure A2.6. Relationship between body morphology, sound producing structure and call characteristics. (A) Relationship between harp area and mirror area. (B) Relationship of pronotum width with pronotum length, wing length, wing width and tibia length. (C) Relationship of harp area with pronotum length, pronotum width, wing length, wing width and tibia length. (D) Relationship of peak frequency with pronotum length, wing length, wing width and tibia length.

Table A2.1. Comparison of temporal variation in calling activity on seasonal and diel scale. ns represents no significant difference.

	P value
Seasonal variation	
SEP-NOV vs DEC-FEB	**<0.01**
SEP-NOV vs MAR-MAY	**<0.01**
SEP-NOV vs JUN-AUG	ns
DEC-FEB vs MAR-MAY	**<0.01**
DEC-FEB vs JUN-AUG	**0.02**
MAR-MAY vs JUN-AUG	**<0.01**
Diel variation	
18:00-21:00 vs 21:00-24:00	**<0.01**
18:00-21:00 vs 24:00-03:00	ns
18:00-21:00 vs 03:00-6:00	**<0.01**
21:00-24:00 vs 24:00-3:00	**<0.01**
21:00-24:00 vs 3:00-6:00	**<0.01**
24:00-3:00 vs 3:00-6:00	**<0.01**

Table A2.2. Descriptive statistics and Analysis of variance of tooth width for three different teeth regions: Apical, Middle and Basal.

TEETH REGIONS	Mean	SD	N
APICAL	42.27	9.54	24
MIDDLE	57.21	5.22	24
BASAL	48.15	5.96	24

Analysis of Variance

	SS	df	MS	SS	df	MS	F	p
TOOTH WIDTH	2717.122	2	1358.561	3540.760	69	51.31537	26.47474	**< 0.01**

Tukey HSD test

	{1}	{2}	{3}
APICAL {1}		**< 0.01**	**< 0.01**
MIDDLE {2}	**< 0.01**		**< 0.01**
BASAL {3}	**< 0.01**	**< 0.01**	

Chapter 3

Effect of natural and artificial light on the calling behaviour of *Acanthogryllus asiaticus*

Illuminated campus of IISER Mohali (Picture credit: Nakul Raj)

3.1 Introduction

Light as an environmental cue plays a ubiquitous role in regulating various life processes of organisms (Foster and Kreitzmann 2004). The rotation of the earth on its axis over ~24 h produces a daily light-dark cycle which in turn synchronises endogenous circadian clocks of organisms, allowing them to adapt to the daily variation in environment and thus optimally organise its behaviour, metabolism and physiology (Foster and Kreitzmann 2004; Gaston et al. 2017). The endogenous circadian clock is not only entrained to the daily environmental cycle but also to the annual cycle of day-length change (photoperiod) caused by the tilting of the earth's axis relative to the sun (Gaston et al. 2017).

In addition to natural variation in the ambient light, rapid urbanization has resulted in an unprecedented increase in artificial lights in our surroundings. Artificial light at night (ALAN) is now an inherent feature of urban spaces. Although the widespread use of ALAN is related to increased affluence, modernity, security and may have enhanced the living standards of humans, currently, it is one of the most pervasive forms of environmental alteration for non-human animals (Longcore and Rich 2004; Davies and Smyth 2018). The rapid global increase of ALAN by 6% every year has fundamentally contaminated earth's nocturnal landscape (Falchi et al. 2016). A recent study reveals that almost 23% of the terrestrial area, including 50% of the United States is under light pollution (Falchi et al. 2016). Exponential growth in population, rapid urbanisation and industrial development have significantly increased light pollution in India as well. Satellite images released by NASA earth observatory using VIIRS shows the drastic increase in light pollution in India from 2012 to 2016 (Figure 3.1).

Various lighting devices contribute to nightscape alteration such as public street lighting, and light from advertising, architecture, domestic sources and vehicles. Of these, street lighting is considered to be the most continuous and intense source of lighting in urban

landscapes (Longcore and Rich 2004; Gaston et al. 2017; Davies and Smyth 2018). The artificial light produced from these sources is different from natural lights sources (moon and sun) based on spectra, intensities and spatial illumination from local to sky glow (Gaston et al. 2013; 2017). Recent studies suggest that increased erosion of natural light-dark cycles due to ALAN impacts foraging (Santos et al. 2010), diel movement (Berge et al. 2009), sleep (Raap et al. 2016), reproduction (van Geffen et al. 2015a) and migration (Riley et al. 2012) in a wide range of taxa. A major focus of studies examining the negative impact of ALAN is on diurnal animals who are impacted during their rest period due to ALAN. For instance, ALAN has been show to affect singing behaviour in zebra finches (Jha and Kumar 2017), American Robins (Miller 2006), Chaffinch, Blue Tit, Great Tit (Kempenaers et al. 2010), cognitive performance in Indian house crows (Taufique and Kumar 2016) and reproductive physiology in blackbirds (Dominoni et al. 2013).

Nocturnal animals are also negatively impacted by ALAN (bats: Stone et al. 2015; insects: Owens and Lewis 2018; Desouhant et al. 2019; birds: Cabrera-Cruz et al. 2018). For instance, ALAN negatively affects foraging and commuting behaviour in bats as it reduces and delays the onset of commuting behaviour (Stone et al. 2015). In nocturnal invertebrates such as fireflies and glow worms, ALAN has been shown to hinder reproduction by disturbing bioluminescent visual signals used in a courtship display to find and attract mates (Firebaugh and Haynes 2016). In male winter moths, *Operophtera brumata*, ALAN was shown to cause chemical disruption of female sex pheromones (Van Geffen et al. 2015a, 2015b). Along with the disruption of visual and chemical cues, alteration in acoustic cues due to ALAN in nocturnal organisms has also been reported. For instance, constant illumination disturbs calling activity in male green frogs (Baker and Richardson 2006) and teleost fish (Feng and Bass 2016). However, till date, there is no evidence for disruption of acoustic communication in nocturnal insects (order: orthoptera) due to ALAN.

It has also been shown that nocturnally-active animals tailor their behaviour according to variation in light conditions associated with the lunar cycle. For instance, birds, rodents, bats and marine animals adjust their foraging behaviour according to changes in the lunar cycle (reviewed in Gaston et al. 2017). Some insects, amphibians and bats are known to reduce their behaviour during full moon conditions to avoid predation (Tuttle et al. 1982; Lang et al. 2006). This behaviour of avoiding full moonlight condition was termed as "lunar phobia" by Morrison (1978). Given the behaviour in organisms can be intensely affected even by natural variation in moonlight levels, the recent intrusion of artificial light at night (ALAN) is likely to impact the behaviour of animals too. In fact, it has been demonstrated to deteriorate the light-dark cycle, lunar, and seasonal rhythms of animals (Longcore and Rich 2004; Gaston et al. 2017) and negatively affect animals at all levels of biological organisation, from molecular to ecosystem levels (Gaston et al. 2013).

The light-dark cycle also synchronises the diurnal rhythm of melatonin concentration, which signals photoperiodic information and regulates various aspects of rhythmic activity in animals (Bentley 2001; Vivien-Roels and Pévet 1993). Presence of light suppresses melatonin production and darkness elicits its synthesis and secretion (Navara and Nelson 2007). For instance, melatonin levels have been reported to reduce due to ALAN in birds (de Jong et al. 2016; Raap et al. 2016) and zebrafish (Khan et al. 2018). In crickets, it has been shown that constant illumination reduces melatonin level and thereby affects immune function (Jones et al. 2015, Durrant et al. 2015). Given that stridulatory activity in crickets is also under circadian control (Loher 1972), it is expected that melatonin may regulate calling behaviour in crickets. Further, an external supply of melatonin has been shown to restore the ALAN-disturbed circadian activity such as nocturnal vocalization in a teleost

fish (Feng and Bass 2016) and locomotor activity in the house cricket, *Acheta domesticus* (Yamano et al. 2001). To the best of my knowledge, studies examining this link between melatonin and calling rhythm of insects are absent.

Male crickets produce a stereotypic intense long-distance mating call to attract sexually responsive conspecific females who are away (Alexander 1962). Females move towards the calling male and use characteristics of this public signal to assess the quality of male (Brown et al. 1996). Among 28410 valid orthopteran species (http://orthoptera.speciesfile.org) which produce acoustic signals for mate finding and mate choice, the effect of night lighting on acoustic signal has been studied in only one species, *Teleogryllus commodus*, a field cricket (Botha et al. 2017). This is a lab-based study which shows no effect of lifetime exposure to artificial light on courtship signals. To my knowledge, there has been no other study which examines the effect of ALAN on calling activity of crickets.

In this study, I examined whether the presence of artificial light at night impact calling activity in *Acanthogryllus asiaticus* and affect their rhythmicity. I also tested the potential role of melatonin in regulating their calling rhythm. The specific objectives were as follows:

i. To examine the vertical and horizontal attenuation in light intensities of streetlights to assess the severity of ALAN in the natural habitat of crickets.
ii. To examine whether light levels are different between full and new moon nights and whether that impacts calling behaviour of males. In other words, do male crickets exhibit 'lunar phobia'?
iii. To examine if areas illuminated by artificial light are brighter than those that are not and whether ALAN impacts calling behaviour of *A. asiaticus*.

iv. To examine if constant illumination affects calling rhythmicity and the role of melatonin in controlling the calling rhythm.

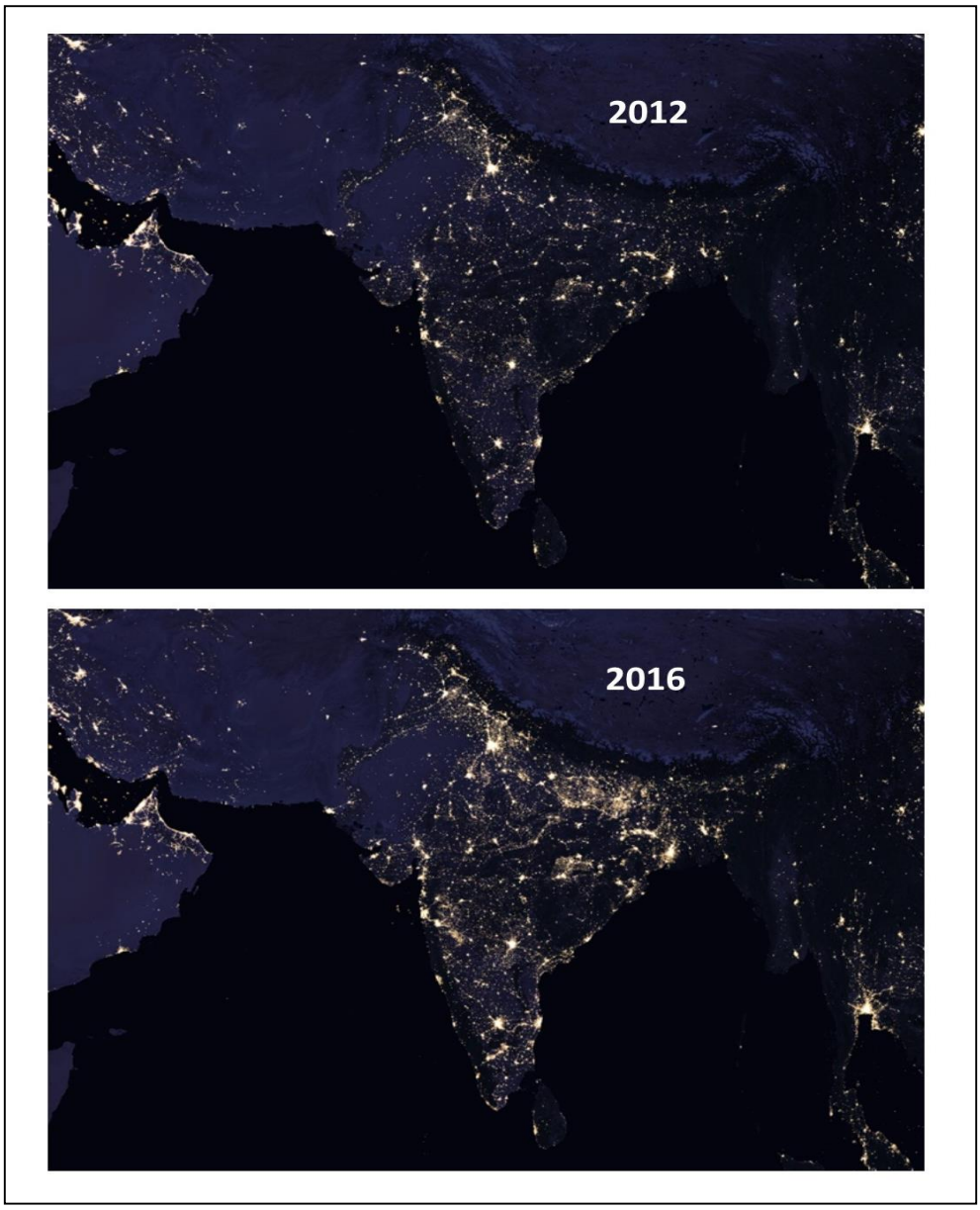


Figure 3.1. Night time view of India in year 2012 and 2016 taken by Joshua Stevens, NASA Earth Observatory using Suomi NPP VIIRS data from Miguel Román, NASA's Goddard Space Flight Center.

3.2 Materials and Methods

3.2.1 Vertical and horizontal attenuation in light intensities

Vertical and horizontal attenuation in light intensities for street lights (N = 3) was measured using a light meter (LX-1108, meet C.I.E. spectrum photopic, Lutron electronic enterprise Co. Ltd., Taiwan) at increasing distance from the light source. Vertical attenuation in light intensities was measured away from the light source at 1m, 2m, 4m and ground, measured directly under the light source. Horizontal spread of light intensities was measured at 0m, 2m, 4m and 8m distance away from the street light at ground level.

3.2.2 Examining calling activity in field

The study was carried out during the month of March – May (peak activity season for *A. asiaticus*) (Singh and Jain 2020) for two years: 2016 and 2017 at Indian Institute of Science Education and Research campus in Mohali (30°39'N, 76°43'E). Three areas each for light and dark conditions of about 225 m^2 each selected (see Figure B3.1). While selecting areas for the two categories of habitats, namely, naturally dark and artificially-lit, I ensured that the vegetation structure is similar. In order to select naturally dark and artificially-lit areas, I needed to control for the habitat-specific difference. It was important to select areas that were similar in vegetation and habitat structure to rule out these confounding effects on calling activity in different area. Thus, all sampling areas had a grassy ground cover with an intermittent canopy of trees such as *Populous deltiodes*, *Ficus religiosa* and *F. glomerata*. The dark areas were about 30 m apart from each other and these areas were devoid of any artificial light sources whereas artificially-lit areas had sodium-vapour street lamps. Light intensities at ground level were measured for these areas at cicket calling site using a light meter (LX-1108, meet C.I.E. spectrum photopic, Lutron electronic enterprise Co. Ltd., Taiwan). Calling activity of *A. asiaticus* was monitored by conducting a census

of calling males in a given area using psychoacoustic sampling. It is a reliable and non-invasive method to monitor orthopteran species diversity in the given area by a trained observer (Diwakar et al. 2007). Census was only done from walking paths along the periphery of plots and not by walking across the plots to avoid disturbing calling animals. Weather parameters such as humidity and temperature were measured just above the ground for every sampling hour using a pocket weather meter (Kestrel 4000, Nielsen-Kellerman, Chester, U.S.A.)

Impact of natural light on calling activity

Light intensity measurements for full moon and new moon days were carried out only in naturally dark areas to avoid the confounding effect of ALAN. These measurements were taken at ground level near cricket calling sites which are naturally occurring cracks under grassy cover. Census for calling activity was carried out for 5 nights for each month for both moon phase conditions between 1900 to 0200 h. Thus, for a given moon phase, the moon phase day and 2 days before and after it were sampled (i.e. full moon day (± 2 days), and new moon day (± 2 days)). Across the 3 dark areas, sampling was conducted over 5 days such that each habitat was sampled at least twice every month, for each moon phase condition.

Impact of ALAN on calling activity

Light intensity measurements for artificially-lit and naturally dark areas were carried out only in new moon days. These measurements were taken at ground level near cricket calling sites which are naturally occurring cracks under grassy cover. For light areas measurement, were done at 5 m away from the street light. Census for calling activity was carried out for 5 nights for each month for each artificially lit and dark areas between 1900 to 0200 h. Thus, for artificially-lit and naturally dark area condition, the new moon day and 2 days

before and after it, were sampled (i.e. new moon day (± 2 days)) each for light and dark area condition. Across the 3 artificially-lit and 3 dark areas, sampling was conducted over 5 days (new moon ± 2 days) such that each habitat was sampled at least twice every month.

3.2.3 Examining the role of melatonin

30 adult male crickets (3-4 weeks old) were pooled from lab culture and divided into three sets, each set containing 10 males. These sets were control, test condition 1 (TC1) and test condition 2 (TC2) (Figure 3.2). These sets were maintained in three different air-conditioned rooms (temperature at 24°C) equipped with white light florescent tubes (150-170 lux). Each cricket was placed individually in a plastic box provided with food and water *ad libitum*. For the first 10 days, all the three sets were exposed to 12:12 h L:D condition. After the completion of 10th day, for the next 10 days, 12:12 h L:L condition was maintained for TC1 and TC2 whereas no change was done for the control set. After the completion of 20th day, crickets in test condition 2 were provided water supplemented with synthetic melatonin (Sigma-Aldrich, India) for the next 10 days while no changes were done for TC1 and control set. Dietary supplementation of melatonin for TC2 was administered through water at 18:00 only for 12 h while only plain water was supplied to TC1 and control set for 12 h only. After the completion of 12 h, cotton from all the sets were removed. This was done every evening for the next 10 days. For dietary supplementation of melatonin, a stock solution was prepared for which50mg melatonin powder was dissolved in 100% ethanol and then stored in the dark at 4°C. While providing melatonin to crickets, dilutions were made fresh from the stock using distilled water to give solutions of 100 µg/ml (Yamano et al. 2001). Calling activity for each individual for every conditions was observed for every hour for 24 hours for all 30 days. For analysis, only last five days for each phases per treatment were considered.

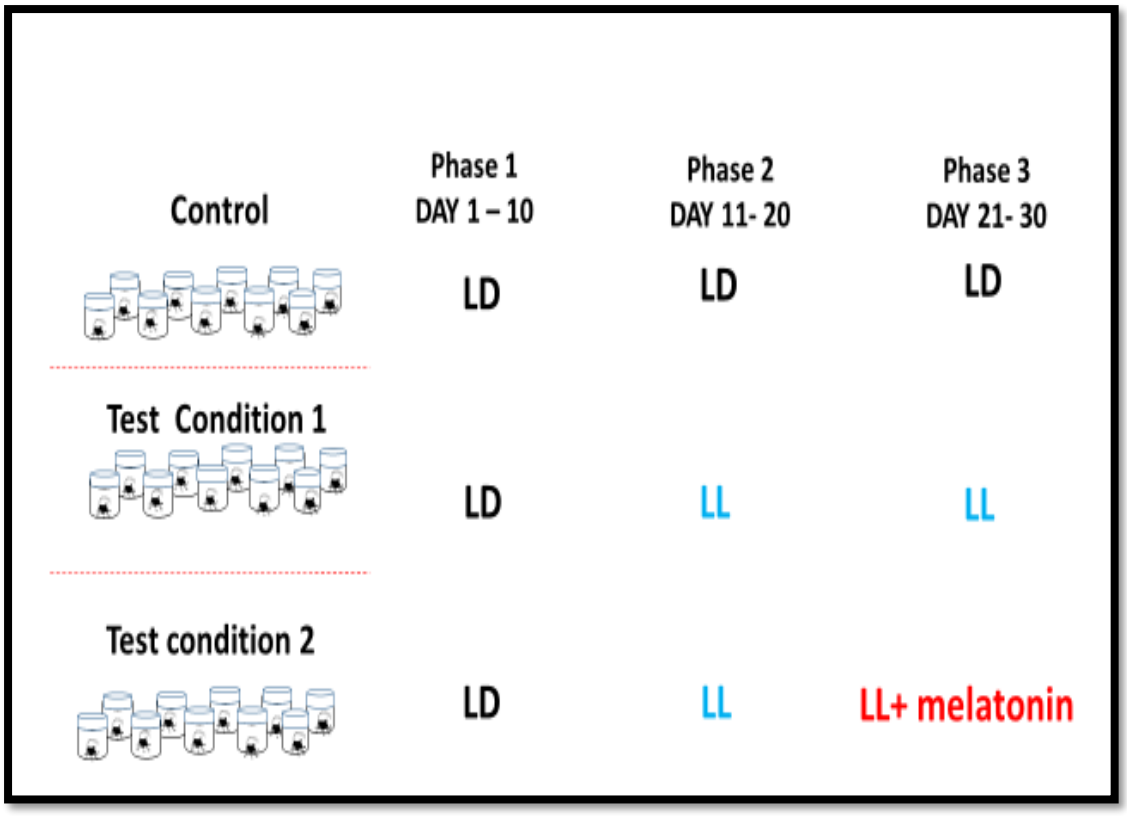


Figure 3.2. Sampling protocol for examining circadian control on cricket calling activity and role of melatonin in controlling calling rhythm.

3.2.4 Statistical analyses

Statistical tests were performed using Statistica 64 (Dell Inc. 2015, Version 12). Data were checked for normality using the Shapiro-Wilk test. Data on light intensities and calling activity in artificially-lit and dark areas and light intensities during different moon phases was compared using Mann-Whitney U-test as data did not follow a normal distribution. Data on calling activity during full moon and new moon were compared using t-test. Calling rhythms for different test condition (Control, TC1 and TC2) for different treatment phases across different time slots were compared using Generalized Linear Model where factorial ANOVA was done considering treatment, phase and time as predictor and number

callers as dependent variable. Later, Tukey-HSD was performed for pair-wise comparison of same timeslots during different phases.

3.3 Results

3.3.1 Light intensities

Vertical and horizontal spread of light from street lamps showed variation in light intensities ranging from top: 145 lux to bottom: 15 lux (vertically) and from 0 m: 15 lux to 8 m away from the pole: 2 lux (horizontally) (Figure 3.3; 3.4A and B). Light intensities for full moon and new moon were not found to be significantly different (Mann Whitney U test, U = 2263, N = 72, P = 0.177; Figure 3.5). Light intensity levels for artificially-lit and naturally dark areas were found to be significantly different (Mann Whitney U test, U = 0.00, N = 72, P < 0.001, Figure 3.6).

3.3.2 Calling activity in field

No significant effect of natural light i.e. full moon and new moon on calling activity of crickets was found (t - test, t = - 0.64, df = 82, P = 0.52; Figure 3.5, Table B3.1). Calling activity in artificially-lit and naturally dark areas was found to be significantly different from each other (Mann Whitney U test, U = 378, df = 82, P < 0.001, Figure 3.6, Table B3.2).

3.3.3 Role of melatonin

Factorial ANOVA analysis showed differences in calling rhythms when tested for interaction between treatments, phases and time slots (F = 2.490, P < 0.001, Figure 3.7, Table 3.1). The interaction of phase with time for each treatment showed significant difference (Control: F = 1.949, P = 0.03; Test condition 1: F = 6.416, P < 0.0001; Test condition 2: F = 4.3835, P < 0.0001; Figure 3.7, Table 3.1 and B3.3). To test whether calling

activity is disrupted each time slots across phases were compared. I found calling activity during 1000-1400h and 1400-1800h for phase 1 with phase 2 and 3 of test condition 1 showed significant difference ($P < 0.001$, Figure 3.7, Table B3.3 & B3.4). Pairwise comparison of the time slots of phase 2 with phase 3 of test condition 1 found to be not significantly different from each other as both phases were exposed to LL condition. However, in test condition 2, calling activity during 1000-1400h and 1400-1800h in Phase 1 was not significantly different with Phase 3 ($P > 0.05$, Figure 3.7, Table B3.3 & B3.4) but was different with phase 2. This is because in test condition 2 animals went arrhythmic during phase 2 but on administering melatonin this arrhythmic behaviour disappeared and natural diel calling pattern resurfaced.

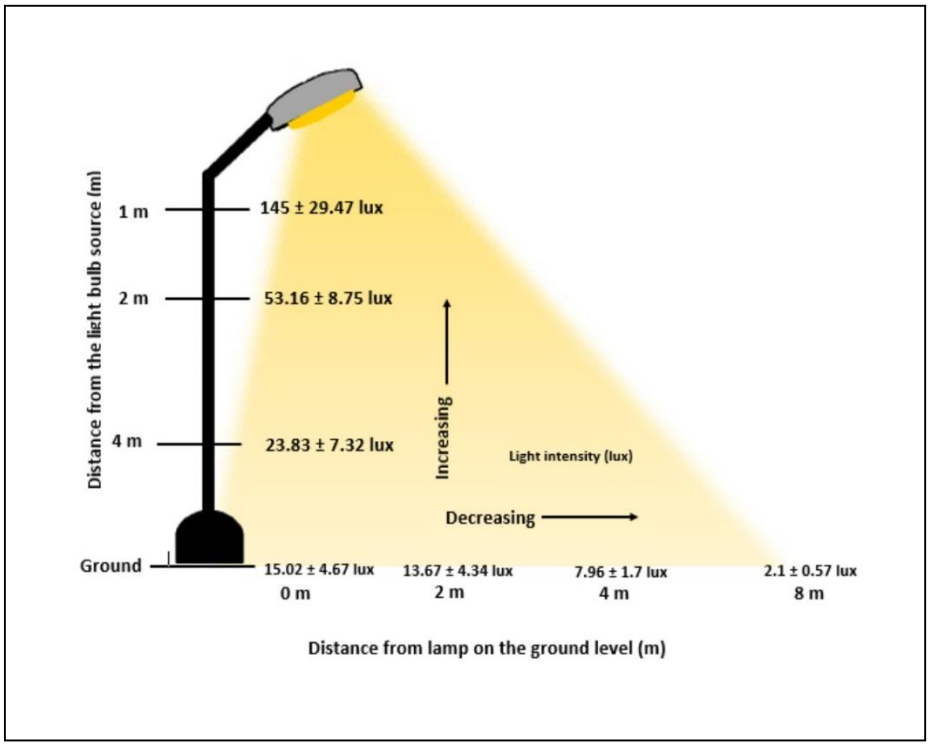


Figure 3.3. Vertical and horizontal gradient of light intensity (lux) from sodium street lamp light source.

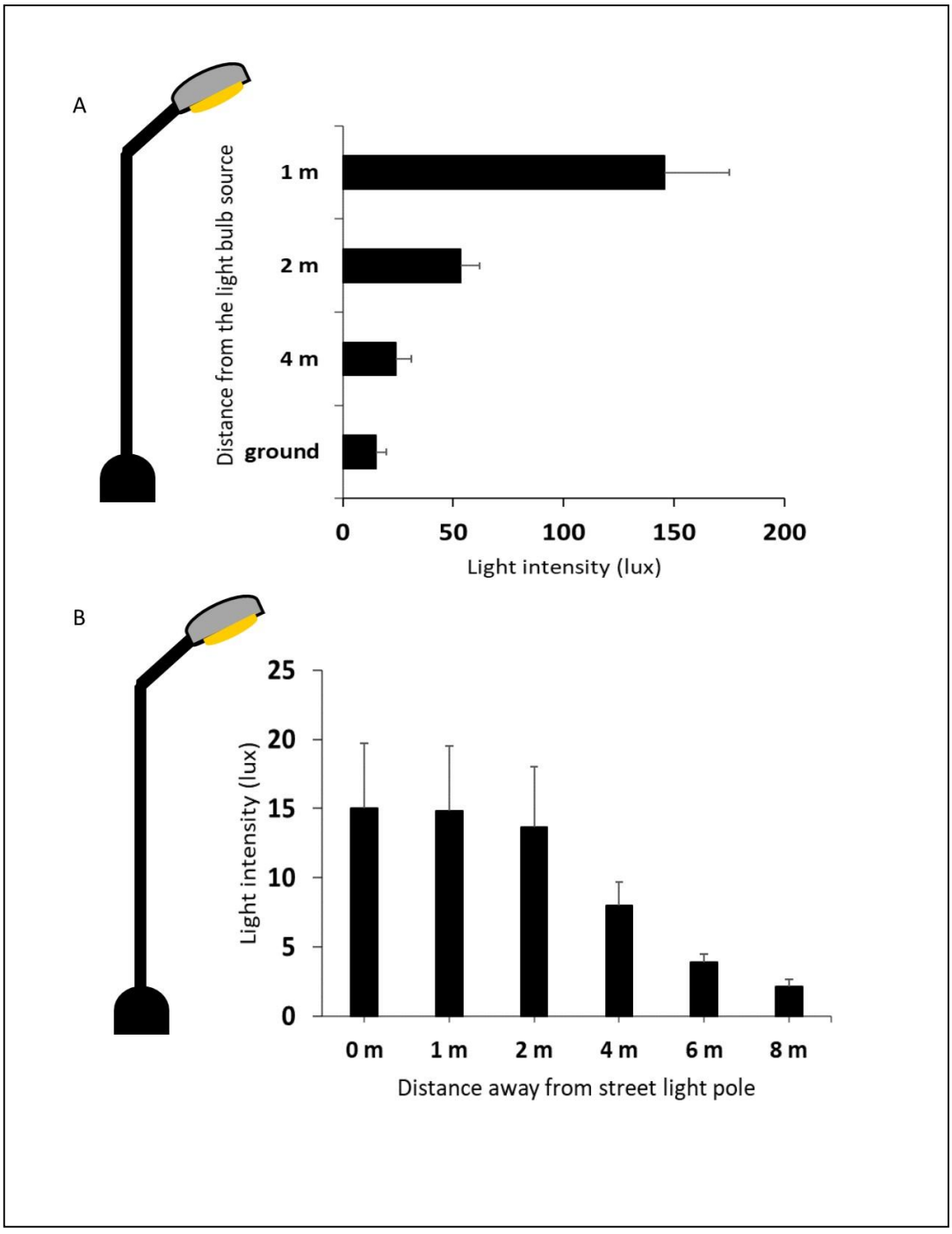


Figure 3.4. A. Vertical and B. Horizontal gradient in light intensities (Mean ± SD; N = 3 per distance).

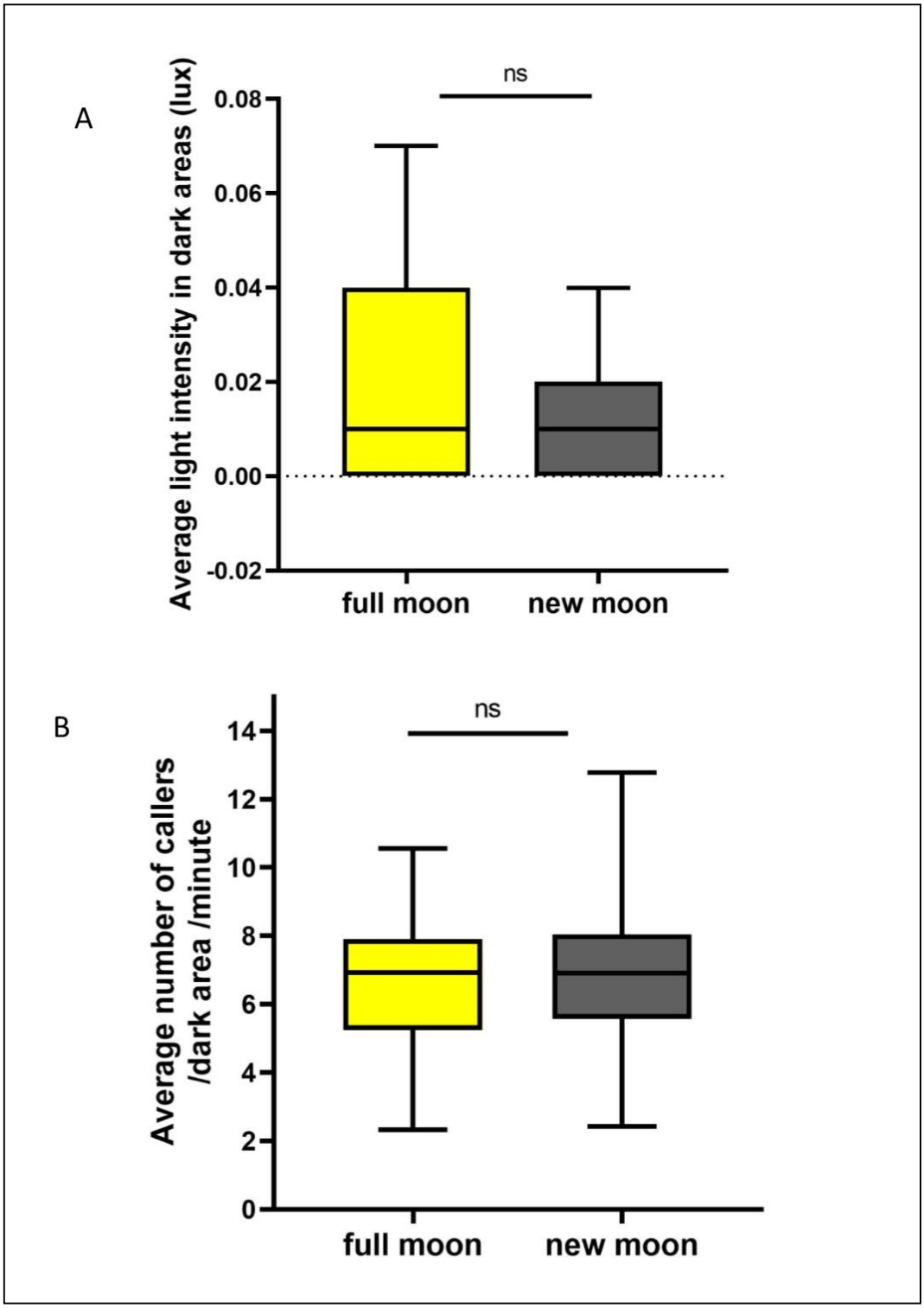


Figure 3.5. Effect of moonlight on calling activity of *A. asiaticus*. A. Light intensities (lux) during full moon and new moon (N = 72 per conditions). B. Calling activity during full moon and new moon. ns represents no significant difference, P > 0.05. Box and whiskers plot (Median; Min-Max). N = 42 replicates per conditions.

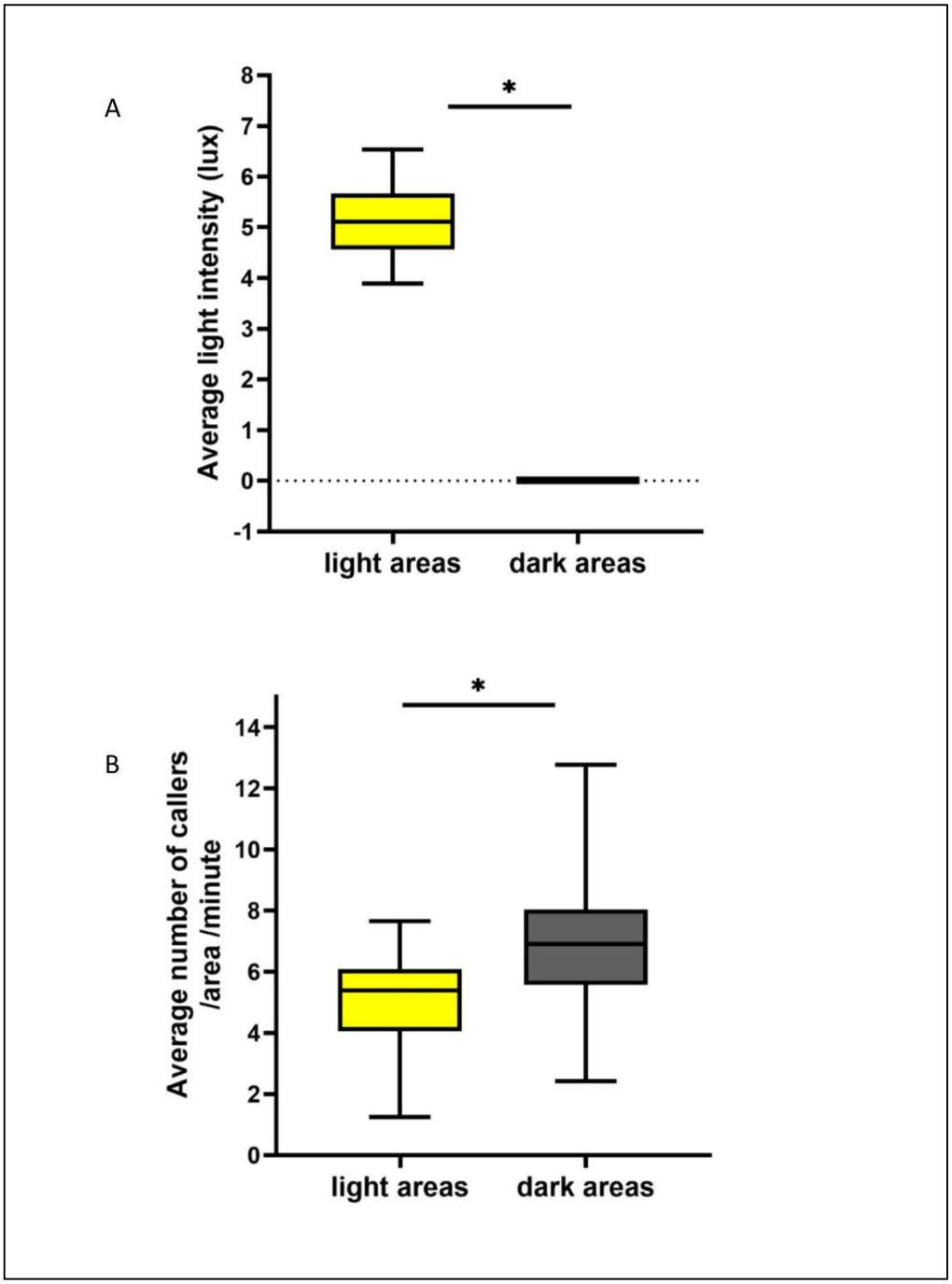


Figure 3.6. Effect of artificial light on calling activity of *A. asiaticus*. A. Light intensities (lux) In dark and light areas (N = 72 per conditions). B. Calling activity in dark and light areas. * represents significant difference, $P < 0.05$. Box and whiskers plot (Median; Min-Max). N = 42 replicates per conditions.

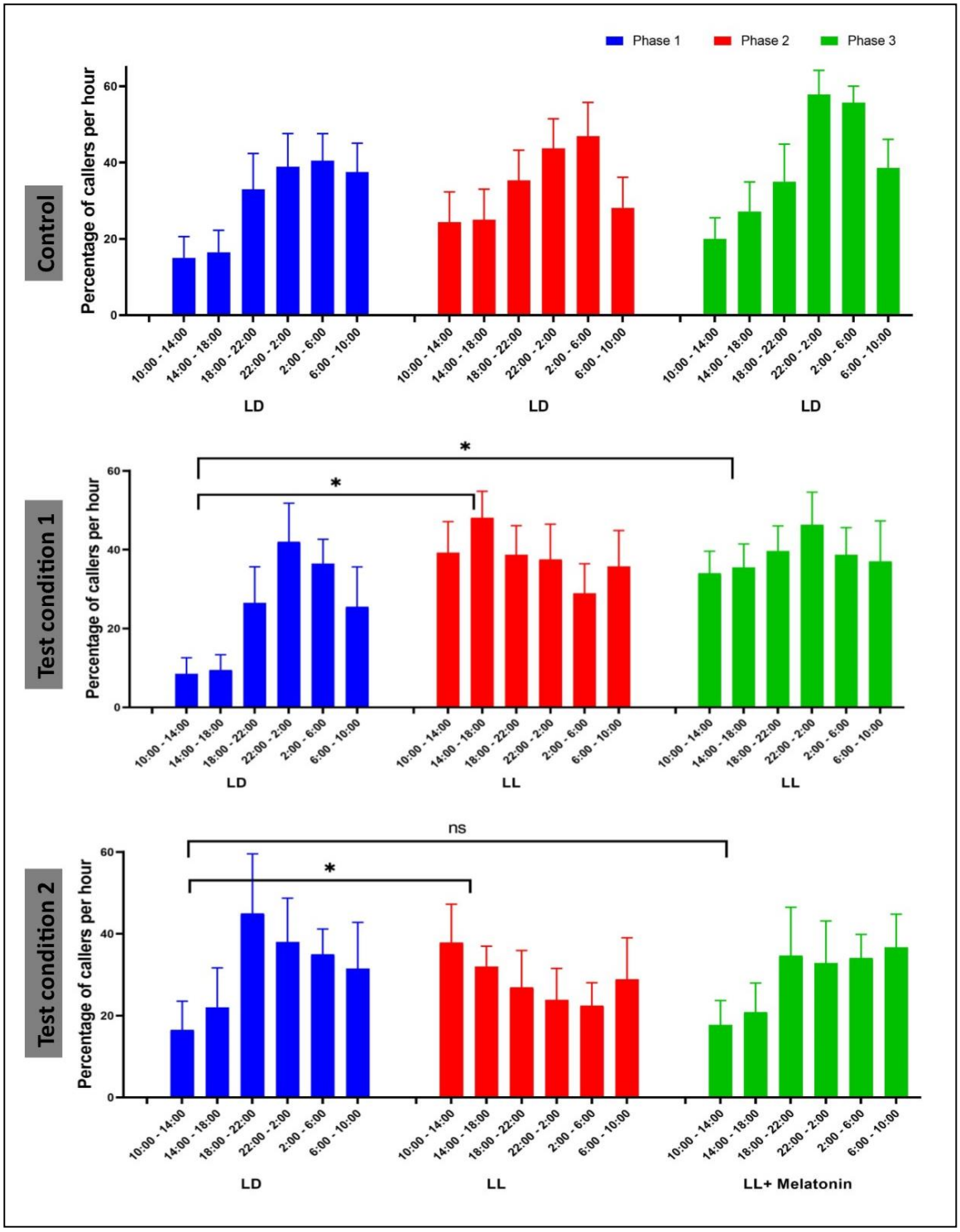


Figure 3.7. Circadian control on calling activity of *A. asiaticus*. Phase 2 of test condition 1 and 2 represents altered calling activity under constant illumination. Phase 3 of test condition 2 represents melatonin restoring calling rhythms. Mean with 95% CI. * represents significant difference, $P < 0.05$ and NS represents no significant difference, $P < 0.05$.

Table 3.1. Factorial ANOVA test showing response of callers during each time slots of phases for each treatments. Significant difference is indicated in bold.

Control, Test condition 1 and 2					
	SS	Degree of Freedom	MS	F	P
Intercept	1138063	1	1138063	3820.508	**< 0.0001**
treatment	4910	2	2455	8.242	**0.000281**
Phase	8837	2	4419	14.834	**< 0.0001**
Time	36889	5	7378	24.767	**< 0.0001**
treatment*Phase	11137	4	2784	9.347	**< 0.0001**
treatment*Time	16049	10	1605	5.388	**< 0.0001**
Phase*Time	23271	10	2327	7.812	**< 0.0001**
treatment*Phase*Time	14867	20	743	2.495	**0.0002**
Error	303542	1019	298		
Total	1072	429901			
Control					
Intercept	420590.6	1	420590.6	1635.221	**< 0.0001**
Phase	4678.1	2	2339.1	9.094	**< 0.0001**
Time	40521.4	5	8104.3	31.509	**< 0.0001**
Phase*Time	5012.9	10	501.3	1.949	**0.038**
Error	86936	338	257.2		
Test condition 1					
Intercept	406543.9	1	406543.9	1517.886	**< 0.0001**
Phase	14594.4	2	7297.2	27.245	**< 0.0001**
Time	7228.9	5	1445.8	5.398	**< 0.0001**
Phase*Time	17184	10	1718.4	6.416	**< 0.0001**
Error	90796.3	339	267.8		
Test condition 2					
Intercept	315067.2	1	315067.2	856.474	**< 0.0001**
Phase	688.1	2	344.1	0.9353	0.393462
Time	5146.3	5	1029.3	2.7979	**0.017**
Phase*Time	16125.3	10	1612.5	4.3835	**< 0.0001**
Error	125810	342	367.9		

3.4 Discussion

This study demonstrates for the first time the significant negative effect of artificial light at night on calling activity of an insect species in natural conditions and role of melatonin in controlling calling rhythmicity. It also demonstrates the lack of support for the 'lunar phobia' hypothesis in this species of field cricket.

3.4.1 Light intensities and calling activity: natural light

Light intensity levels during full moon and new moon at cricket calling site in dark areas were not found to be significantly different. Average light intensity during the full moon was found to be around 0.02 ± 0.02 lux whereas, during the new moon, it was around 0.01 ± 0.01 lux in dark areas. Kyba et al. (2017) observed that light intensity during the full moon to be between 0.05 to 0.1 lux at temperate latitudes during the summer. Kyba et al. (2017) discussed the incorrect values reported in other studies regarding light intensity during the full moon night, such as 0.5–1 lux (Bruce-White and Shardlow 2011) and 2 lux (Yorzinski et al. 2015). I found the maximum light intensity during the full moon at cricket calling site to be around 0.07 lux. Given the observation were taken at the ground level near the cricket calling site, these values are likely to be the most biologically meaningful for a ground insect species as opposed to measurements made at higher positions. Field crickets mostly call from cracks or other shelter places covered by grass or leaf litter. Such vegetation can create shadow and obscure the moon light at ground level which might have resulted in no significant difference in light levels between full moon and new moon. No difference in the calling behaviour of *A. asiaticus* was observed in dark areas during the full moon and new moon, thereby clearly demonstrating the lack of support to the 'lunar phobia' hypothesis. Contrary to this, a study on *T. oceanicus* observed reduced calling behaviour on full moon day, but this study lacks quantitative measurement of light intensity

and calling behaviour (Loher and Orsak, 1985). A study by Lang et al. (2006) in the Barro Colorado Island, Panama showed that during full moon, calling activity of katydids found to be reduced as background noise level declined which in turn decreased the foraging activity of the perch-hunting bats, *Lophostoma silvicolum*.

3.4.2 Light intensities and calling activity: ALAN

Vertical attenuation of light intensities from street lights showed that even at the ground level, light intensity was found to be around 15 lux (at the street light) which could be severe for organisms found near a street light. Horizontally, the light level decreased, but even at the distance of 8 m light intensity was found to be around 2 lux which is 200 times brighter than the natural dark areas. Light levels measured in the two habitat types (artificially-lit and naturally dark) during the night showed a significant difference between them, clearly demonstrating that street lamps are the prominent sources of light pollution during night time. In fact, artificially-lit areas were 500 times brighter than dark areas during night time. Such variation in light intensity level was sufficient to cause less calling activity in lit areas as compared to the dark ones. Reduced calling activity in artificially-lit areas may also reflect an anti-predator response as it has been showed that animals reduce their activity to minimize predation risk from visual predators under illuminated environments (Kramer and Birney 2001). Reduced calling activity in artificially-lit areas demonstrates the significant negative affect artificial lighting may have on mating success of these insects in urban environments. However, a lab-based study on *T. commodus* showed that chronic lifetime exposure to light at night increased the probability of successful mating but disturbed the rate of post-copulatory mating behaviour. Furthermore, this study also showed no effect of chronic exposure to different light intensities (0, 1, 10 and 100 lux) on the number of courtship calls produced or on their

signal structure (Botha et al. 2017). In another study, on the same species, a lab-based experiment showed that artificial light at night did not affect the movement pattern of virgin females towards broadcasted call from the speaker, although the initiation of movement was slower compared to the unlit environment (Thompson et al. 2019). Advertisement calls were also found to be reduced in male green frogs when exposed to ALAN (Baker and Richardson 2006). A study conducted on 6 frog species: Northern Cricket Frogs (*Acris crepitans*), Gulf Coast Toads (*Incilius nebulifer*), Cliff Chirping Frogs (*Eleutherodactylus marnockii*), Spotted Chorus Frogs (*Pseudaaris clarkii*), American Bullfrogs (*Lilhobates catesbeianus*) and Rio Grande Leopard Frogs (*Lilhobates berkandieri*), also showed reduced calling activity on the introduction of acute artificial lighting to the natural condition (Hall 2016).

3.4.3 Role of melatonin in regulating calling rhythmicity

The lab experiment in my study showed that constant illumination altered cricket calling activity while in LD conditions cricket showed synchronized calling behaviour as peak calling activity was observed during the night and reduced calling activity was observed during day time. Individuals in both the test conditions, when exposed to LL condition, showed an arrhythmic pattern in calling activity. Similarly, in a study on calling behaviour in the dark–active males of *Gryllus campestris* showed random activity pattern when the males were switched from LD to LL treatment (Honegger 1981). Such arrhythmic singing behaviour has also been observed in zebra finches when exposed to LL conditions (Jha and Kumar 2017). In this study, when melatonin was provided as supplement to the individuals in test condition 2, peak calling activity during night time, was found to be restored. A similar finding has been shown in a vertebrate system where a study on teleost fish reported the decline of courtship vocalization during night time under constant illumination

condition, but the exogenous supply of melatonin restored the rhythmicity (Feng and Bass 2016). This study suggested that melatonin act as a cue for regulating vocalization timing in nocturnally active fish. A study on locomotor activity of *A. domesticus* showed that addition of melatonin synchronized the altered behaviour in LD cycles and improved the free-running rhythm in DD condition (Yamano et al. 2001). Although melatonin was found to restore the calling rhythmicity in *A. asiaticus*, the underlying mechanism needed to be examined further to understand the link between melatonin and cricket calling activity. Itoh et al. (1995) measured melatonin concentration in different body organs of *G. bimaculatus* and found that melatonin levels in the compound eye, brain, and palp were found to be significantly higher during the dark period than during the light period as compared to other body parts. Given that the stridulatory pattern-generating networks are housed within the thoracic ganglia but are controlled by the brain (Hedwig 2000), such variation in melatonin level in the brain is expected to control the calling behaviour. Exogenous supply of melatonin has also been reported to influence immunity in field crickets. For instance, in *T. commodus*, constant illumination negatively affected haemocyte concentration and lytic activity (Durrant et al. 2015) but supplementation of dietary melatonin at different concentrations: 0, 10 or 100 mg in their drinking water, over four weeks, improved haemocyte concentration and lysozyme-like activity (Jones et al. 2015).

This study provides evidence that it is not a moonlight but an artificial light of 5 lux, which can alter calling behaviour of crickets. One possibility is that the melatonin levels of insects under bright street lamps were different from those found in darker areas, resulting in differences in calling levels. This, however remains to be tested. This decrease in calling activity, by even a small percentage, can severely affect mating success due to a reduction

in the probability of attracting a female which may consequently, lead to a significant decrease in population. A study conducted in Germany reported about the drastic decline in biomass of flying insect by 75% in the protected areas of the country and scientists referred to such sudden as 'Ecological Armageddon' (Hallmann et al. 2017). ALAN can be speculated to be a potential stressor and a possible causal factor for such biodiversity loss. Increased research interest on the ecological impact of ALAN has raised concerns among researchers and policy makers to provide mitigation measures and management options to control and reduce the ecological effects of nighttime light pollution. Gaston et al. (2013) suggested five management options to prevent light pollution: (a) maintenance and increase in dark areas by removing unnecessary artificial lightings (b) reducing light trespass by using improved designed lighting devices (c) reducing the intensity of artificial lighting emissions; (d) part-night lighting by using light for limited time and (e) incorporating light of proper spectra that provide sufficient human benefit while curtailing other biological impacts such as the introduction of broads spectrum street lamps could alter the balance of species interactions in the artificially-lit environment. We urgently need to review the situation of ALAN in India and implement policy level changes to bring back the dark night.

3.5 References

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3.6 Appendix B

Table B3.1 Calling activity of *A. asiaticus* (Mean ± SD) during moon phases for 5 nights for each month (Mar- May) for two years (2016-2017) in dark area only. N represents total number of replicates per month

Month-Year	Full moon	New moon	N
Mar-16	7.71 ± 1.36	6.60 ± 1.49	7
Apr-16	7.76 ±1.48	8.18 ± 2.48	7
May-16	7.62 ± 1.33	7.36 ± 2.60	7
Mar-17	4.47 ±1.18	6.49 ± 0.92	7
Apr-17	7.55 ± 1.42	8.78 ± 2.11	7
May-17	5.36 ± 1.51	4.82 ± 1.74	7

Table B3.2 Calling activity of *A. asiaticus* (Mean ± SD) in artificially-lit and dark area for 5 nights for each month (Mar- May) for two years (2016-2017) around new moon days only. N represents total number of replicates per month

Month-Year	Light area	Dark area	N
Mar-16	5.81 ± 0.90	6.60 ± 1.49	7
Apr-16	5.96 ± 0.56	8.18 ± 2.48	7
May-16	6.47 ± 0.46	7.36 ± 2.60	7
Mar-17	2.79 ± 1.00	6.49 ± 0.92	7
Apr-17	4.71 ± 1.09	8.78 ± 2.11	7
May-17	4.20 ± 0.60	4.82 ± 1.74	7

Table B3.3. Descriptive statistics for calling activity of *A. asiaticus* for each time slots of phases per treatment.

A. Control	**Phase**	**Time**	**Percentage of Callers - Mean**	**CI -95.00%**	**CI +95.00%**	**N**
1	1	6:00 - 10:00	37.500	30.446	44.554	20
2	1	10:00 - 14:00	15.000	7.946	22.054	20
3	1	14:00 - 18:00	16.500	9.446	23.554	20
4	1	18:00 - 22:00	33.000	25.946	40.054	20
5	1	22:00 - 2:00	38.947	31.710	46.185	19
6	1	2:00 - 6:00	40.500	33.446	47.554	20
7	2	6:00 - 10:00	28.125	21.071	35.179	20
8	2	10:00 - 14:00	24.375	17.321	31.429	20
9	2	14:00 - 18:00	25.000	17.946	32.054	20
10	2	18:00 - 22:00	35.294	27.643	42.945	17
11	2	22:00 - 2:00	43.750	36.696	50.804	20
12	2	2:00 - 6:00	46.875	39.821	53.929	20
13	3	6:00 - 10:00	38.571	31.517	45.625	20
14	3	10:00 - 14:00	20.000	12.946	27.054	20
15	3	14:00 - 18:00	27.143	20.089	34.197	20
16	3	18:00 - 22:00	35.000	27.946	42.054	20
17	3	22:00 - 2:00	57.857	50.803	64.911	20
18	3	2:00 - 6:00	55.714	48.660	62.768	20
B. Test condition 1	Phase	Time	Percentage of Callers - Mean	CI -95.00%	CI +95.00%	N
1	1	6:00 - 10:00	25.500	18.302	32.698	20
2	1	10:00 - 14:00	8.500	1.302	15.698	20
3	1	14:00 - 18:00	9.500	2.302	16.698	20
4	1	18:00 - 22:00	26.500	19.302	33.698	20
5	1	22:00 - 2:00	42.000	34.802	49.198	20
6	1	2:00 - 6:00	36.500	29.302	43.698	20
7	2	6:00 - 10:00	35.714	28.516	42.912	20
8	2	10:00 - 14:00	39.206	32.008	46.404	20
9	2	14:00 - 18:00	48.095	40.897	55.293	20
10	2	18:00 - 22:00	38.655	30.848	46.463	17
11	2	22:00 - 2:00	37.540	30.342	44.738	20
12	2	2:00 - 6:00	28.968	21.770	36.166	20
13	3	6:00 - 10:00	37.000	29.802	44.198	20
14	3	10:00 - 14:00	34.000	26.802	41.198	20
15	3	14:00 - 18:00	35.500	28.302	42.698	20
16	3	18:00 - 22:00	39.667	32.469	46.865	20
17	3	22:00 - 2:00	46.333	39.135	53.531	20
18	3	2:00 - 6:00	38.667	31.469	45.865	20

C. Test condition 2	Phase	Time	Percentage of Callers - Mean	CI -95.00%	CI +95.00%	N
1	1	6:00 - 10:00	31.500	23.064	39.936	20
2	1	10:00 - 14:00	16.500	8.064	24.936	20
3	1	14:00 - 18:00	22.000	13.564	30.436	20
4	1	18:00 - 22:00	45.000	36.564	53.436	20
5	1	22:00 - 2:00	38.000	29.564	46.436	20
6	1	2:00 - 6:00	35.000	26.564	43.436	20
7	2	6:00 - 10:00	28.849	20.414	37.285	20
8	2	10:00 - 14:00	37.837	29.402	46.273	20
9	2	14:00 - 18:00	31.964	23.529	40.400	20
10	2	18:00 - 22:00	26.867	14.402	31.273	17
11	2	22:00 - 2:00	23.810	15.374	32.245	20
12	2	2:00 - 6:00	22.421	13.985	30.856	20
13	3	6:00 - 10:00	36.667	28.231	45.102	20
14	3	10:00 - 14:00	17.738	9.302	26.174	20
15	3	14:00 - 18:00	20.833	12.398	29.269	20
16	3	18:00 - 22:00	34.643	26.207	43.078	20
17	3	22:00 - 2:00	32.857	24.422	41.293	20
18	3	2:00 - 6:00	34.048	25.612	42.483	20

Table B3.4 Post-hoc analyses of response of callers during each time slots of phases for each treatment for testing the effect of melatonin on rhythmicity of cricket calling activity using Tukey's HSD test.

A

Control																		
	Phase	Time	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}
1	1	06-10h																	
2	1	10-14h	**< 0.01**																
3	1	14-18h	**< 0.01**	ns															
4	1	18-22h	ns	**< 0.01**	ns														
5	1	22-02h	ns	**< 0.01**	**< 0.01**	ns													
6	1	02-06h	ns	**< 0.01**	**< 0.01**	ns	ns												
7	2	06-10h	ns	ns	ns	ns	ns	ns											
8	2	10-14h	ns	ns	ns	ns	ns	ns	ns										
9	2	14-18h	ns	ns	ns	ns	ns	ns	ns	ns									
10	2	18-22h	ns	**< 0.01**	ns	ns	ns	ns	ns	ns	ns								
11	2	22-02h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	**< 0.01**	**< 0.01**	ns							
12	2	02-06h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns						
13	3	06-10h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns					
14	3	10-14h	ns	ns	ns	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	**< 0.01**	**< 0.01**	**< 0.01**				
15	3	14-18h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**< 0.01**	ns	ns			
16	3	18-22h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
17	3	22-02h	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	
18	3	02-06h	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns	ns	**< 0.01**	**< 0.01**	**< 0.01**	ns

Table B3.4 Post-hoc analyses of response of callers during each time slots of phases for each treatment for testing the effect of melatonin on rhythmicity of cricket calling activity using Tukey's HSD test.

B

Test control 1																		
	Phase	Time	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}
1	1	06-10h																	
2	1	10-14h	ns																
3	1	14-18h	ns	ns															
4	1	18-22h	ns	ns	ns														
5	1	22-02h	ns	**< 0.01**	**< 0.01**	ns													
6	1	02-06h	ns	**< 0.01**	**< 0.01**	ns	ns												
7	2	06-10h	ns	**< 0.01**	**< 0.01**	ns	ns	ns											
8	2	10-14h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns										
9	2	14-18h	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns	ns	ns									
10	2	18-22h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns								
11	2	22-02h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns							
12	2	02-06h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	**< 0.01**	ns	ns						
13	3	06-10h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns					
14	3	10-14h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns				
15	3	14-18h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
16	3	18-22h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
17	3	22-02h	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
18	3	02-06h	ns	**< 0.01**	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table B3.4 Post-hoc analyses of response of callers during each time slots of phases for each treatment for testing the effect of melatonin on rhythmicity of cricket calling activity using Tukey's HSD test.

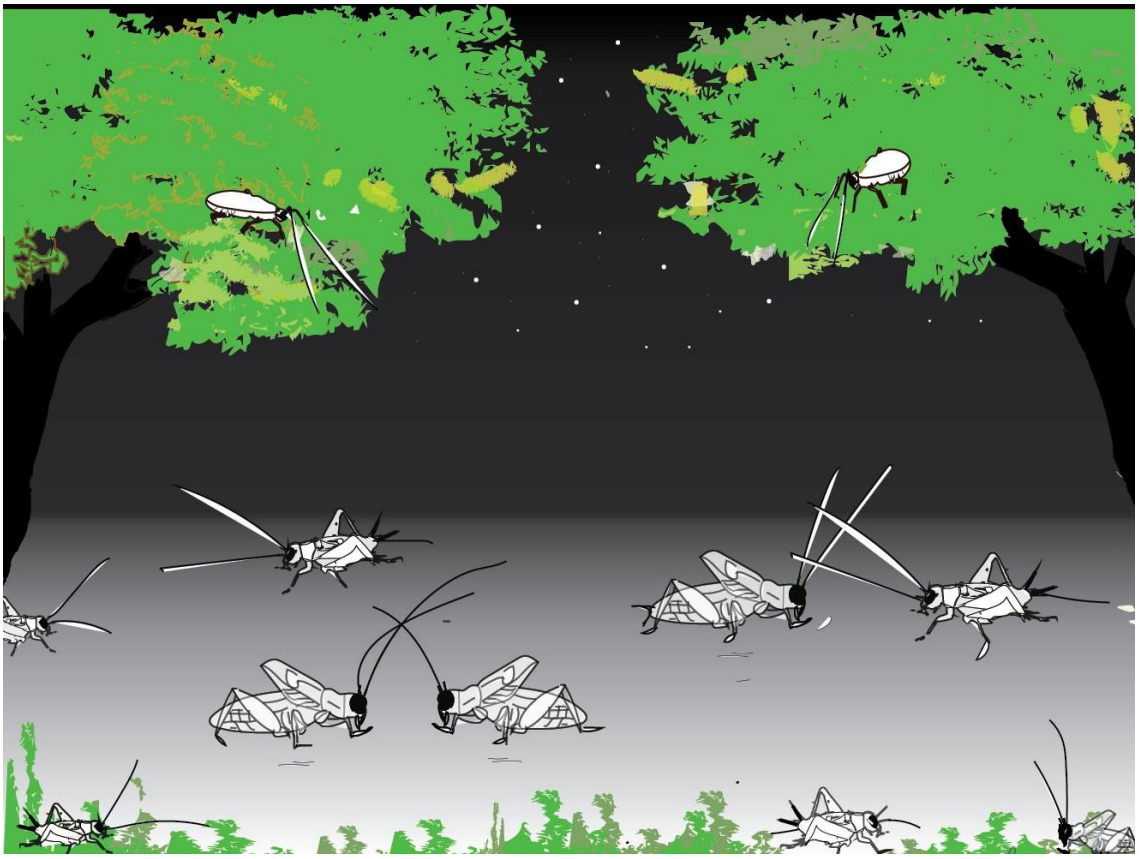
C

Test control 2																		
	Phase	Time	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	{11}	{12}	{13}	{14}	{15}	{16}	{17}
1	1	06-10h																	
2	1	10-14h	ns																
3	1	14-18h	ns	ns															
4	1	18-22h	ns	**< 0.01**	**< 0.01**														
5	1	22-02h	ns	**< 0.01**	ns	ns													
6	1	02-06h	ns	ns	ns	ns	ns												
7	2	06-10h	ns	ns	ns	ns	ns	ns											
8	2	10-14h	ns	**< 0.01**	ns	ns	ns	ns	ns										
9	2	14-18h	ns	ns	ns	ns	ns	ns	ns	ns									
10	2	18-22h	ns	ns	ns	**< 0.01**	ns	ns	ns	ns	ns								
11	2	22-02h	ns	ns	ns	**< 0.01**	ns	ns	ns	ns	ns	ns							
12	2	02-06h	ns	ns	ns	**< 0.01**	ns	ns	ns	ns	ns	ns	ns						
13	3	06-10h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns					
14	3	10-14h	ns	ns	ns	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns				
15	3	14-18h	ns	ns	ns	**< 0.01**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
16	3	18-22h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
17	3	22-02h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
18	3	02-06h	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Figure B3.1. Study map showing light and dark habitats.

Chapter 4

Spatial distribution, masking interference and acoustic interactions in males of *Acanthogryllus asiaticus*



An illustration showing aggregation in ensifera

4.1 Introduction

"Love of rhythm is not a solely human trait for it is also inherent in many species of insect." -Fulton, 1934

Efficacy of acoustic signalling depends on the signal characteristics, habitat through which it travels, ambient noise levels of the surrounding, proximity of senders and receivers and the ability of the receiver to detect the signal and extract information from it (Forrest 1994). Animals often signal in the vicinity of other signalling individuals of the same and/or different species. These aggregations may be driven by habitat requirements, predation pressure or the need to get mating advantages such as the higher probability of encounters with mates or as a hotspot for mate attraction which generate a communal display (Gerhardt and Huber 2002; Bradbury and Vehrencamp 2011; Greenfield 2015). Collective displays of these signalling animals are considered to be some of the ''great spectacles of the living world'' (Wilson 1975). Such communal acoustic displays are also known as 'choruses' which include multiple acoustically active participants that can be conspecific or heterospecific. The dawn choruses of songbirds, sound produced by aggregation of anurans and insect choruses are among some of the well-known examples (reviewed in Hulse 2002). As a result of simultaneous signalling, overall sound amplitude of such choruses is very high. This in turn may serve to attract more potential mates but also predators and parasites (Gerhardt and Huber 2002; Greenfield 2015).

4.1.1 Acoustic masking interference

While choruses can act as a supernormal stimulus due to the increased overall amplitude, simultaneous signalling from many individuals at the same place can cause degradation and acoustic interference of the signal (Romer 1998). This impairs the ability of receivers in detecting, recognizing and localising relevant signals in the presence of high levels of

masking noise (Bee and Micheyl 2008; Brumm and Slabbekoorn 2005; Hulse 2002). This leads to the problem of acoustic masking interference or popularly known as cocktail party problem (Cherry 1953) which describes the difficulty of human listeners in perceiving speech in a noisy social environment. There has been a longstanding interest for several decades in finding an answer to "How do we recognize what one person is saying when others are speaking at the same time?" (Bronkhorst 2015). This problem is not only common to humans. Numerous studies on nonhuman animals in different taxa have shown that they face similar problems in increasing signal detection thresholds and the inability of signal recognition and discrimination in masking noisy conditions (Bee and Micheyl 2008; Brumm and Slabbekoorn 2005; Hulse 2002). For instance, in bats, anurans, songbirds, several species of acoustically signalling insects (primarily crickets, katydids and cicadas) calling at the same time and place, face the similar problem (Hase et al. 2018; Bee and Micheyl 2008; Brumm and Slabbekoorn 2005; Hulse 2002; Gerhardt and Huber 2002). However, most species communicate successfully under noisy conditions. For example, echolocating bats avoid jamming (Hase et al. 2018), frogs produce loud advertisement calls when aggregating in a mixed-species chorus (Gerhardt 1975), songbirds are able to communicate in dawn choruses (Langemann and Klump 2001) and colonial bird species reunite with chicks in a large and noisy colony using acoustically mediated parent-offspring recognition (Aubin and Jouventin 2002). These examples clearly suggest that animals are able to solve the cocktail party problem. Comparable studies on insect choruses also suggest that they use various solutions to this common problem (reviewed in Romer 2013; Balakrishnan 2016). The structure of acoustic signals and the behaviour of signallers represent adaptations that have evolved as a result of selection pressures associated with improving masking problems for receivers (Brumm and

Slabbekoorn 2005). I have described various strategies used by insects in the following section (Figure 4.1).

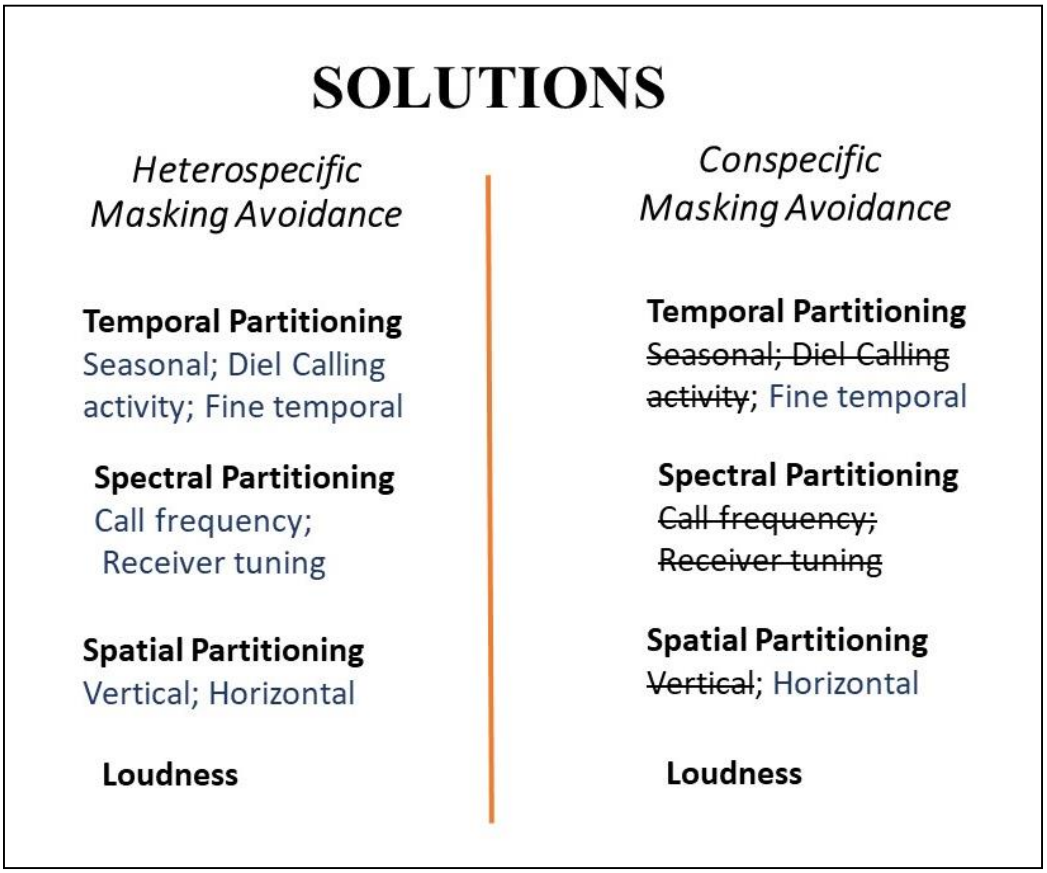


Figure 4.1 Strategies used to avoid heterospecific and conspecific masking

4.1.2 Common problem, different solutions

Given that this may have detrimental consequences on the fitness of both senders and receivers, it is expected that both senders and receiver employ a wide variety of behavioural adaptations as diverse solutions to the common problem of masking interference. Insects in a mixed-species chorus could face two types of masking problems, heterospecific masking interference from different species and conspecific masking interference from same species. It is expected that potential solution strategies used by senders and recievers could vary for these two kinds of masking interference. These strategies are discussed below:

Receiver strategy

a) *Frequency Tuning*

Tuning of receiver auditory is one of the most fundamental and ubiquitous solutions to avoid masking interference at receiver side in case of ensiferan insects (Jain et al. 2014; Schmidt and Balakrishnan 2015; Balakrishnan 2016). This allows the matching of frequency band between the signaller's call and receiver sensitivity, thereby improving signal-to-noise ratio (Simmons 2013). A study on the paleotropical rain forest assemblage of crickets and katydids in India found that tuned receivers faced low levels of effective acoustic interference (Jain et al. 2014). A study in the rainforest of Panama revealed sharply tuned frequency selectivity in cricket species living within a large assemblage of acoustically co-active species (Kostarakos et al. 2008, 2009; Schmidt et al. 2011, 2013; Schmidt and Römer 2011; Romer 2013). This kind of strategy is only applicable in avoiding masking in heterospecific assemblages or habitat-induced noise. In case of conspecific masking, it is not valid due to similar frequency range of signals of conspecific signallers which leads to complete spectral overlap.

b) *Spatial release from masking*

Spatial release from masking as one of the solutions by receivers to detect signal in noisy conditions of hetero and conspecific masking avoidance (Schmidt and Römer 2011). This refers to the situation when two auditory objects (e.g. conspecific signal and masker) are spatially separated, the detection of a sound signal will be improved (Bee 2008). A study on the cricket *Paroecanthus podagrosus* showed that signal detection thresholds of receiver significantly improved by 6–9 dB when the signal (i.e. conspecific calling song) and masker (i.e. nocturnal background noise) were spatially separated by 180° (Schmidt and Römer 2011).

c) *Selective attention*

To overcome acoustic masking interference from both hetero and conspecific signallers, receivers in crickets and katydids have neuronal mechanism of selective attention wherein only the loudest call (after filtering by the ear) on each side is represented (Pollack 1988; Römer and Krusch 2000). More precisely, this mechanism can allow preferential representation and selective attention to louder signal even in the presence of spectral overlap in signals. Such a mechanism was first reported for the omega neuron in crickets, which selectively encodes the more intense signal in the presence of a signal with lower intensity, based on combined synaptic activity of inhibitory-excitatory effects (Pollack 1988).

Sender strategy

a) *Spectral partitioning*

One of the axes of segregation from the sender's side can be spectral partitioning.

Heterospecific masking avoidance: Various studies on insect acoustic assemblages have shown overall low levels of pairwise spectral overlap between signals of different species (Schmidt et al. 2013; Jain et al. 2014). Such low levels of spectral overlap are driven by selection for spectral partitioning (Schmidt et al. 2013). Species with a high level of spectral overlap are likely to partition their call temporally (Römer 2013). However, a study on a paleotropical rain forest assemblage of crickets in India by Jain et al. (2014) found no negative correlations between spectral and temporal overlaps implying that species with higher temporal overlaps do not evolve greater spectral segregation. Schmidt et al. (2016) found no significant difference between acoustic signals of species pairs that called together/used similar calling frequencies and acoustic signals of species that were spatially/temporally segregated/used different calling frequencies.

Conspecific masking avoidance: This kind of partitioning is applicable in avoiding heterospecific masking and not conspecific masking due to similar frequency range of signals of conspecific signallers which leads to complete spectral overlap.

b) *Lombard effect*

In order to counteract masking, one of the most obvious mechanisms is to increase signal amplitude as noise level increases (Zollinger and Brumm 2011). This phenomenon is termed as the Lombard effect and was first discovered in human speech (Lombard 1911). This effect has been observed in birds, bats and mammals (reviewed in Zollinger and Brumm 2011; Brumm and Slabbekoorn 2005). However, so far, there is no such case reported for insects. Several studies do indicate that females have a strong preference for louder calls such as fruitflies, mole crickets, crickets and katydids prefer louder signallers and insects use resonators, amplification burrows and baffles to achieve an increased loudness (review in Römer 1998; 2013).

c) *Spatial partitioning*

To avoid masking interference, signallers can distance themselves. Spatial release from masking improves the detection and discrimination of signals in noise for the receiver (Bee 2008).

Heterospecific masking avoidance: Spatial partitioning can be employed at both horizontal and vertical scales. However, in heterospecific insect assemblages, it seems that spatial partitioning has a smaller role to play as the two studies which examined the same did not find evidence for horizontal spatial separation between individuals of different species to reduce interspecific acoustic interference (Schmidt et al. 2013; Jain et al. 2014; Balakrishnan et al. 2014). This could be possible as other levels of segregation such as spectral partitioning (sender strategy) and frequency tuning (receiver strategy) effectively

solve masking interference. The vertical stratification of heterospecific insects has been reported in rain forest assemblage (Sueur 2002; Diwakar and Balakrishnan 2007; Schmidt et al. 2013) but whether vertical stratification solves the masking interference problem is still unexplored.

Conspecific masking avoidance: Horizontal spacing can strongly determine the severity of conspecific masking interference. In conspecific field crickets, horizontal spacing has been reported in *Acanthogryllus fortipes* and *Plebeiogryllus guttiventris* (Cade and Otte 1982; Mhatre and Balakrishnan 2006).

d) Temporal Partitioning

Temporal partitioning can be accomplished at different timescales for different species choruses (reviewed in Schmidt and Balakrishnan 2015; Balakrishnan 2016).

Heterospecific masking avoidance: Hetero-specific signallers can decrease temporal overlap in their signals by (1) avoiding same breeding seasons; (2) calling at different times of the day or night (diel partitioning: Diwakar and Balakrishnan 2007); (3) calling in the same diel period but in non-overlapping bouts (Gross temporal partitioning (GTO): Jain et al 2014); or (4) calling simultaneously in the same bout but placing individual calls in the silent gaps between the calls of heterospecifics (Fine temporal partitioning (FTO): Jain et al 2014; Gerhardt and Huber 2002). A study from the paleotropical rainforest assemblage of cicada, cricket and katydid showed that cicada partitioned their call on diel scale to avoid masking from crickets and katydids (Diwakar and Balakrishnan 2007). Another study from the same paleotropical rainforest assemblage suggested that species calling within diel period (GTO) (within 5-min time windows) are more likely to possess signal temporal structures (FTO) that led to less level of overlapping (Jain et al. 2014).

Conspecific masking avoidance: Although masking interference is mostly known in heterospecific signallers, insects face a similar problem of masking with their conspecific neighbours (Romer 2013). In such dense aggregates, the overlap between male calls mask temporal pattern and impair females' ability to recognize relevant signals (Greenfield 1994). This is solved when fine-scale signal partitioning is done where intermittently singing individuals either synchronise or alternate with the signals of their neighbours, i.e. when the phase angles are approximately either 0° or 180°, respectively (Greenfield 2015). This fine-scale temporal partitioning has been supported by various evolutionary explanations: (1) maintenance of the species-specific temporal pattern (Walker 1969), (2) avoiding acoustically orienting predators and parasitoids (Otte 1977) and (3) emergence of group synchrony and alternation as by-products of basic signal interactions between neighbours (Greenfield 1994). With respect to fine temporal patterns of acoustic interactions, synchrony has been shown to preserve species-specific temporal patterns which attract females in katydids (Nityananda and Balakrishnan 2009; Hartbauer et al. 2014). In addition, synchrony also increases the group's overall sound amplitude, a phenomenon known as the 'Beacon effect' (Hartbauer et al. 2014), confuses acoustically orienting natural predators and parasites as it hampers their ability to localize single signaller in a group due to sound coming from all directions simultaneously (Tuttle and Ryan 1982). Some grasshopper species also use call alternation to avoid masking (Greenfield and Minckley 1993). Alternation allows males calling in vicinity of signalling neighbour(s) to clearly assess the rival neighbour's calls and adjust its own signalling accordingly (active avoidance), e.g., matching or exceeding neighbour's call features (Greenfield and Minckley 1993). Additionally, alternating calls can be clearly perceived and evaluated by conspecific females. Female crickets are known to prefer calls with higher chirp rates and longer chirps (Wagner 1996) and alternation also serves to double the call

rate for a listener. Males would, therefore, be expected to alter the temporal structure of their calling songs, producing longer chirps at higher rates, in response to the song of a competing neighbour.

Overall strategies used to avoid conspecific masking: Solutions used to avoid heterospecific masking are different in case of conspecific masking avoidance. For example, frequency tuning (receiver strategy) and spectral partitioning (sender strategy) for conspecific masking avoidance, were completely ruled out due to similar frequency ranges of conspecific signals and complete spectral overlap. In addition, sender strategies of vertical spatial partitioning, seasonal and diel temporal partitioning are not applicable for conspecific masking avoidance. The only types of segregation which can be expected to be used for conspecific masking avoidance are increase in loudness, horizontal spacing and fine temporal partitioning of chirps.

No study has so far examined all levels of segregation used by senders to avoid acoustic masking interference in conspecifics. For instance, only fine temporal partitioning was studied in a tarbush grasshopper in field conditions (Minckley et al. 1995) and in a bushcricket species *Mecopoda* 'Chirper' in lab conditions (Nityananda and Balakrishnan 2007). In a field cricket, *P. guttiventris*, Mhatre and Balakrishnan (2006) looked at the presence of spatial partitioning and fine temporal partitioning in the lab environment while Cade and Otte (1982) described spacing pattern and examined fine temporal partitioning in *A. fortipes* in field conditions. No study in my knowledge has overall examined the spatial partitioning, temporal partitioning and the presence of Lombard effect with respect to conspecific masking avoidance in an insect species. Also, the studies on field crickets are conducted either in lab or field. In this study, I investigated the problem of conspecific acoustic masking interference in male field crickets (*Acanthogryllus asiaticus*) and the strategies they use to solve it. In this chapter, I examined the following aspects of acoustic

signalling in *A. asiaticus* in the context of conspecific acoustic masking interference: (1) male spacing in the field to examine if males aggregate in choruses during signalling; (2) potential of acoustic masking interference by examining the degree of overlap of signal broadcast areas of signalling males in a given habitat; (3) the nature of acoustic interactions (if any) of focal male with nearest conspecific neighbours in field and lab environment; (4) presence of any change in call features when calling in solo vs calling in interaction and (5) use of the Lombard effect to avoid masking interference.

4.2 Materials and methods

The entire study was carried out from 2016 to 2018. Site for field study was IISER Mohali campus, Punjab, India. (For geography, vegetation and climate, see Chapter 2).

4.2.1 Sound pressure level and signal attenuation

I psychoacoustically located (Diwakar et al. 2007) calling individuals in the field during their peak calling hours and measured the sound pressure level (SPL) of the males to calculate the species-specific average. SPL (LAF in 1/3-octave bands; bandpass filter centred at 5 kHz) was measured using a Brüel & Kjær ½″ microphone, Type 4189 (20 Hz to 20 kHz) attached to a Sound Level Meter, Type 2270 (Brüel & Kjær, Naerum, Denmark). While the microphone is directional, additional care was taken to ensure SPL of only one male was registered during measurements. Towards this only those calling males were selected which were distant apart from the other calling individual otherwise any other male near the focal male was interrupted during SPL measurement. All measurements were made at a distance of 50 cm above a calling male and values were averaged across multiple readings to arrive at SPL at source for a given male. Measurements are expressed as dB SPL (re 2 X 10^{-5} $N/m^{2)}$. To determine the average SPL of *A. asiaticus* song at source, I measured the SPL of 20 males in their natural habitat.

I then examined the distance over which the signal of a calling male was likely to propagate, given the habitat characteristics. Towards this, SPL attenuation was measured by broadcasting a representative call of *A. asiaticus* (chosen based on various acoustic features reflecting population means; Singh and Jain 2020) from a loudspeaker (JBL GO 2, Harman International) placed on the ground (to simulate a calling male) connected to a laptop (Thinkpad T480, Lenovo) at average calling SPL (dB level measured at 50 cm from the speaker). 3 SPL readings were taken at increasing distance from the speaker: 50 cm, 1m, 2 m, 3 m 4 m, 6 m, 7 m, 8 m, 9 m, 10 m, 16 m and 32 m. This was done in three different natural habitats (where the species are naturally found to signal) and average SPL values were taken. This SPL attenuation (total attenuation) experiment was conducted during the night time of the non-peak calling season to avoid signal interference and to match with the temperature of peak season. However, the ambient noise level of these habitats was measured during peak calling season.

4.2.2 Male spatial distribution

A survey of calling males of *A. asiaticus* was carried out in the study site during peak calling time and all areas where animals were actively signalling across different nights were identified. 10 sites were selected for further fieldwork and at each site, all calling individuals were psychoacoustically located and their positions were flagged with a unique ID for each caller. Care was taken to not to disturb the animals when planting the flags. These sites were then revisited the next mornings and distance between flagged males were measured using a meter tape or using the triangulation method (Jain et al. 2014). Every site was sampled just once and the spacing consistency of any given site across different nights was not examined. Using the spatial data, choruses were reconstructed (Gnuplot, version 5.2). Nearest neighbour and next neighbour measurements were made for all the calling males across all the choruses.

4.2.3 Acoustic interaction with the neighbour

Acoustic interaction in the field condition

To examine the acoustic interactions of individuals in the field, a pair of calling individuals were located and their calls were recorded simultaneously using two directional microphones (Sennheiser ME66), each facing one of the two callers, attached to a dual-channel linear PCM recorder (Tascam DR-40, TEAC Professional, USA) (Figure 4.1). Following this, SPL of these individuals and the distance between the two individuals were also recorded. Ambient temperature and wind were noted for reference. This was done for 23 pairs of individuals across different habitats in the study site.

Acoustic interaction in the laboratory environment

Playback experiments were performed to examine the acoustic interaction between males in the laboratory environment. For this experiment, lab-reared individuals were used. Adult males were placed in plastic box container (diameter-12 cm; height 6 cm) covered with cloth mesh. All animals were maintained at 24°C, 40 - 70 % humidity, 12:12 h light: dark condition and food and water were provided *ad libitum*. Prior to the commencement of every experiment, solo call along with SPL was recorded for each individual. After this, a simulated neighbour call (*A. asiaticus* call with average values of parameters recorded at 24°C; Singh and Jain 2020) was broadcasted from the speaker at species-specific mean SPL of 62 dB. The focal male was given a couple of minutes to acclimatize to the presence of a simulated neighbouring signaller. Following this, calls of the focal individual placed in nylon mesh box were recorded again to assess its response/interaction, if any. Finally, the SPL of the focal male during the interaction was measured after turning off the speaker briefly. This was done for 25 focal individuals. All the recordings were analyzed using sound analysis software Raven Pro 1.4 (Bioacoustics Research Program, Cornell

Laboratory of Ornithology, Ithaca, NY). Call characteristics such as chirp duration, chirp period, syllable duration, syllable period, number of syllables per chirp, number of chirps per second, peak frequency were compared for 10 chirps for all the calls recorded during solo and duet condition for each male.

Phase angle calculations

Phase angle measurements were performed for the above-collected recordings by following the method used by Mhatre and Balakrishnan (2006). To examine phase relations between the chirps of two chorusing males in field, one of the two males in a chorus was randomly selected as focal male and then the phase relations of 10 chirps with respect to those of the neighbour during the chorus was measured. To examine phase relations for acoustic interaction of focal male in the laboratory environment, phase of 10 chirps with respect to a simulated neighbour was measured. The phase angle of each chirp of the focal male (Figure 4.2) was calculated using the formula given below:

$$[(Tf-TsB)/(TsA-TsB)] \times 360°$$

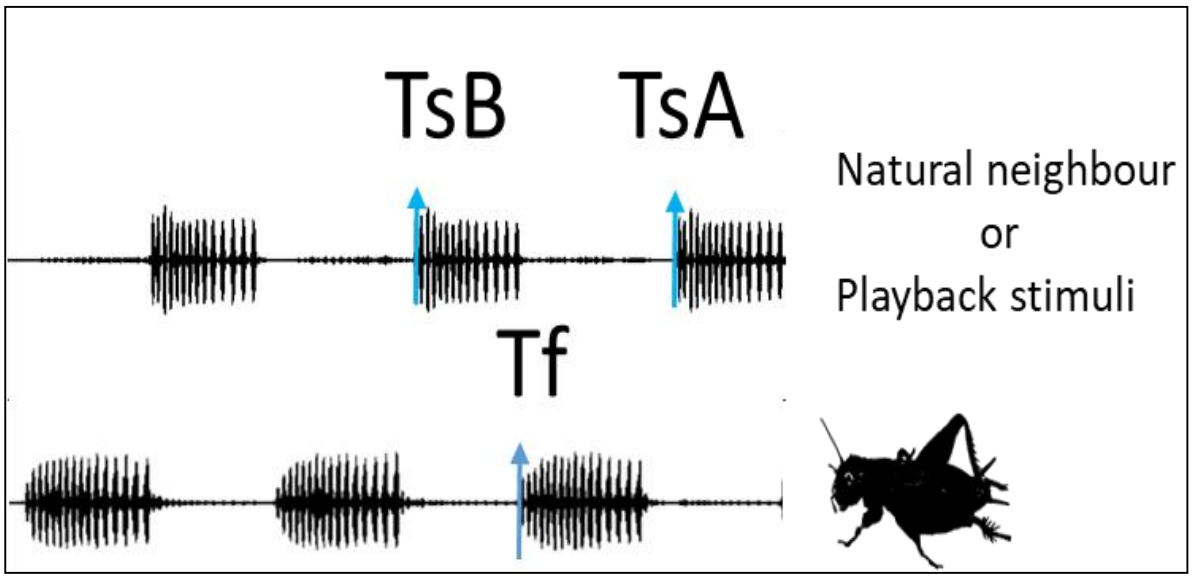


Figure 4.2. Schematic diagram to represent phase relationship calculation.

where Tf = time of onset of the *i*th chirp of the focal male; TsB = time of onset of the chirp of the neighbour that occurred immediately before or at the same time as the *i*th chirp of the focal male; TsA = time of onset of the chirp of the neighbour of the focal male that occurred immediately after the *i*th chirp of the focal male. Chirps with phase angles lying at 0° considered to be perfect synchrony (e.g., Walker 1969), whereas chirps with phase angle 180° considered to be the perfect alternation (e.g., Shaw 1968) (Figure 4.3).

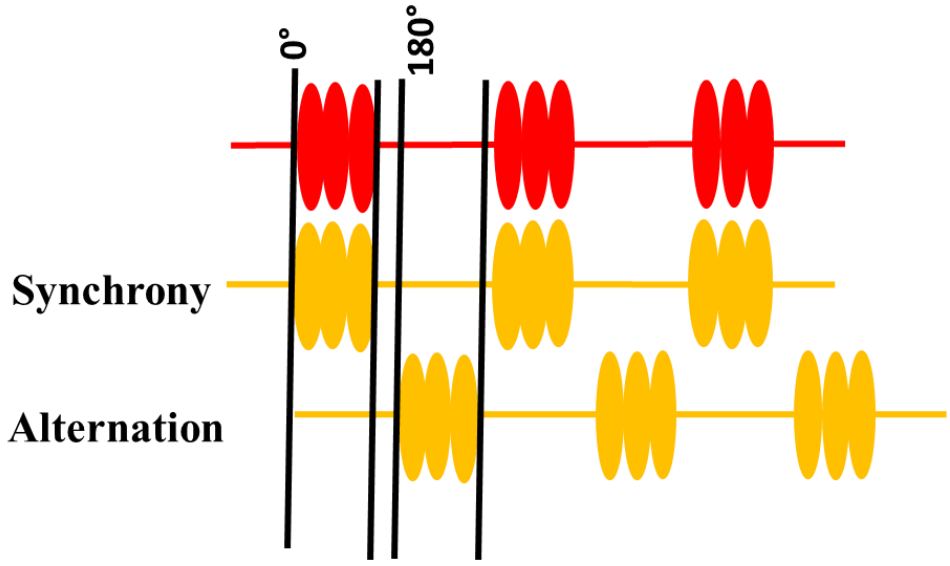


Figure 4.3. An example of phase alignment of chirps showing phase angle = 0° considered to be perfect synchrony and phase angle = 180° considered to be the perfect alternation.

4.2.4 SPL modulation

Playback experiments were conducted for 31 laboratory-bred adult males of *A. asiaticus*. Each individual was exposed to simulated neighbour call (*A. asiaticus* call with average values of parameters recorded at 24°C, Singh and Jain 2020) broadcasted as a stimulus from the speaker. Commencement of calling of a focal male marked the starting of a trial. SPL was recorded for the solo calling individual. After 2 minutes, LOUD (5 dB higher than actual call) or SOFT (5 dB lower than actual call) stimulus was played for 2 minutes. The

speaker was turned off briefly (a few seconds) to measure the SPL of the focal male during the interaction and then restarted. Again, after a break of 2 minutes, response for the same individual was tested against LOUD/ SOFT stimulus (whichever not tested earlier) for 2 minutes. The order of first exposure to LOUD or SOFT test call was randomized across trials.

4.2.5 Masking probability analyses

On reconstructed choruses, masking probability for each male across all the choruses was calculated by following methods given by Jain et al (2014). Unlike heterospecific masking, for conspecific masking, Gross Temporal Overlap (the probability that two masking individuals call together in a 5 min window) is 1, probability of Spectral Overlap (calls with similar spectral features will have a higher chance to overlap in frequency hence the value will be closer to 1) is also 1. This leaves only three lines of segregation, for masking avoidance from conspecific maskers: spatial segregation to minimize Active Space Overlap, minimize Fine Temporal Overlap (see below; Jain et al. 2014) and to modulate SPL in a manner to increase SNR. I investigated each of these three strategies to examine the severity of conspecific masking interference and to test whether they employ these strategies to counter the problem.

Active space overlap (ASO)

Active space is defined as the volume of space around a focal male where it can be heard by a specific receiver (Jain et al. 2014). For a field cricket, in the context of conspecific masking, both the sender and the receiver are on the ground and thus the active space is effectively the total circular area of a circle with the focal caller at the centre and a radius ‘r’ that is determined by the maximum distance signal gets transmitted in ambient noise condition. I assumed female hearing threshold likely to be similar to ambient noise level.

Any intersection of two or more such acoustic areas of calling individuals amounts to active space overlap. Therefore, ASO was calculated by measuring the proportion of an individual's broadcast area that was overlapped by the broadcast area of a neighbour (following Jain et al. 2014 with custom-written scripts in Java version 8 by Jimmy Bahuleyan).

Fine temporal overlap (FTO)

Fine Temporal Overlap (FTO) is a temporal overlap between the calls of two individuals calling at the same time, assuming no acoustic interaction between them. The proportion of this total time that was overlapped by the call of neighbour was calculated (following Jain et al. 2014 with custom-written scripts in Java version 8 by Jimmy Bahuleyan) to obtain the FTO which ranged from 0 (no overlap) to 1 (100 % overlap).

Effective acoustic overlap (EAO)

Effective Acoustic Overlap (EAO) for a focal individual was calculated (following Jain et al. 2014 with custom-written scripts in Java version 8 by Jimmy Bahuleyan) by multiplying the ASO and FTO values since GTO and SO probabilities are equals to 1. This is the total overlap suffered by a calling individual whose acoustic space overlaps with the calling neighbour as well as its call is also getting overlapped by the call of the neighbour falling acoustic area of the focal male.

4.2.6 Statistical analyses

Circular statistics for acoustic interaction was performed using Oriana (version 4.02). I used the Rayleigh test to check for uniform distribution around the circle and used the V-test to determine for significant alternation or synchrony (Batschelet 1981). Other statistical tests were performed using Statistica 64 (Dell Inc. 2015, Version 12). To compare the temporal and spectral features during solo calling and chorusing as well as SPL responses for the

modulation experiment, paired t-test were performed after being checked for normality using the Shapiro-Wilk test.

4.3 Results

4.3.1 SPL measurement and Signal attenuation

Average SPL for calling individuals in the field was found to be 62 dB (LAF in 1/3-octave bands; bandpass filter centred at 5 kHz). Ambient noise for habitats during peak season was found to be 38.02 dB (LAF in 1/3-octave bands; bandpass filter centred at 5 kHz). The signal transmission was found to be till 3 m which was measured during the nights of non-peak calling season. Thus, at this distance, the signal gets completely masked by ambient noise (Figure 4.4). The hearing threshold of female was assumed to be around 38.02 dB.

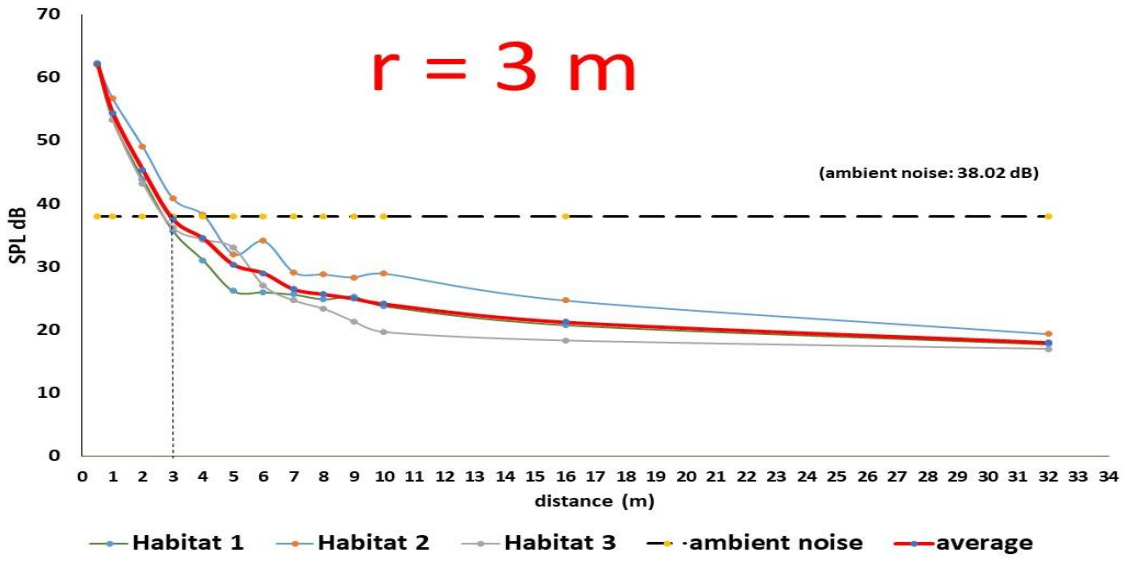


Figure 4.4. Total attenuation profile of calls of *A. asiaticus*. Each line represents the attenuation profile at different habitats along with the red line representing average attenuation.

4.3.2 Spatial distribution and masking

Nearest neighbour distance across all the choruses was ranged 0.24 m to 14.89 m whereas next neighbour distance was found to be varying between 1.41 m to 24.63 m (Figure 4.5, Table 4.1). Nearest neighbour distance was found to around median 2.95 (1.73-4.62) and next neighbour distance was found to be median 5.23 (3.52-8.93). ASO for each focal male on an average was found to be median 0.408 (0.135-0.789) whereas EAO was found to be 0.168 (0.014-0.355) (Figure 4.5, Table 4.1, Table C4.1). The median number of maskers was found to be 2 and the median number of audible maskers was found to be 1 (Figure 4.6, Table 4.1). In order to calculate effective masking, ASO of nearest audible neighbour was not considered as later in acoustic interaction experiments, I found focal males to be alternating their call with the nearest audible neighbour to alternate. Therefore, FTO was considered to be zero in this case. With the next nearest masker, FTO was set to 0.7 because in *A. asiaticus*, average chirp duration of the call is 0.3 s and average chirp period is 0.9 s (Singh and Jain 2020). Therefore, there is probability that 0.6 s of chirp period will be masked and only 0.3 s will escape masking. Under these circumstances, given the temporal structure of call, in absence of active resetting, the masking probability with another calling individual (who is calling at a random phase angle with focal male) is 0.7. This means that there is 70% chance of fine temporal overlap (in absence of active resetting and alternation). Only a 30% chance of escaping masking. This 30% chance of escaping masking is also lost when even one more masker is added to the chorus. Hence, in presence of more than 1 masker (apart from the nearest neighbor with whom it is actively resetting) FTO was set to 1.

Table 4.1. Active space overlap (ASO), Effective Acoustic overlap, maskers, nearest neighbour (NN) and next nearest neighbour (NNN) distances across 10 choruses (Median (Inter quartile range)).

Chorus	ASO	EAO	Maskers	Audible masker	NN	NNN
Chorus 1	0.347 (0-0.68)	0.063 (0-0.244)	2 (0-2)	0 (0-1)	4.85 (2.8-10.34)	5.07 (4.85-14.65)
Chorus 2	0.368 (0.34-0.39)	0.26 (0.24-0.27)	2 (2-2)	0 (0-1)	3.64 (2.84-3.64)	5.59 (5.59-5.64)
Chorus 3	0.031 (0-0.34)	0 (0-0.21)	1 (0-1)	0	5.47 (3.29-7.24)	8.93 (8.93-9.19)
Chorus 4	0.709 (0.36-0.96)	0.347 (0.16-0.644)	6 (2-7)	1 (0-2)	2.17 (1.58-3.94)	3.7 (2.25-5.89)
Chorus 5	0.131 (0-0.131)	0.091 (0-0.09)	1 (0-1)	0	4.6 (4.6-6.79)	11.02 (6.79-11.02)
Chorus 6	0.400 (0.17-0.42)	0.036 (0-0.119)	1 (1-2)	1 (0-1)	2.95 (2.9-4.47)	6.04 (5.46-10.48)
Chorus 7	0.185 (0-0.215)	0.129 (0-0.16)	1 (0-2)	0	4.08 (2.96-6.28)	7.04 (5.23-11.94)
Chorus 8	0.680 (0.49-0.807)	0.245 (0.21-408)	3 (2-4)	1 (0-2)	1.96 (1.73-3.69)	4.1 (2.83-4.7)
Chorus 9	0.987 (0.95-0.99)	0.814 (0.46-0.83)	6 (6-6)	4 (2-4)	0.29 (0.24-0.71)	1.63 (1.47-2.6)
Chorus 10	0.408 (0.058-0.76)	0.041 (0.019-0.062)	1.5 (1-2)	0.5 (0-1)	2.1 (1.43-3.85)	3.58 (3.175-4.85)
Overall	**0.408 (0.135-0.789)**	**0.168 (0.014-0.355)**	**2 (1-4)**	**1 (0-1)**	**2.95 (1.73-4.62)**	**5.23 (3.52-8.93)**

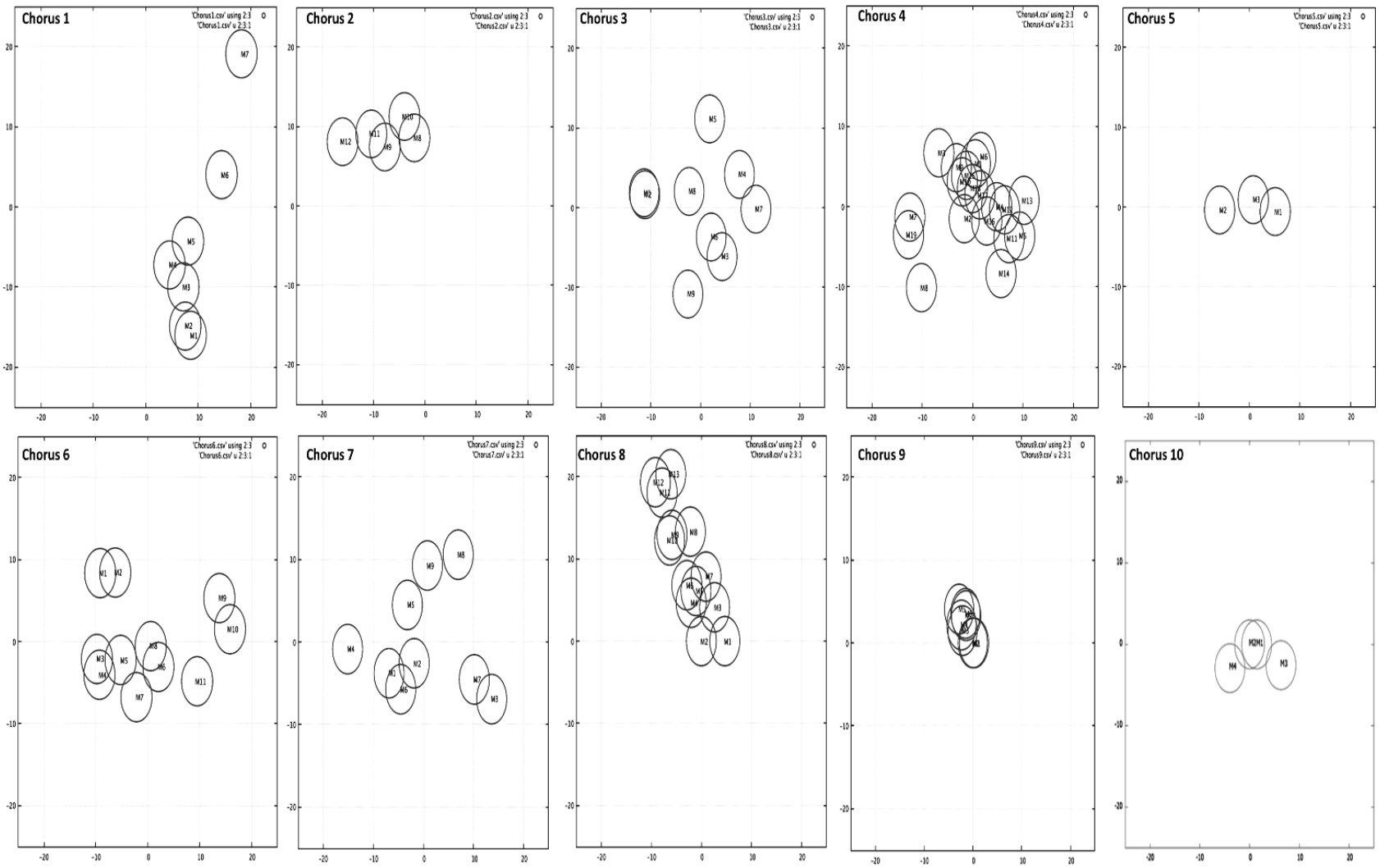


Figure 4.5. 10 choruses observed at different nights. Circles in each chorus belong to different individuals. Each circle depicts the acoustic range of an individual with a radius of 3 m.

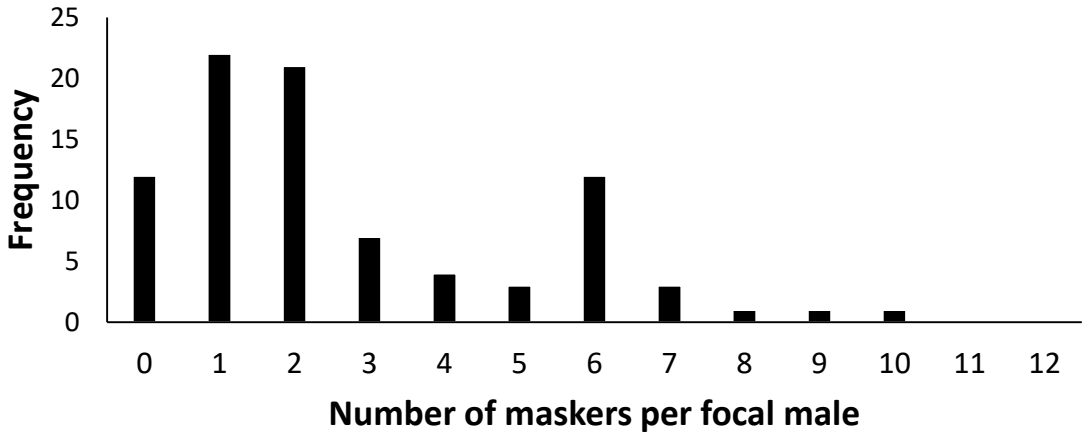


Figure 4.6. Frequency of total number of maskers for every focal male across the 10 choruses examined.

4.3.3 Acoustic interaction with the neighbour

I found active alternation in both field and lab experiment. In field condition, mean vector of phase angles was found to be 184.69° with circular standard deviation 88.63° (V test, $P < 0.01$; Rayleigh test, $P < 0.01$, Figure 4.7 & 4.8, Table 4.2) which indicate alternation with the neighbour. In lab playback experiments, mean vector of phase angles was found to be 217.29° with circular deviation 92.76° which also indicate alternation (V test, $P < 0.01$; Rayleigh test, $P < 0.001$, Figure 4.9 & 4.10, Table 4.3).

4.3.4 Changes in call temporal features

Calls of focal males when singing in solo was found to have significantly different chirp periods and chirp rate when compared to calls recorded during interaction with the simulated neighbour (Paired t-test, $P < 0.05$, Figure 4.11, Table C4.2). Other temporal and spectral features along with SPL did not change while interacting with the simulated neighbour (Paired t-test, $P > 0.05$, Figure 4.11, Table C4.2).

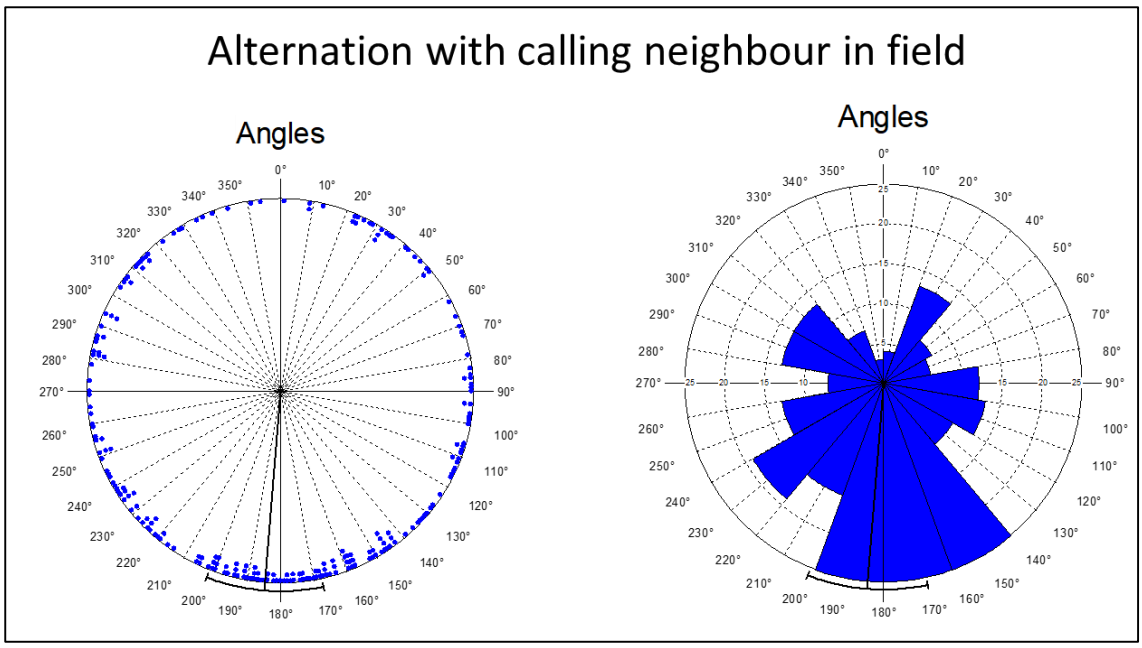


Figure 4.7. Circular plots showing phase relations of the chirps produced during acoustic interaction between focal male and its natural neighbour. Left plot shows all raw data points pooled across all the males and right plot represents frequency distribution of all phase angles of interactions of all males. Mean vector of population is 184.69° with circular standard deviation 88.63°. N = 23 males

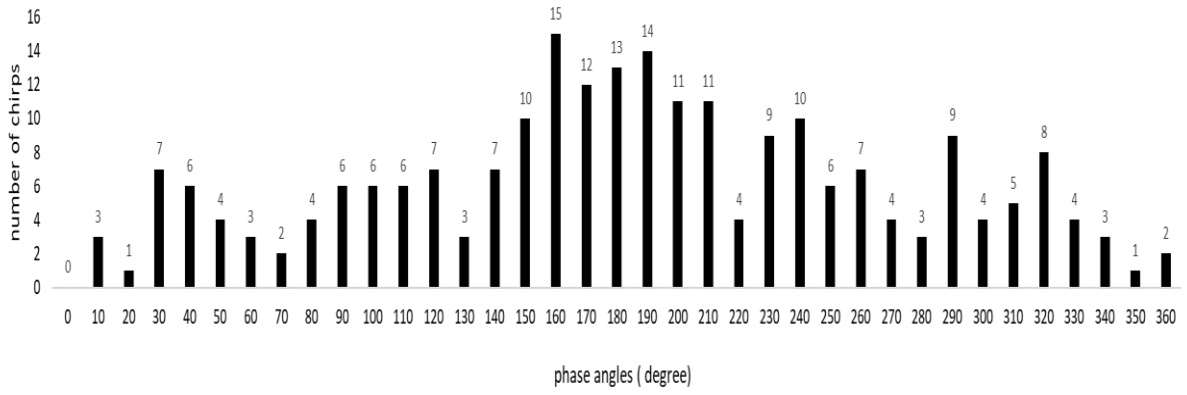


Figure 4.8. Frequency distribution of phase angles for the chirps produced by a focal male while acoustically interacting with a natural neighbour in the field.

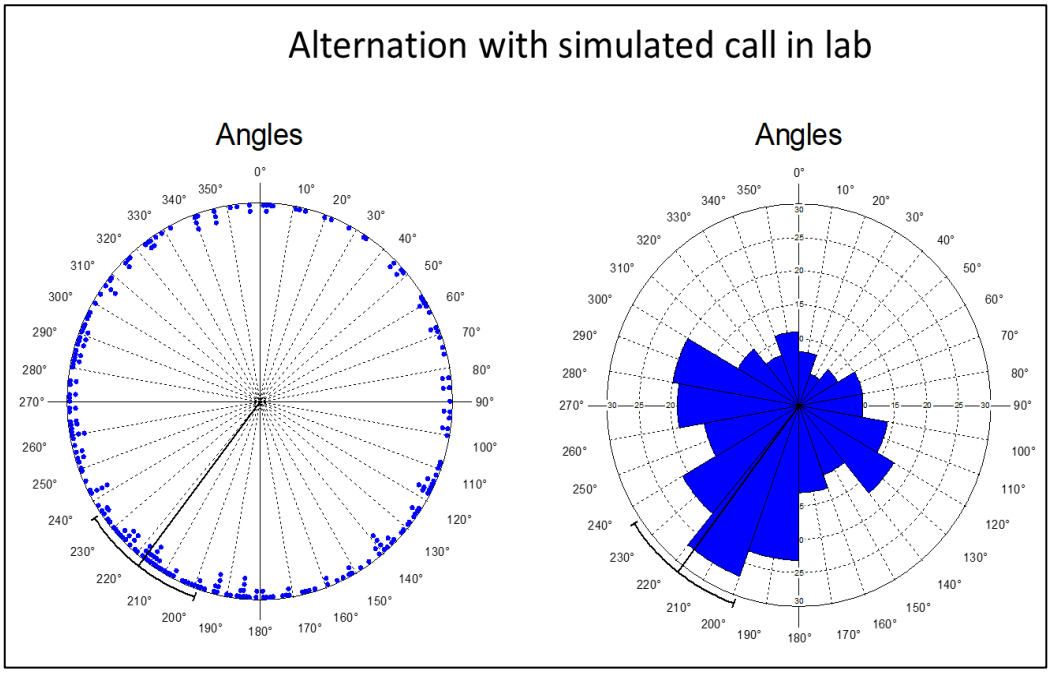


Figure 4.9. Circular plots showing phase relations of the chirps produced during acoustic interaction between focal male and its simulated neighbour. Left plot shows all raw data points pooled across all the males and right plot represents frequency distribution of all phase angles of interactions of all males. Mean vector of population is 217.29° with a circular deviation of 92.76°. N = 25 males.

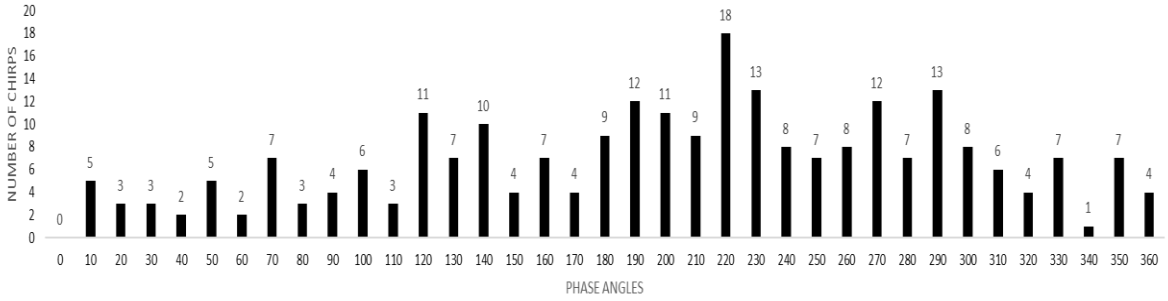


Figure 4.10. Frequency distribution of phase angles for the chirps produced by a focal male while acoustically interacting with a simulated neighbour in laboratory condition.

Table 4.2. Statistical analysis for acoustic interaction of a focal male with a natural neighbour in the field.

Variable	Angles
Data Type	Angles
Number of Observations	230
Mean Vector (μ)	**184.692°**
Length of Mean Vector (r)	**0.302**
Median	179.947°
Concentration	0.634
Circular Variance	0.698
Circular Standard Deviation	**88.629°**
Standard Error of Mean	8.628°
95% Confidence Interval (-/+) for μ	167.778°
	201.607°
99% Confidence Interval (-/+) for μ	162.465°
	206.92°
Rayleigh Test (Z)	21.016
Rayleigh Test (p)	7.46E-10
Rao's Spacing Test (U)	151.094
Rao's Spacing Test (p)	< 0.01
Watson's U^2 Test (Uniform, U^2)	1.141
Watson's U^2 Test (p)	< 0.005
Kuiper's Test (Uniform, V)	3.246
Kuiper's Test (p)	< 0.01
V Test (u)	6.461
V Test (p)	< 0.01

Table 4.3. Statistical analysis for acoustic interaction of a focal male with a simulated neighbour in the lab.

Variable	Angles
Data Type	Angles
Number of Observations	250
Mean Vector (μ)	**217.396°**
Length of Mean Vector (r)	**0.27**
Median	215.961°
Concentration	0.56
Circular Variance	0.73
Circular Standard Deviation	**92.762°**
Standard Error of Mean	9.324°
95% Confidence Interval (-/+) for μ	199.117°
	235.675°
99% Confidence Interval (-/+) for μ	193.376°
	241.417°
Rayleigh Test (Z)	18.179
Rayleigh Test (p)	1.27E-08
Rao's Spacing Test (U)	143.767
Rao's Spacing Test (p)	< 0.05
Watson's U^2 Test (Uniform, U^2)	0.949
Watson's U^2 Test (p)	< 0.005
Kuiper's Test (Uniform, V)	3.064
Kuiper's Test (p)	< 0.01
V Test (u)	4.79
V Test (p)	< 0.01

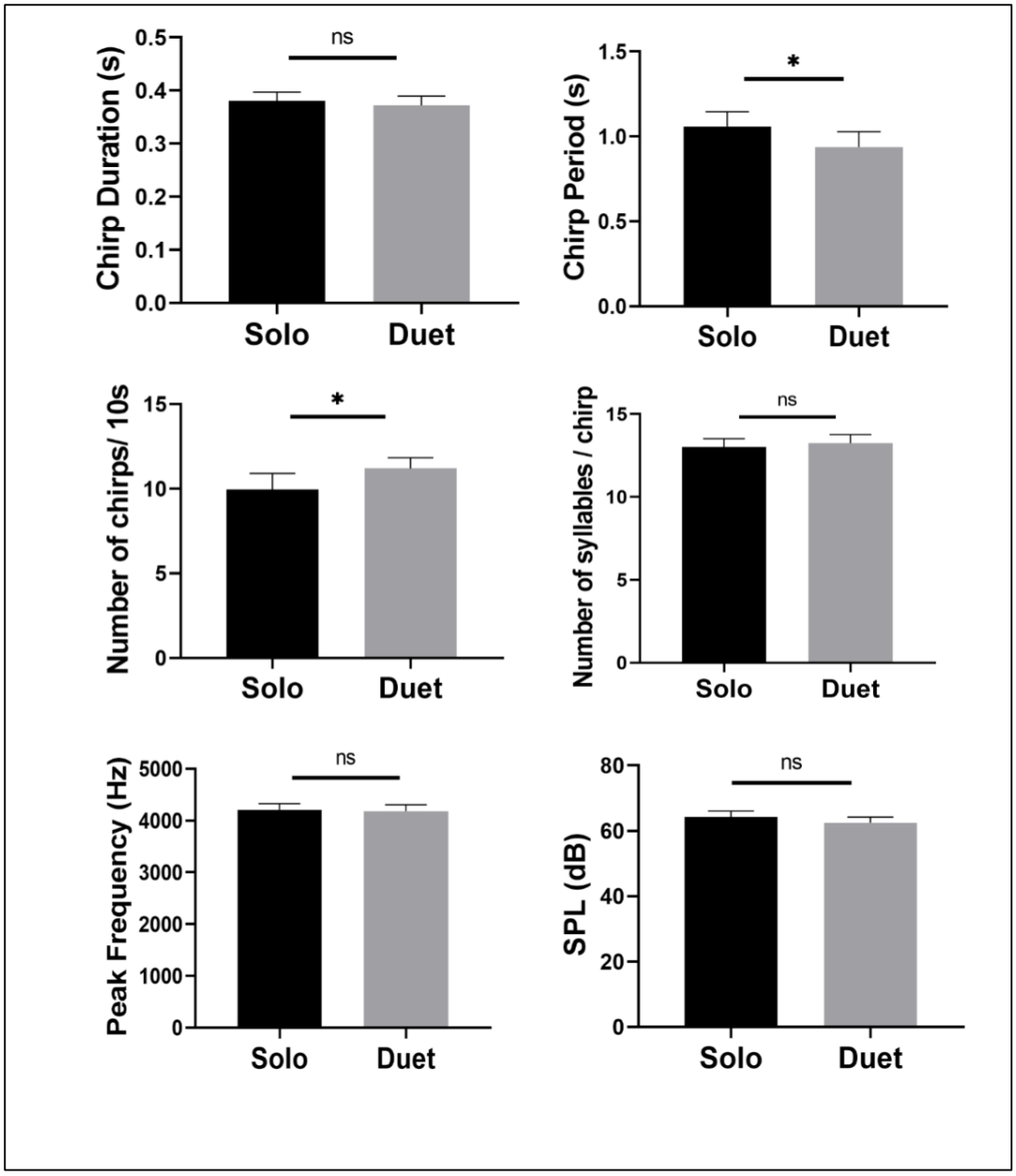


Figure 4.11. Comparison of temporal and spectral features of focal male calling in solo and interacting with a simulated neighbour. * signifies $P < 0.05$. N = 25 males.

4.3.5 SPL modulation

I found that *A. asiaticus* shows SPL modulation wherein, when the male is exposed to softer call, individuals decreased SPL and then later increased it when exposed to loud call (Paired t-test, Control vs Soft: $P < 0.05$, Soft vs Loud: $P < 0.05$, Control vs Loud: $P > 0.05$; Figure 4.12A, Table C4.3). However, when first exposed to a louder neighbour, the focal male decreased it SPL and showed no effect when exposed to softer call (Paired t-test, Control

vs Loud: P < 0.05, Loud vs Soft: P > 0.05, Control vs Soft P < 0.05; Figure 4.12B, Table C4.3).

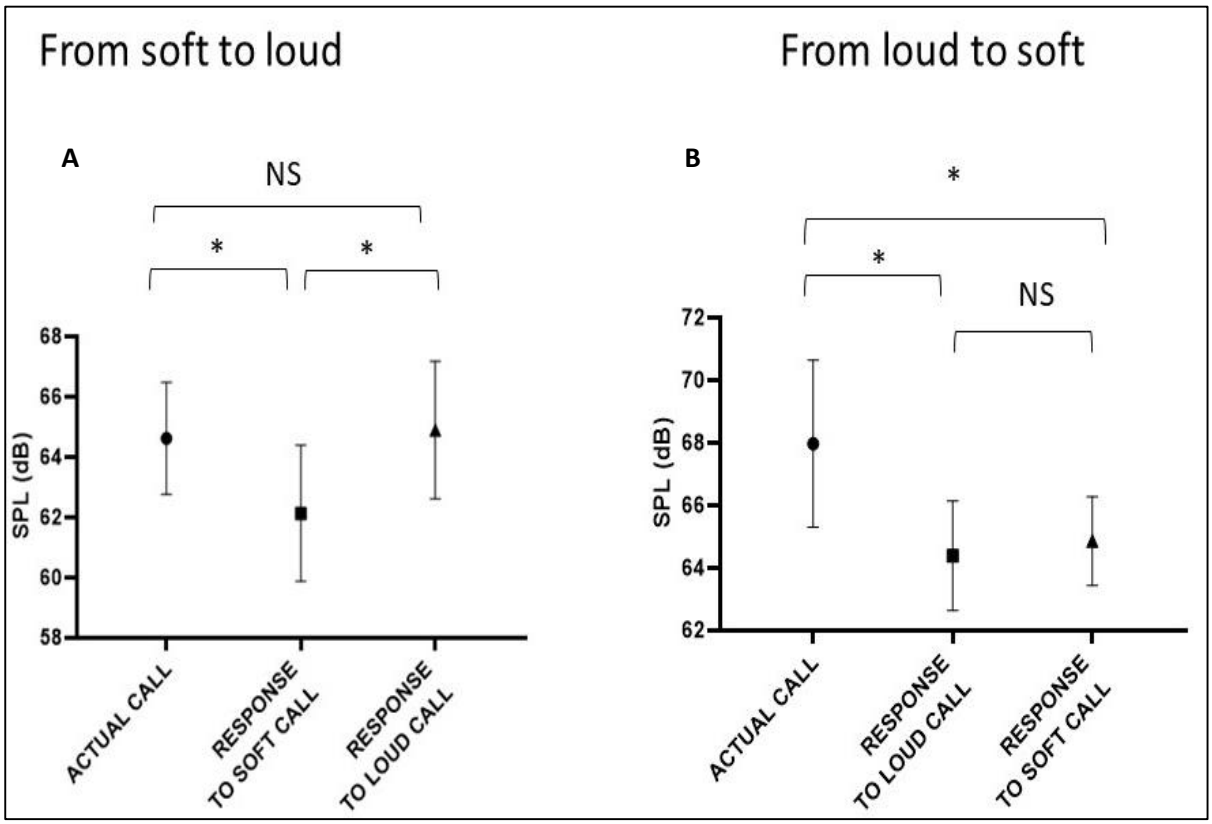


Figure 4.12. SPL modulation responses of individuals. A. individual responses when exposed to soft neighbour followed by a loud neighbour. B. individual responses when exposed to loud neighbour followed by a soft neighbour. * signifies P < 0.05.

4.4 Discussion

This study examines three levels of segregation: spatial partitioning, fine temporal partitioning and SPL modulation and provides evidence males of *A. asiaticus* employ multiple techniques to avoid conspecific acoustic masking by spatial partitioning and temporal partitioning. My findings reveal that males call from spatial aggregations with a signaling neighbour within approximately 3 m. Further, males on an average have 2 maskers of which 1 is audible. This demonstrates that there is a potential for masking interference by multiple maskers. Of them, males can potentially hear one masker and this

allows them to actively avoid call overlap by employing fine-temporal partitioning (via alternation). Alternation with their nearest neighbour clearly shows that males largely escape masking from the nearest (and most significant) masker. The study also provides insights into the potential for a female to sample two or more males in the field.

4.4.1 Spatial distribution and acoustic masking interference

Calling *A. asiaticus* males showed an aggregated distribution in space similar to that seen in other field crickets (Cade and Otte 1982, Mhatre and Balakrishnan 2006) and bushcrickets (Weidemann et al. 1990; Arak and Eiriksson 1992). These aggregates formed functional conspecific choruses, that is, the calling males of *A. asiaticus* within the aggregates were spaced such that their acoustic ranges overlapped to a large extent. Similar observations have been made in some bushcricket species such as *Mygalopsis marki, Decticus verrucivorus* and *Tettigonia viridissima* (Romer and Bailey 1986). The nearest-neighbour analysis demonstrates that male *A. asiaticus* are usually aggregated, a characteristic spacing pattern in many acoustical insects. Among crickets, *A. fortipes, Gryllus integer, G. veletis, Teleogryllus commodus* and the mole crickets (Cade 1981; Cade and Otte 1982; Campbell and Shipp 1979; Kleyla and Dodson 1978) are known to have aggregated calling males. However, a study on the tree cricket *Oecanthus Henryi* showed that males did not form active choruses as males were spaced quite apart with minimal ASO (Deb and Balakrishnan 2014).

In my study, I found that ASO across 10 choruses of *A. asiaticus* can range from 0.08 to 0.95 and on an average becomes as low as 0.40. Similarly, EAO which ranges from 0.06 to 0.69, due to natural spacing this value on an average becomes 0.17. This explains that despite having multiple masking conspecifics, these noisy choruses, by virtue of natural

spacing of the males, signal transmission in the habitat and behavioural strategies employed by males, are not so noisy after all. While in heterospecific masking avoidance, it has been shown that spatial partitioning doesn not play any role (Balakrishnan et al. 2014), my work suggests that spatial partitioning significantly contribute in conspecific masking avoidance in *A. asiaticus*. Given the distribution of males of *A. asiaticus*, it makes it possible for females to simultaneously sample males (potential for active female choice) without travelling more, thereby, investing less energy (Real 1990) and time (Kagel et al. 1986) as found in *P. guttiventris* (Mhatre and Balakrishnan 2006). On an average, a male was found to have two maskers, of them, one audible masking neighbour. This provides a male the opportunity to exhibit active masking avoidance by employing fine-temporal partitioning of their call by alternating their chirps with the audible masker.

4.4.2 Acoustic interaction with the nearest neighbour

My study shows that in both field and lab environment, *A. asiaticus* alternate their chirps with either real male neighbours in the field or simulated neighbours in the lab. Such kind of temporal partitioning of calls at fine temporal scales over seconds, with one individual calling in the silent inter-bout intervals of the other probably functions in maintaining the distinctive characteristics of a signal which are attractive to females. Alternation in field crickets has also been reported only in *A. fortipes* (Cade and Otte 1982). This study showed that two adjacent males alternated their chirps such that an individual called during the silent period in a neighbour's song. This was also valid when they did playback with the recorded song of *A. fortipes* song (Cade and Otte 1982). A study on conspecific masking in other field cricket, *Plebeiogryllus guttiventris*, showed no significant alternation or synchrony of calls (Mhatre and Balakrishnan 2006). Acoustic interaction with neighbours in *Ligurotettix planum*, a tarbush grasshopper, showed crude alternation in which by calling

at the ends of silent intervals, males may actively compete with neighbours (Minckley et al. 1995). However, alternation is not persistent as it is occasionally interrupted by synchrony when fluctuations in call periods result in two males, sometimes, start calling simultaneously for short periods (Cade and Otte 1982; Minckley et al. 1995). A study on katydid shows that by default, males also synchronize with their second and third, nearest neighbours (Party et al. 2015). Alexander (1975) proposed that males that alternate their calls should occur close together and thus facilitate signal interactions.

4.4.3 Change in temporal call features

Acanthogryllus asiaticus, while interacting with a simulated neighbour, altered some features of the temporal pattern of their calling song. Heiligenberg (1969) observed an increase in the chirp rate of an individual of the species *A. domesticus* in response to playbacks. Males of *A. asiaticus,* also lowered their chirp period and increased their chirp rates. This means that while interacting with the nearest neighbour, they produced more energetically expensive calls than they would have done when calling in solo. Female crickets are known to prefer males with higher chirp rates and longer chirps, that is, males with songs that are energetically more expensive to produce (Wagner 1996). In the study by Mhatre and Balakrishnan (2006), it was found that *P. guttiventris* males either increased the length of their chirps or increased their chirp rates or both. All males produced songs that were more energetically expensive than the song of neighbour by at least one feature: they either called more quickly or had longer chirps (Mhatre and Balakrishnan 2006). Contrarily, Otte and Cade (1982), in *A. fortipes* showed that individuals alternated their call with a simulated neighbour and produced lower chirp rate than non-alternating males. Such temporal adjustments have also been observed during synchrony in *Mecopoda* “Chirper”

by Nityananda and Balakrishnan (2007), where they have found that males have a solo intrinsic chirp period that differs from their duet chirp period because of adjustments made during interactions with other males and the male with the faster intrinsic chirp rate leads more than 50% of the partner's chirps (Nityananda and Balakrishnan 2007). In addition, males of *M.* 'Chirper' also show selective attention by spacing in the field, which means they interacted only with a subset of neighbours (Nityananda et al. 2007). Given that, in *A. asiaticus*, with the first audible masker, a focal male avoids masking by alternating, therefore EAO for this becomes nil. However, there is still one more masker with which male has to deal with. I found that EAO is as low as 0.17, which means that there is only a 17% chance that a male will face effective acoustic masking interference from a neighbour, despite calling from dense choruses of conspecifics.

4.4.4 SPL Modulation

My study shows that while interacting with a simulated neighbour, which is either 5 dB louder or softer, males of *A. asiaticus* decreased their SPL and did not call louder. The first-time exposure to either louder or softer neighbour led to decrease in SPL of the focal male, however the second time exposure to the louder neighbour increased the SPL of the focal male (equals to original SPL) and second time exposure to softer neighbour did not change the SPL. This explicitly shows the presence of order effect which is expected to be present in natural condition as well. My study implies that *A. asiaticus* males do not show the Lombard effect to avoid masking. This is in line with other reviews which shows no evidence of the Lombard effect in insects (Romer 2013; Zollinger and Brumm 2011; Brumm and Slabbekoor 2005).

In conclusion, this study suggests that males of *A. asiaticus* are spatially aggregated and form active choruses. The acoustic ranges of males overlapped significantly on average with two maskers. However, with the closest audible masker, males show alternation by changing temporal call features. In addition, they did not call louder in response to masking neighbor. Overall, the study implies that such spatial and acoustic organization of males might influence the female mate choice. Whether females actually exhibit active mate choice is yet to be tested.

4.5 References

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4.6 Appendix C

Table C4.1. masking probability of each individual across all the 10 choruses.

Chorus	Focal male	Active space overlap (ASO)	Maskers	Audible masker	Effective Acoustic overlap (EAO)	NN	NNN
Chorus 1	M1	0.676	1	1	0.000	2.8	5.95
	M2	0.766	2	1	0.063	2.8	4.62
	M3	0.347	3	0	0.244	4.62	4.85
	M4	0.377	2	0	0.265	4.85	5.07
	M5	0.133	2	0	0.094	4.88	5.07
	M6	0.000	0	0	0.000	10.34	14.65
	M7	0.000	0	0	0.000	14.89	24.63
	Median	**0.347**	**2**	**0**	**0.063**	**4.850**	**5.070**
Chorus 2	M8	0.336	2	0	0.235	2.84	5.59
	M9	0.412	3	1	0.288	3.64	5.59
	M10	0.368	2	0	0.258	2.84	5.64
	M11	0.387	2	1	0.271	3.64	5.53
	M12	0.014	1	0	0.010	5.53	8
	Median	**0.368**	**2**	**0**	**0.258**	**3.640**	**5.590**
Chorus 3	M1	0.940	1	1	0.000	0.28	9.06
	M2	0.940	1	1	0.000	0.28	8.93
	M3	0.338	1	0	0.237	3.29	8.93
	M4	0.031	1	0	0.021	5.47	9.19
	M5	0.000	0	0	0.000	9.19	9.99
	M6	0.338	1	0	0.237	3.29	7.24
	M7	0.031	1	0	0.021	5.47	9.65
	M8	0.000	0	0	0.000	7.24	8.93
	M9	0.000	0	0	0.000	8.57	8.93
	Median	**0.031**	**1**	**0**	**0.000**	**5.470**	**8.930**
Chorus 4	M1	0.990	6	3	0.574	1.43	2.25
	M2	0.363	5	0	0.321	4.1	4.3
	M3	0.228	2	0	0.161	3.94	5.89
	M4	0.962	7	2	0.538	1.58	2.67
	M5	0.674	4	1	0.192	2.17	4.45
	M6	0.701	6	0	0.288	1.43	3.68
	M7	0.542	1	1	0.000	2.21	9.08
	M8	0.000	0	0	0.000	7.08	9.08
	M9	0.866	7	2	0.644	2.11	2.23
	M10	0.942	8	3	0.837	1.18	2.11
	M11	0.854	6	1	0.347	2.17	3.7
	M12	0.981	6	1	0.550	1.58	3.7
	M13	0.316	4	0	0.244	4.04	4.51
	M14	0.125	1	0	0.087	4.64	6
	M15	1.000	7	4	0.927	1.18	2.01
	M16	0.709	6	1	0.509	2.67	3.52
	M17	0.964	10	1	0.768	1.69	3.52
	M18	1.000	9	3	0.830	1.69	2.01
	M19	0.542	1	1	0.000	2.21	7.08
	Median	**0.709**	**6**	**1**	**0.347**	**2.170**	**3.700**
Chorus 5	M1	0.130	1	0	0.091	4.6	11.02
	M2	0.000	0	0	0.000	6.79	11.02

	M3	0.130	1	0	0.091	4.6	6.79
	Median	**0.130**	**1**	**0**	**0.091**	**4.600**	**11.020**
Chorus 6	M1	0.408	1	1	0.000	2.9	10.48
	M2	0.408	1	1	0.000	2.9	10.7
	M3	0.633	2	1	0.094	1.98	4.577
	M4	0.647	2	1	0.104	1.98	4.47
	M5	0.224	3	0	0.184	4.47	5.46
	M6	0.420	2	1	0.014	2.95	5.61
	M7	0.051	2	0	0.036	5.46	5.61
	M8	0.400	1	1	0.000	2.95	6.04
	M9	0.171	1	0	0.120	4.32	11.01
	M10	0.171	1	0	0.120	4.32	8.97
	M11	0.000	0	0	0.000	7.67	8.97
	Median	**0.400**	**1**	**1**	**0.036**	**2.950**	**6.040**
Chorus 7	M1	0.400	2	1	0.293	2.96	5.23
	M2	0.215	2	0	0.163	2.96	5.23
	M3	0.185	1	0	0.130	4.08	18.22
	M4	0.000	0	0	0.000	8.78	11.6
	M5	0.000	0	0	0.000	6.28	7.04
	M6	0.538	2	1	0.389	2.96	2.96
	M7	0.185	1	0	0.130	4.08	12.14
	M8	0.000	0	0	0.000	6.33	11.94
	M9	0.000	0	0	0.000	6.28	6.33
	Median	**0.185**	**1**	**0**	**0.130**	**4.080**	**7.040**
Chorus 8	M1	0.235	2	0	0.168	4.6	4.7
	M2	0.260	3	0	0.187	4.7	4.9
	M3	0.504	5	0	0.409	4.6	4.9
	M4	0.807	5	2	0.548	1.73	2.3
	M5	1.000	4	3	0.734	1.73	1.96
	M6	0.688	3	2	0.477	1.96	2.3
	M7	0.559	4	1	0.355	2.65	4.1
	M8	0.270	2	0	0.241	3.69	4.29
	M9	0.946	3	1	0.208	0.84	5.51
	M10	0.823	2	1	0.122	0.84	4.29
	M11	0.781	3	2	0.315	1.84	2.83
	M12	0.680	2	1	0.245	1.84	3.23
	M13	0.486	2	1	0.245	2.83	3.23
	Median	**0.680**	**3**	**1**	**0.245**	**1.960**	**4.100**
Chorus 9	M1	0.952	6	2	0.427	0.24	2.8
	M2	0.987	6	2	0.460	0.24	2.6
	M3	0.960	6	6	0.822	0.71	1.95
	M4	0.993	6	4	0.849	0.71	1.47
	M5	0.775	6	4	0.680	1.41	1.63
	M6	0.987	6	4	0.814	0.29	1.41
	M7	0.994	6	4	0.832	0.29	1.47
	Median	**0.987**	**6**	**4**	**0.814**	**0.290**	**1.630**
Chorus 10	M1	0.727	2	1	0.019	1.43	2.77
	M2	0.789	2	1	0.062	1.43	3.58
	M3	0.027	1	0	0.019	2.77	3.58
	M4	0.089	1	0	0.062	4.92	6.13
	Median	**0.408**	**2**	**1**	**0.041**	**2.100**	**3.580**

Table C4.2. Statistical analysis for comparison of temporal and spectral features of focal male calling in solo and interacting with a simulated neighbor using paired t test.

Call parameters	Solo (Average ±SD)	Duet (Average ±SD)	N	t	P
Chirp duration (s)	0.38 ± 0.04	0.37 ± 0.04	25	-0.76	0.455
Chirp period (s)	1.05 ± 0.21	0.94 ± 0.21	25	-2.365	**0.026***
Number of chirps	9.96 ± 2.28	11.2 ± 1.53	25	2.599	**0.015***
Number of syllables per chirp	13.024 ±1.19	13.238 ±1.26	25	0.754	0.458
Peak frequency (Hz)	4205.24 ± 292.72	4182.95 ± 298.14	25	-0.663	0.514
SPL (dB)	64.15 ± 4.75	62.45 ± 4.32	25	-1.661	0.11

Table C4.3. Statistical analysis for SPL modulation responses of individuals using paired t test. A. individual responses when exposed to soft neighbor followed by a loud neighbor. B. individual responses when exposed to loud neighbor followed by a soft neighbor. Significant differences are represented by *.

	Average ± SD (1)	Average ± SD (2)	N	t	P
A. Exposure type: Soft followed by loud call					
1 VS 2					
Control VS Soft	64.62 4.08	62.13 ± 4.95	21	3.62	**0.0017***
Control VS Loud	64.62 4.08	64.90 ± 5.01	21	0.32	**0.0018***
Soft VS Loud	62.13 ± 4.95	64.90 ± 5.01	21	3.59	0.744
B. Exposure type: Loud followed by soft call					
Control VS Loud	67.97 ± 5.02	64.39 ± 3.3	16	3.09	**0.007***
Control VS Soft	67.97 ± 5.02	64.86 ± 2.67	16	2.33	**0.034***
Loud VS Soft	64.39 ± 3.3	64.86 ± 2.67	16	0.57	0.57

Chapter 5

Effect of traffic noise on cricket calling behaviour

Measurement of traffic noise level at a noisy site - Tribune Chowk, Chandigarh

5.1 Introduction

The world is not a quiet place for acoustically-active organisms. The presence of signalling conspecifics and heterospecific (Römer 2013), the sound of flowing water (Feng et al. 2006), wind (Lengagne et al. 1999), or dense foliage (Mathevon 2005) result in acoustic masking interference (as detailed in Chapter 4). This, in turn, influences the signalling behaviour of these organisms as it deters their ability to detect and distinguish relevant signals from irrelevant ones (Wiley 2006). In addition to natural biotic and abiotic sounds, noise created by human activities—anthropogenic noise is a relatively novel form of background noise which is affecting acoustic communication in both marine (Slabbekoorn et al. 2010) and terrestrial environments (Brumm 2004; Brumm and Slater 2006; Barber et al. 2010; Kight and Swaddle 2011) by overlapping or masking animal signals that occur in the similar frequency range. Road traffic noise is one of the most pervasive human-generated noise which has a potential to affect acoustic communication of various species inhabiting near the roadside and may significantly reduce the reproductive success and/or survival (Barber et al. 2010; Halfwerk et al. 2011). Various studies have investigated the impact of traffic noise on a wide range of taxa and demonstrated that animals could change their signalling behaviour to avoid masking (reviewed in Barber et al. 2010). To counter the masking problem, animals may exhibit long-term adaptations such as changes in signal characteristics over evolutionary time scale or they may exhibit short-term adaptations such as adjustments of signal traits in response to temporary changes in the background noise (reviewed in Brumm and Slabbekoorn 2005). One of the short-term adjustments animals may exhibit is to increase the amplitude of signaling in noisy environments, referred to as 'Lombard effect' (Lombard 1911; Zollinger SA and Brumm 2011; Brumm 2004; Lowry et al. 2012). Such modification in signalling improves the signal-to-noise ratio, which is required to detect and distinguish the required signal from various sounds (Wiley 2006).

Other adjustments include shifting the frequency components of the signal (birds: Bermúdez-Cuamatzin et al. 2009; frog: Parris et al. 2009), increase the calling rate (birds: Brumm and Slater 2006; frogs: Kaiser and Hammers 2009; Sun and Narins 2005), change time of calling to avoid masking by traffic noise (birds: Bergen and Abs 1997) or avoid noise spatially by moving away from the noisy areas (birds: Bayne et al. 2008; McLaughlin and Kunc 2013).

While a plethora of studies exist on the impact of anthropogenic noise on animal signalling, the problem has mostly been examined in vertebrates and studies on invertebrates are limited (Morley et al. 2014; Shannon et al. 2016). Invertebrates comprise 97% of animal species on Earth and play a significant role in various ecological processes, despite this, only 4% of the work on noise has been carried out on invertebrates (Shannon et al. 2016). Among invertebrates, insects are the oldest taxa that evolved communication via airborne sound signals. Most notably, orthopteran insects are known for their conspicuous acoustic signals and many species are found along a gradient of urban landscapes making them susceptible to be affected by the recent intrusion of traffic noise. Only recently, a handful of studies have been reported on the impact of traffic noise on insect acoustic communication (see Table 5.1). Studies on nocturnal insects, crickets, have mostly examined the effect of noise pollution on male signalling at the individual level while studies at the population level are lacking. With rapid urbanization and an ever sprawling road network, it is likely that a drastic increase in ambient noise is also widespread. It is important to examine the effect of traffic noise across populations to understand the broader ecological implications of traffic noise on animal communication. Further, most of these studies (Table 5.1) are lab-based, thus, whether and how insects solve the problem of masking from traffic noise in their natural environment is still a question. Moreover, it is

also essential to investigate whether the animals exhibit long-term or short-term adaptation to avoid signal masking from traffic noise exposure.

In this study, I examined the effect of chronic and short-term exposure to traffic noise on male signalling in *Acanthogryllus asiaticus,* a broadly distributed species that occurs along an urbanization gradient. The species is found all over India (See Chapter 1) and occurs in rural, semi-urban and urban habitat including roadside footpaths, urban gardens, highways and even railway tracks (*personal observations*). The males produce a stereotypic long-distance mating call with a relatively low fundamental frequency ranging from 3300 and 5500 Hz (Average: 4687 ± 482 Hz at 24°C) to attract females (Singh and Jain 2020). Depending on the nature of background noise from traffic, animal signals would be susceptible to masking if there is sufficient spectral overlap between signals and background noise. Hence, for this chapter I examined the impact of road traffic noise on the calling behaviour of male *Acanthogryllus asiaticus* with the following objectives:

1) To acoustically characterize profiles of ambient noise in regions of the very low and high incidence of traffic in areas where animals are present.

2) To compare the acoustic features of calls of males from populations from 'noisy habitats' with chronic traffic noise and those from 'quiet habitats' without traffic noise.

3) To examine whether naïve males make any adjustments to short term traffic noise exposure.

5.2 Materials and Methods

5.2.1 Study sites and animals used

A total of 9 areas in Chandigarh and Mohali, India were selected (Table 5.2, Figure D5.1) wherein 5 were prone to heavy night time traffic (called 'noisy habitats') while the other four had a very low incidence of traffic (called 'quiet habitats'). These regions were selected after ascertaining that males of *A. asiaticus* were found to be present in these areas. For all lab-based playback experiments, lab-bred individuals were used. Individuals were placed in a plastic container (diameter-12 cm, height-6 cm) covered with cloth mesh. All animals were maintained at 24°C, 40 - 70 % humidity, 12:12h light: dark condition and food and water were provided *ad libitum*. Field study (5.2.2 and 5.2.3) was carried out in the month of March, August, September and November of 2018 and lab-based playback experiment (5.2.4) was carried out in March 2019.

5.2.2 Ambient noise level measurements

Ambient noise for all noisy and quiet habitats (Table 5.2) was measured as Sound pressure level (SPL) dBA (LAF in 1/3-octave bands) using a Brüel & Kjær ½″ microphone, Type 4189 (20 Hz to 20 kHz) attached to a Sound Level Meter, Type 2270 (Brüel & Kjær, Naerum, Denmark). Noise level at a distance of 50 cm from the previously marked calling site (see 5.2.3) of males of *A. asiaticus* in three out of five noisy areas was measured at night during traffic peak hours between 1900 to 2200 h. This measurement was carried out during the off-season (November 2018; when no crickets were present) from previously marked male calling sites to control for any confounding changes in the spectra of ambient noise due to the insect calls.

5.2.3 Population level difference in signals

50 adult males of *A. asiaticus* were recorded from the roadside across five different traffic-prone noisy areas in Chandigarh, India (Table 2). Singing males were approached and calls were recorded as 16-bit WAV file at a sampling rate of 44.1 kHz using Tascam, Linear PCM Recorder (DR-20 Mk II, TEAC Professional, USA) and SPL dBA (LAF in 1/3-octave bands) was measured using a Brüel & Kjær ½″ microphone, Type 4189 (20 Hz to 20 kHz) attached to a Sound Level Meter, Type 2270 (Brüel & Kjær, Naerum, Denmark) at a distance of 50 cm from the calling male. While taking SPL of cricket calls in traffic-prone noisy areas, it was ensured that no vehicle was passing by. This was done by taking readings when traffic signals were red. The male positions were marked and their distance from the edge of the road was measured to estimate the proximity of *A. asiaticus* to the roadside. A total of 48 adult males were recorded from quiet areas of which SPL of 40 individuals and calls of 21 individuals were recorded. Calls and SPL of the population from noisy and quiet areas were compared (Table 5.2). Temperature for all the call recordings was in the range of 28-30°C.

5.2.4 Short-term noise exposure

To examine whether males make short-term adjustments to their signal parameters in response to exposure to traffic noise, playback experiments were performed with 30 naïve lab-bred males (that were never exposed to traffic noise). A focal individual was exposed to a control silent treatment of noise level 25 dBA SPL and recorded once it started calling. This was followed by traffic noise treatment where the focal individual was exposed to noise level 74 dBA SPL (LAF in 1/3-octave) for 2 minutes. 74 dBA SPL was an average SPL of traffic noise measured at the male calling positions in traffic areas in the frequency

band 100 Hz-20 kHz. The latency to resume calling on the exposure of traffic noise was also recorded using an IC recorder (Sony corporation, China) which was switched on during the entire trial. After this, the speaker was turned off and the call and SPL of the focal individual was recorded. Therefore, recordings of each individual were made in two phases: (a) a silent pre-noise exposure to assess natural call characteristic and (b) a silent post-noise exposure period. All trials were performed at 24° C in a dark room with a red headlight. Each individual was placed 2 m away from the traffic noise broadcasting loudspeaker (JBL GO 2, Harman International) connected to a laptop (Thinkpad T480, Lenovo).

5.2.5 Call analyses

All the recordings were analyzed using sound analysis software Raven Pro 1.4 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY). Call parameters used for the analyses of population level differences in signal characteristics include chirp duration, chirp period, peak frequency, number of syllables per chirp and number of chirps per 10 second. Syllable duration and syllable period were not measured to avoid any discrepancy as high level of masking at syllable level was observed for the calls recorded in the traffic prone areas. For playback experiment, call parameters used in analyses were chirp duration, chirp period, syllable duration, syllable period, number of syllables per chirp, number of chirps per 10 second, peak frequency which were compared for before and immediately after noise exposure phase. I also examined, if exposure to traffic noise disrupted calling in male *A. asiaticus* and the latency to resume calling on exposure to traffic noise.

5.2.6 Statistical analyses

Statistical tests were performed using Statistica 64 (Dell Inc. 2015, Version 12). Data were checked for normality using the Shapiro-Wilk test. For data collected to examine population level difference in signals, Mann-Whitney U-test was performed on SPL values as it did not follow a normal distribution, whereas, for other call characteristics, t-test was done to compare the calls from the two sets of populations. For playback experiments, Wilcoxon signed-rank matched pair test for loudness was performed for before and after traffic noise exposure phases as it did not follow a normal distribution whereas, for other call characteristics which followed a normal distribution, paired t-test was performed.

Table 5.1. Literature review of studies carried out to examine the effect of traffic noise on insect acoustic communication.

Insect	**Species**	**Sex**	**Study conducted**	**Study**	**Result**	**References**
Tree cricket	*Oecanthus pellucens*	Male	Lab	male signalling in response to the fluctuation in traffic noise over a short timescale	males shortened calls (echemes) and paused singing with a higher probability with increasing noise level	Orci et al. 2016
Field cricket	*Teleogryllus oceanicus*	Female	Lab	pre-reproductive experience with noise on adult performance in noisy environments	Hindered mate location ability in females	Gurule-Small and Tinghitella 2018
Tree cricket	*Oecanthus argentinus, O.celerinictus, O. forbesi, O.fultoni, O.quadripunctatus and O. texensis*	Male and Female	Lab	male signalling and female phonotaxis	no effect on male signals and female response	Costello and Symes 2014
Field cricket	*Gryllus bimaculatus*	Female	Lab	female phonotaxis	hindered mate location ability in females	Schmidt et al. 2014
Field cricket	*Gryllus bimaculatus*	Female	Lab	female phonotaxis	hindered mate location ability and searching latency	Bent et al. 2018
Field cricket	*Gryllus bimaculatus*	Male	Field	male calling	decrease in chirp rates	Gallego-Abenza et al. 2019

Field cricket	*Anaxipha sp., Gryllus sp., Podoscirtinae species*	Male	Field	male calling	calling interruption for all the species with change in frequency	Duarte et al. 2019
Grasshopper	*Chorthippus biguttulus*	Male	Lab	male calling	elevate frequency of courtship song in noisy condition; developmental plasticity to minimize masking	Lampe et al. 2014
Cicada	12 species	Male	Field	diel and seasonal calling activity pattern	low species diversity in city and less temporal partitioning than in mountains with high species diversity	Shieh et al. 2015
Grasshopper	*Chorthippus biguttulus*	Male	Lab	male calling	elevate frequency of courtship song in noisy area	Lampe et al. 2012
Cicada	*Cryptotympana takasagona*	Male	Field	male calling	increase in frequency with noise level	Shieh et al. 2012

Table 5.2. Geographic coordinates and mean ambient noise (dB SPL) of five Traffic-prone habitats and four low traffic habitats. All measurements were taken on weekdays (Monday–Friday between 7 PM – 10 PM)

Location	Habitat type	Geographic coordinates	Average background noise loudness dBA broadband (n = 3)	Average background noise loudness dBA at 5kHz (n = 3)	Number of males recorded to be calling
Sector 49	noisy	30°41'50.0"N 76°45'33.2"E	71.55	56.15	7
Tribune Chowk	noisy	30°42'10.3"N 76°47'28.7"E	80.03	60.03	10
Sector 43	noisy	30°42'55.4"N 76°44'36.7"E	80.78	64.04	10
Sector 34	noisy	30°43'18.1"N 76°45'40.0"E	77.42	57.04	12
Sector 10	noisy	30°45'07.3"N 76°47'38.2"E	78.74	59.67	11
IISER Mohali	quiet	30°39'53.7"N 76°43'42.6"E	48.34	38.35	17
ISB Mohali	quiet	30°40'15.4"N 76°43'37.8"E	49.03	39.57	10
Phase 9	quiet	30°39'56.5"N 76°44'22.5"E	52.34	42.67	10
NIPER Mohali	quiet	30°40'57.1"N 76°43'52.1"E	50.34	40.04	3

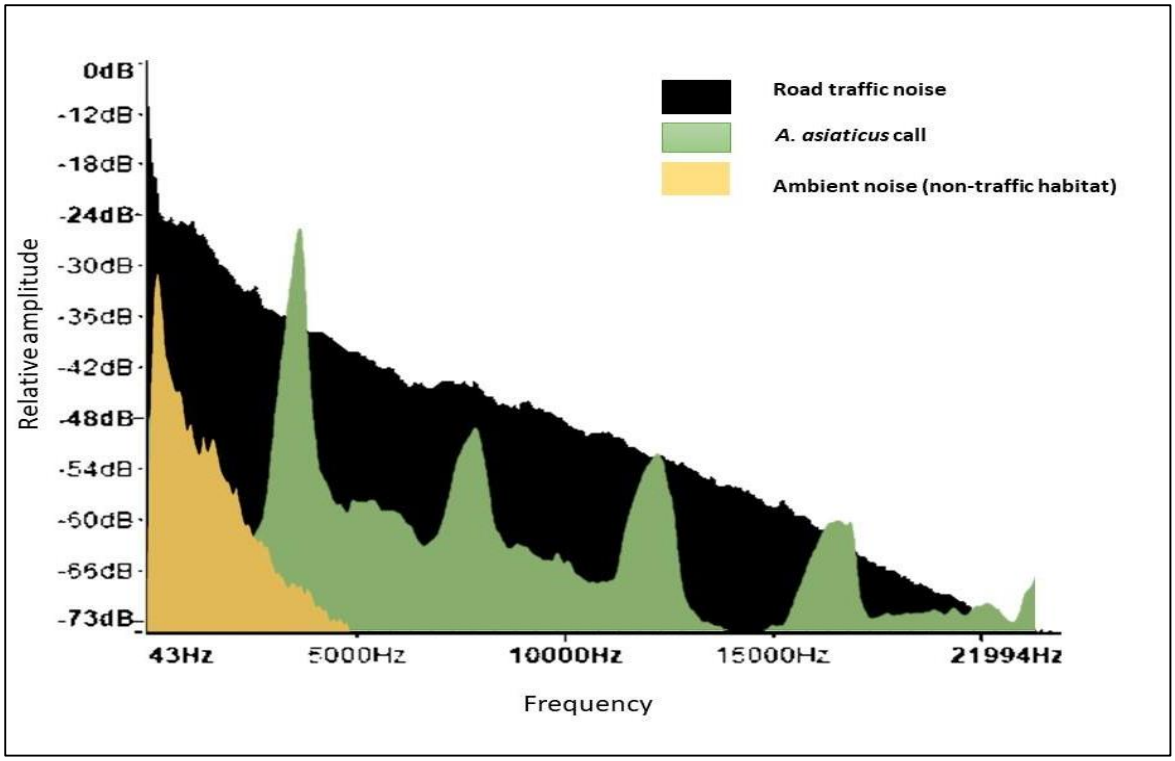

Figure 5.1. Power spectra of ambient noise of traffic-prone areas (black), the ambient noise of non-traffic habitats (yellow) and *A. asiaticus* call (green).

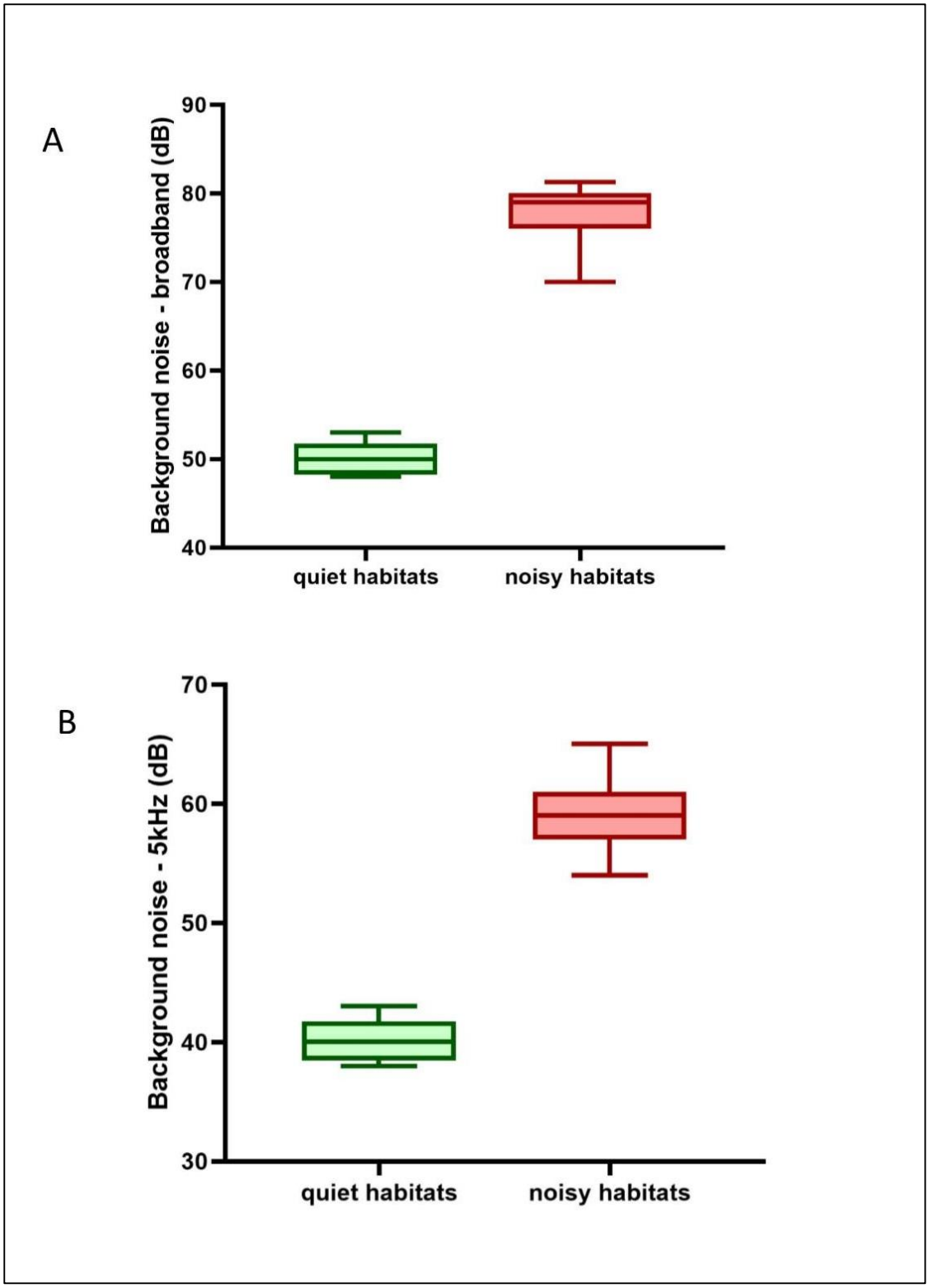

Figure 5.2. Ambient noise at A. broadband and B.5 kHz in quiet and noisy habitats.

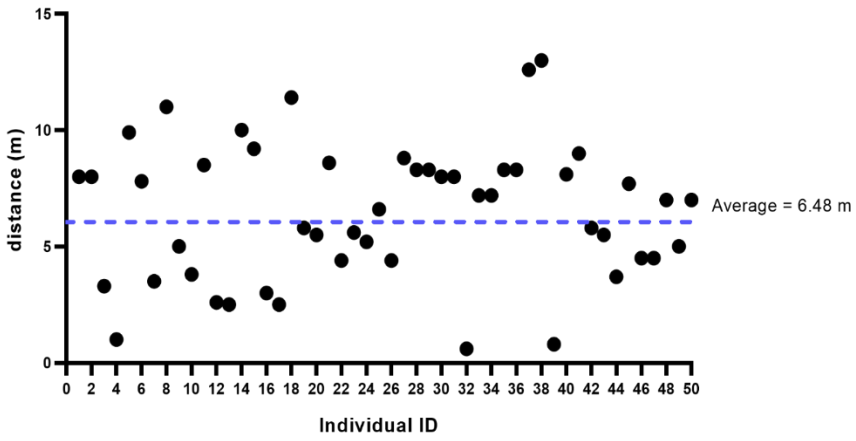

Figure 5.3. Spatial distribution of individuals (distance (m) from road edge) in traffic noise habitats.

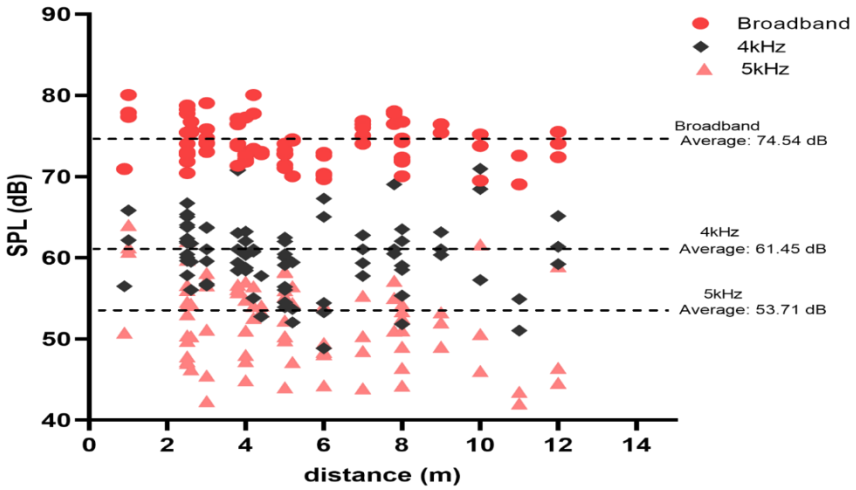

Figure 5.4. Traffic noise level measured at male calling positions in three noisy sites between 7 PM to 10 PM. (X-axis indicate the distance of the male calling site away from the road).

5.3 Results

5.3.1 Ambient noise level measurements

My findings clearly suggest that the ambient noise profiles of the two kinds of habitat are very different from each other (Figure 5.1). The power spectra of ambient noise in traffic-prone areas and that of *A asiaticus* overlap greatly and this indicates a high potential of masking of signals of these insects due to traffic noise (figure 5.1). Ambient noise at 5 kHz for noisy and quiet habitats was found to be 40 ± 2 dB and 60 ± 3 dB, respectively. These were found to be significantly different between the two populations (t-test, at 5 kHz, $t = 24.05$, $df = 25$, $P < 0.01$; Figure 5.2). Populations in the five noisy habitats were found to be distributed along the roadside in the range of 0.5 m - 12 m with the average at 6.4 m (Figure 5.3). SPL of ambient noise at caller positions in traffic-prone areas was found to be 74.5 dB (broadband), 61.5 dB (4 kHz) and 53.7 dB (5 kHz) (Figure 5.4).

5.3.2 Population level difference in signals

I found that the populations of roadside noisy habitats were louder than quiet habitats' populations by 5 dB (Mann - Whitney U- test, $P < 0.01$; Figure 5.5, Table D51). Roadside calling males from noisy habitats were found to produce calls with shorter chirp period (t-test, $P < 0.01$; Figure 5.5, Table D51) and higher chirp rates as compared to silent habitats (t-test, $t = 2.87$, $P < 0.01$; Figure 5.5, Table D51). Other temporal properties were not found to be different from the population of silent habitats (Figure 5.5). Since the study was done in the field and no crickets were collected, hence, no account of age or weight of crickets was taken.

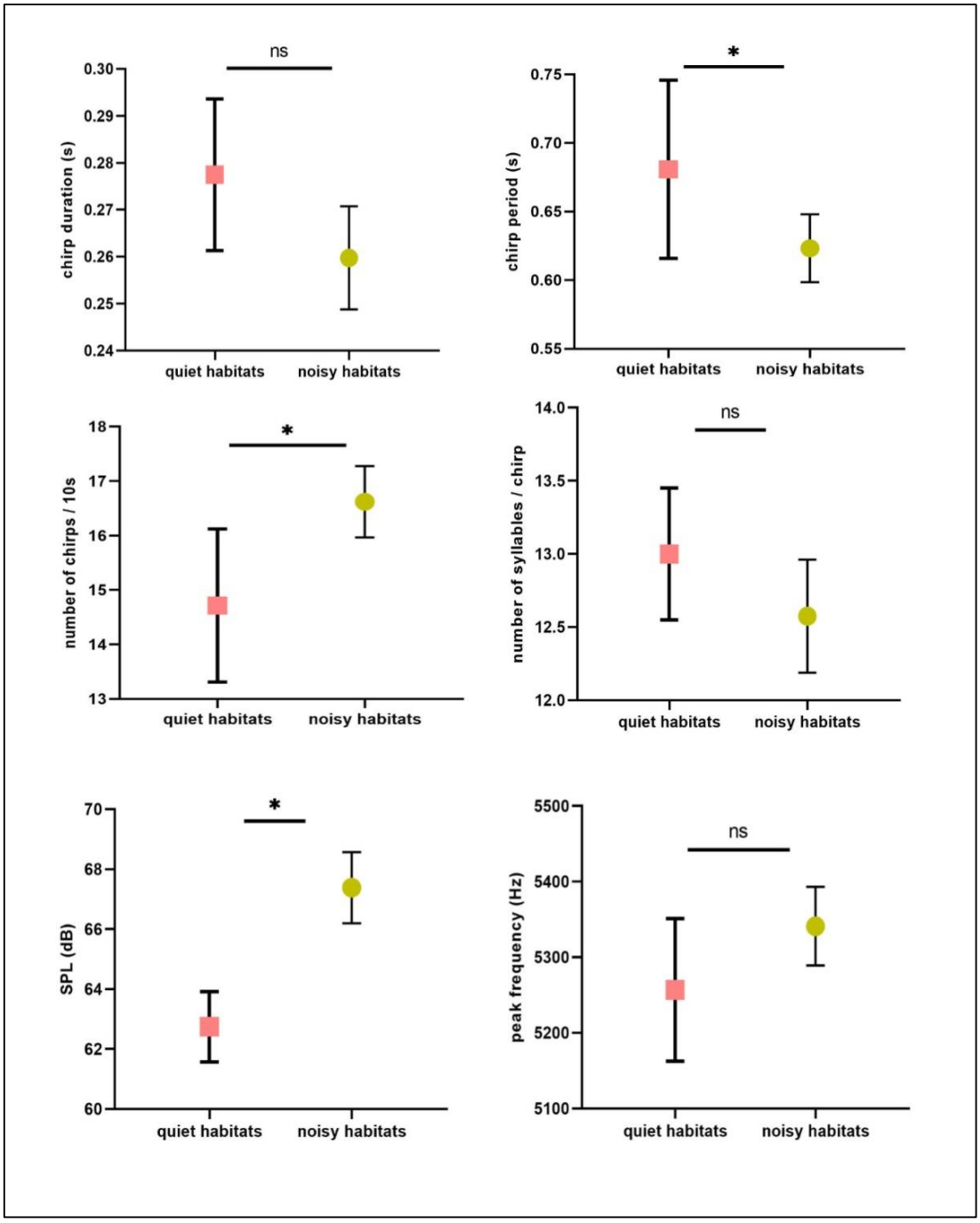

Figure 5.5. Comparison of call properties for population in noisy and quiet habitats. * signifies P <0.05 and ns signifies P > 0.05.

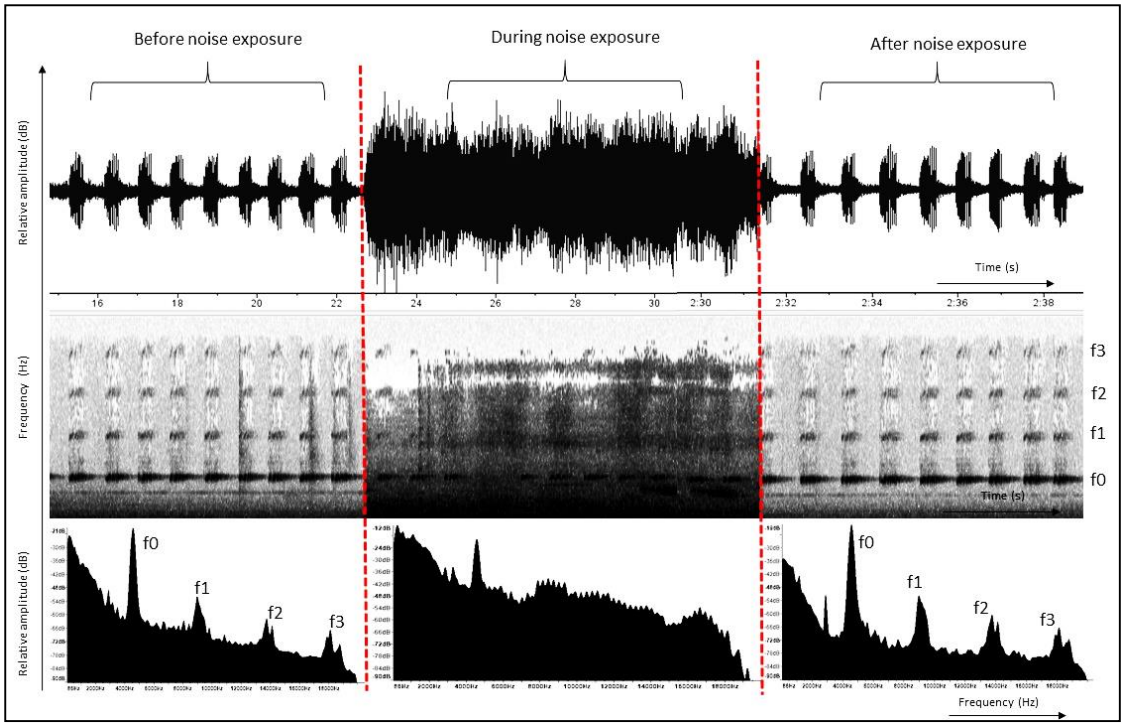

Figure 5.6. Oscillogram, spectrogram and power spectra for three phases: before, during and after noise exposure in playback experiment.

5.3.3 Short-term adjustment to noise exposure

Short-term noise exposure to traffic noise masked the call of *A. asiaticus* (Figure 5.6) as during exposure, SNR likely got reduced and harmonics were not found to be visible as compared to before noise exposure. 19 out of 30 crickets called within 10 seconds when traffic noise was played (Figure 5.7), implying that traffic noise exposure for short duration did not deter probability of calling. Further, exposure to traffic noise did not have any effect on loudness (Wilcoxon Matched Pairs Test, P = 0.085; Figure 5.8, Table D52). It also did not alter any temporal or spectral properties (Figure 5.8 Table D52).

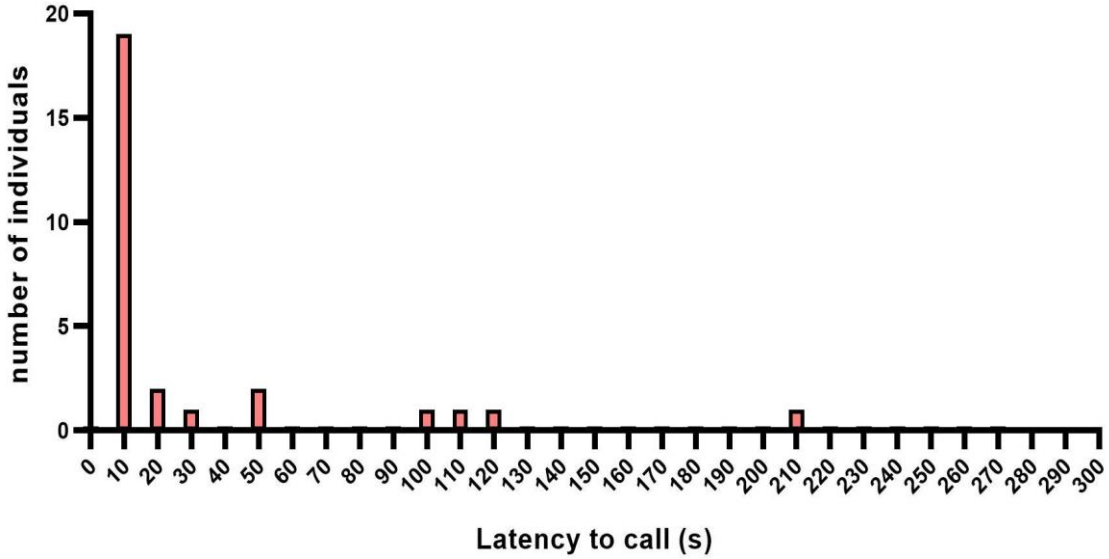

Figure 5.7. Latency to resume calling on exposure to traffic noise playback (N=30).

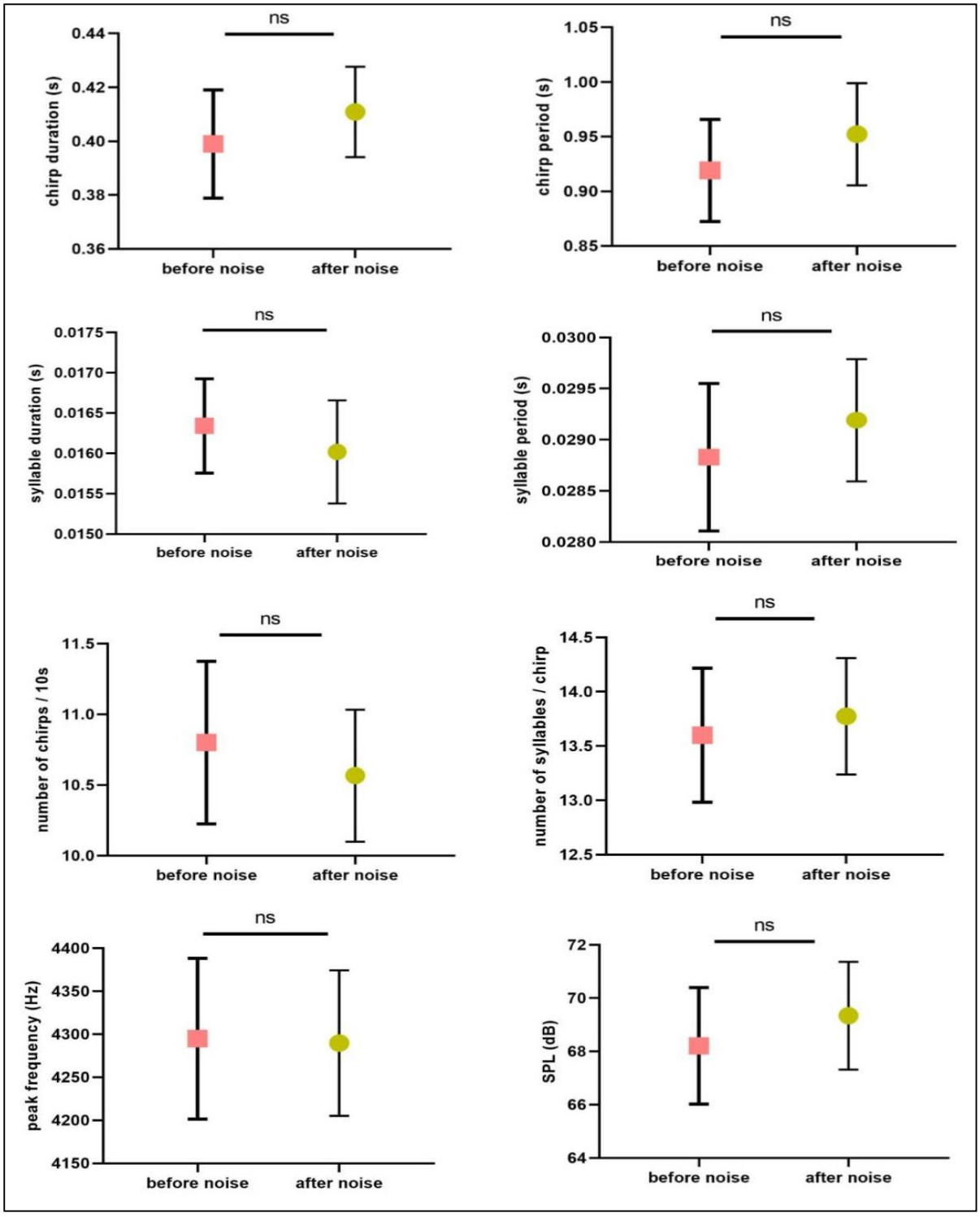

Figure 5.8. Comparison of call properties of individuals before and after noise exposure. * signifies $P < 0.05$ and ns signifies $P > 0.05$.

5.4 Discussion

My study provides evidence that traffic noise does impact the acoustic communication in a field cricket, *A. asiaticus*. This is the first study which examines the impact of traffic noise on a species at population and individual level and shows population level difference in signals recorded in traffic prone areas and quiet areas. However, no evidence of quick adjustment in signals by individuals on short-term noise exposure was found.

5.4.1 Ambient noise profile

I found that ambient noise levels for traffic prone areas to be 20 dB louder than the quiet areas. A study conducted in Melbourne, Australia which examined the impact of traffic noise on Noisy miners showed the difference of 15 dB in ambient noise levels of noisy and quiet roads (Lowry et al. 2012). Also, a study conducted in Berlin, Germany showed that ambient noise levels for less noisy to the noisiest location to be ranging between 40 and 64 dB (Brumm 2004). Such a high level of background noise is likely to decrease signal-to-noise ratio which can degrade the communication between signaller and receiver (Wiley 2006). My study suggests that such high levels of traffic noise masks the signals of *A. asiaticus* which were found to be present at an average distance of 6.8 m away from the road in traffic prone areas.

5.4.2 Population level difference in signals

I found that the males of the population in traffic-prone areas were, on an average, 5 dB louder (68.3 dB) than the quiet areas (63.4 dB). This loudness of signal might allow males to compensate for the signal masking by traffic noise. It has been reported in birds that

those living in noisier locations call significantly more loudly than those living in quiet locations (Brumm 2004, Lowry et al. 2012). For instance, Brumm (2004) found that free-ranging nightingales produce 14 dB louder calls in noisier locations compared to less noisy locations. In Noisy miner, 9 dB louder calls were reported from the individuals recorded at arterial roads compared to residential roads (Lowry et al. 2012). There has been no evidence indicating that insects possess a mechanism to modulate the loudness of their call (Zollinger and Brumm 2011). However, several cricket species increase the intensity of their call either by baffling from the leaf hole (eg. *Oecanthus*; Mhatre et al. 2017) or by calling from burrows (eg. Mole crickets; Forrest 1991). Recently, it has been shown that males of *Anurogryllus muicus* use anthropogenic sites such as walls of houses, concrete stairs or open concrete rain drains to increase the intensity of the call (Erregger and Schmidt 2018). Individuals of *A. asiaticus* mostly found to be calling from crevices and it can be speculated that population in traffic prone noisy area may select their calling crevice more efficiently on the basis of increasing call intensity than the ones in habitat without noise. It is also possible that individuals of *A. asiaticus* on the roadside, being exposed to chronic traffic noise over several generations, have evolved into calling loudly so that their signal gets efficiently detected by a receiver. A study from my lab shows that females of *A. asiaticus* shift their behavioural hearing threshold from 46 dB to 66 dB on the introduction of loud and fluctuating traffic noise (Jain 2019, MS Thesis). This negative impact of anthropogenic noise on female hearing may get balanced by altering signals by male crickets. I also predict that the shift in BHT could be the possible evolutionary driving factor which shaped the altered male calling behaviour. This likely maintains effective communication between signaller and the receiver in altered soundscapes. Studies have shown that exposure to traffic noise during development can also affect signalling behaviour in crickets. For instance, nymphs of *Chorthippus biguttulus*, which were exposed to road noise during

development, produced signals with higher frequency compared to those reared under quiet conditions, revealing that developmental plasticity can be potential mechanism to avoid masking from traffic noise (Lampe et al. 2014). Moreover, another study showed that mate location ability of female field cricket (*Teleogryllus oceanicus*) bred in traffic noise got impaired compared to those bred in silent conditions (Gurule-Small and Tinghitella, 2018). The chronic exposure of traffic noise also found to affecting lifetime strategy of crickets by changing their development time and adult lifespan (Gurule-Small and Tinghitella, 2019). In several animals, contemporary evolution (less than a few hundred generations) has been reported which means rapid evolution in population in response to environmental change, predation pressure, anthropogenic perturbation (Stockwell et al. 2003). For instance, contemporary evolution has been reported in guppies, *Poecilia reticulate* due to predator pressure and in Pitcher plant mosquitoes due to global warming (reviewed in Stockwell et al. 2003). It is expected that traffic noise which is altering the environment can potentially act as human-induced selection pressure and may lead to rapid evolutionary change by influencing various behavioural and physiological traits (Swaddle et al. 2015).

Additionally, I found that the population in traffic noise habitats produce high duty cycle calls with shorter chirp periods and higher chirp rates as compared to those in quiet habitats. Similar findings have been reported in vertebrates where birds and frogs have increased their calling rates to avoid traffic noise (birds: Brumm and Slater 2006; frogs: Kaiser and Hammers 2009; Sun and Narins 2005). In crickets, it has been reported that calls of such characteristics with higher chirp rates tend to be energetically expensive with high metabolic costs for males (Hoback and Wagner 1997). Therefore, in order to avoid masking from traffic noise, crickets may be driven to produce costly signals which require more metabolic energy. In contrast to my result, a recent study on *G bimaculatus* showed that

male crickets reduce their chirp rates in response to passing cars (Gallego-Abenza et al. 2019). Some studies have reported that insect in noise prone areas show in higher peak frequency. For instance, males of *C. biguttulus* from roadside habitats produced higher frequency of call compared to those from quiet habitats (Lampe et al. 2012). Similarly, in cicada species *Cryptotympana takasagona*, peak frequency was found to be increased for those found near roadside (Shieh et al. 2012). Duarte et al. (2019) found that near mining site, *Gryllus* sp. produced call with higher frequencies, average power, and larger bandwidth and *Podoscirtinae* species produced calls with lower minimum frequencies, higher average power, and large bandwidth. No evidence of difference in frequency in noisy and quiet areas was found in *A. asiaticus*. In order to avoid masking from traffic noise, animal can spatially move away from the noise prone areas. For example, such spatial movement away from traffic noise has been shown in European Robins as they moved away from their original position when exposed to the loud noise of 70 – 90 dB (Mclaughlin and Kunc 2013). However, in my study, I found that the average distance at which callers were found was to be at 6.5 m away from the road edge. An important consideration to be made, however, is that the available stretch of habitat in urban landscapes that are crisscrossed by roads is not too big anyway, thereby restricting spatial avoidance. Inter-male spacing may further restrict the possibility of males closer to the road edge to move further away from the noise. These aspects, however, were not examined and can be explored further.

5.4.3 Short-term adjustment to noise exposure

Acanthogryllus asiaticus did not show any short – term adjustment to traffic noise exposure for the short duration. Although, signals were found to be masked as harmonics were not visible during exposure phase. This implies that traffic noise even for a short duration is

likely to reduce SNR drastically. However, the playback experiments done on naïve crickets which were exposed to noise for the first time, showed that traffic noise did not significantly disrupt their calling behaviour as 19 out of 30 immediately started calling within 10 seconds of noise exposure. In these 19 individuals, 13 of them called within less than 5 seconds of noise exposure. This is unlike in the case of 6 species of tree crickets, *Oecanthus* (see Table 5.1) in which males significantly paused calling with increase in traffic noise level (Costello and Symes 2014). My result also shows that the call parameters such as chirp period, chirp duration, syllable period, syllable duration, peak frequency and loudness did not change when exposed to noise for a short duration. This is in agreement with the study on tree crickets which reported that these call parameters did not change when males were exposed to noise for 8 hours (Costello and Symes, 2014). In contrast, in another study, it is shown that tree cricket males decrease their chirp duration and signalling effort with an increase in noise level (Orci et al. 2016). An important distinction between previous studies and my study is that individuals used in experiments in these studies were collected from noisy areas and are likely to have been exposed to traffic noise not only through their adulthood but also through their developmental stages. In my study, crickets were lab-bred, naïve adults and were never exposed to traffic noise. Their first exposure to traffic noise as a novel sound did not modify the call parameters. In this way, this study clearly rules out short-term adjustments to masking by traffic noise.

In conclusion, my study provides evidence of population level difference in signals of *A. asiaticus* found in traffic prone areas compared to quiet areas. However, individuals cannot make quick adjustment of signals upon instant exposure to traffic noise to avoid the interference from anthropogenic noise masking. Studies examining the effect of anthropogenic noise exposure on invertebrates are crucial to our understanding of how such

alterations in environment impact the behaviour and biology of organisms and how it may be altering our ecosystems. A recent study showed that in areas, exposed to traffic noise, species richness and abundance of acoustically oriented birds, grasshoppers and odonates get reduced compared to quiet areas (Senzaki et al. 2020). Bunkley et al. (2017) showed in their study which was carried out near a gas field station at Mexico, USA, that arthropod diversity gets affected in noisy areas as abundance of five arthropod families and one genus found to be decreased with increased background noise level. In addition to this, potential synergistic effects on roadside inhabitants need to be investigated as along with traffic noise, other stressors such as light and chemical pollution are also present. Given that insects are a significant component of biodiversity and play a fundamental role in various ecosystem services, more studies are required to be done to understand the effect of traffic noise on their signalling which ultimately affects their survival and reproduction (Morley et al. 2014). Such studies will enhance our understanding of the ecological and evolutionary consequences of anthropogenic noise on the behaviour and sensory systems of invertebrates both at the individual and population level.

5.5 References

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5.6 Appendix D

Table D5.1 Comparative analysis of call features of noisy and quiet habitats

Call features	Habitat	N	Average	Stdev	Statistics
Chirp duration (s)	Noisy	50	0.26	0.04	t test, t = 1.80, P > 0.05
	Quiet	21	0.28	0.04	
Chirp period (s)	Noisy	50	0.62	0.09	t test, t = 2.08, P < 0.05
	Quiet	21	0.68	0.14	
No. of chirp per 10 s	Noisy	50	16.62	2.30	t test, t = 2.73, P < 0.05
	Quiet	21	14.83	2.97	
No. of syllables per chirp	Noisy	50	12.57	1.36	t test, t = 1.98, P > 0.05
	Quiet	21	13.25	1.19	
Peak frequency (Hz)	Noisy	50	5341	183	t test, test = 1.90, P > 0.05
	Quiet	21	5251	182	
SPL (dB)	Noisy	50	68.30	1.36	Mann-Whitney, U = 389, P <0.05
	Quiet	40	63.40	0.99	

Table D5.2. Comparative analysis of call features of 'Before' and 'After' noise treatment

Call features	Noise treatment	N	Average	Stdev	Statistics
Chirp duration (s)	Before	30	0.40	0.05	Paired t = 1.72, P > 0.05
	After	30	0.41	0.05	
Chirp period (s)	Before	30	0.92	0.13	Paired t = 1.95, P > 0.05
	After	30	0.95	0.13	
No. of chirp per 10 s	Before	30	10.80	1.54	Paired t = 0.97, P > 0.05
	After	30	10.57	1.25	
No. of syllables per chirp	Before	30	13.60	1.65	Paired t = 0.93, P > 0.05
	After	30	13.77	1.43	
Syllable duration (s)	Before	30	0.02	0.00	Paired t = 1.04, P > 0.05
	After	30	0.02	0.00	
Syllable period (s)	Before	30	0.03	0.00	Paired t = 1.91, P > 0.05
	After	30	0.03	0.00	
Peak frequency (Hz)	Before	30	4294.84	250.37	Paired t = 0.31, P > 0.05
	After	30	4289.67	226.72	
SPL (dB)	Before	30	68.20	5.80	Wilcoxon Matched, T= 149, P > 0.05
	After	30	69.10	5.50	

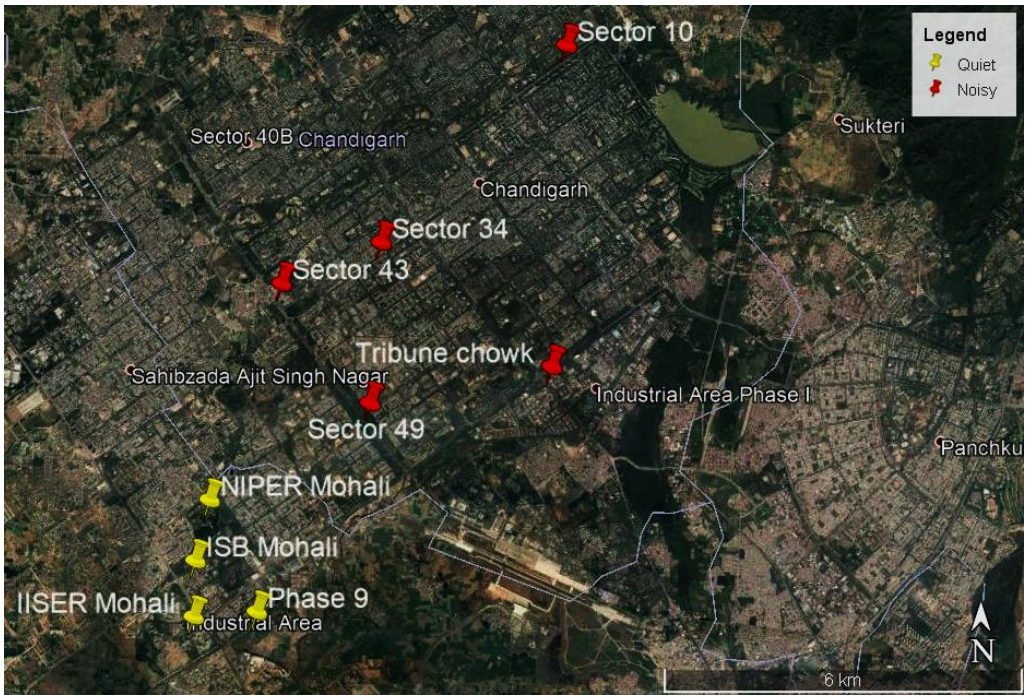

Figure D5.1. Study map showing noisy and quiet habitats.

Chapter 6

Effect of temperature on life history traits and calling behaviour of *Acanthogryllus asiaticus*

Nymph of *Acanthogryllus asiaticus*

Singh R, Prathibha P, Jain M. 2020. Effect of temperature on life-history traits and mating calls of a field cricket, *Acanthogryllus asiaticus*. bioRxiv 2020.06.06.137869; doi: 10.1101/2020.06.06.137869.

6.1 Introduction

All levels of biological organization are dependent on temperature. Various biological processes, such as metabolism, development, growth, movement, and reproduction, are governed mainly by temperature (Grigaltchik et al. 2012). Both increase and decrease in ambient temperature can significantly impact the behaviour and physiology of animals. Changes in ambient temperature may be gradual or rapid and the nature of response of animals to this would be determined by how severely their biological processes are affected. For instance, a transient increase in temperature during a particular time of the day or year may drive animals to seek shade and would determine microhabitat selection if the pattern of temperature changes were predictable (Whitman 1988). A more sustained but gradual increase or decrease in temperature may drive long-term adaptations while a rapid and unpredictable increase that is not transient may significantly impact the survival and reproduction of animals (Prop et al. 2015).

Given the important role of temperature in the biology of organisms, it is foreseeable that a phenomenon like global warming will have adverse effects on diverse life forms of earth. Global warming has profoundly altered environmental conditions and has increased the magnitude of diel and seasonal variation in temperature (Paaijmans et al. 2013; Leonard 2014). The rapid increase in temperature poses a serious risk at organismal, population, community and ecosystem levels (Coumou and Rahmstorf 2012). A special report by IPCC in 2018 suggests that the surface temperature due to global warming is projected to increase by 1.5°C above pre-industrial levels between 2030 and 2052 if it continues to grow at the current rate (Hoegh-Guldberg et al. 2018).

Organisms that are most likely to be significantly affected by the rise in temperature are ectotherms (invertebrates, as well as vertebrates such as fish, amphibians and reptiles)

(Abram et al. 2017). This is because they do not maintain constant body temperature and most of their physiological functions are regulated by ambient temperature (Bartholomew and Tucker 1963). Thus, an increase in temperature would impact the rate of metabolic processes, which would, in turn, affect the physiology and behaviour of these organisms (Abram et al. 2017). Various studies have predicted that the deleterious impact of global warming to be likely more on ectotherms in tropics, even though the rate of warming is slower in the tropics than at higher latitudes (Deutsch et al. 2008).

Among terrestrial ectotherms, insects are the largest group and put together, terrestrial insect in the tropics are likely to be severely affected by the global rise in temperature. However, insects are known to perform thermoregulation by behavioural and/or physiological adjustments, which allows them to perform essential functions such as foraging, movement, mating (by maximizing muscle performance) and also insulates them from the real dangers of overheating or freezing (May 1985; Woods et al. 2015).

Temperature acts as a critical determinant of life-history traits in insects as it strongly influences the rate of development. This is further related to the morphological change and growth, which is associated with an increase in body mass (Jarosık et al. 2004). According to ‘‘temperature-size rule’’, ectotherms reared at low temperatures take longer to develop but have larger bodies at equivalent developmental stages than ectotherms reared at high temperatures (Atkinson 1994). Therefore, investigating the ideal range of temperature for the growth and development of an organism helps in understanding its biology.

Temperature also influences the sound production in ectotherms. Several studies across a wide range of taxa have shown that various properties of acoustic signals are temperature dependent. For instance, studies in insects (Martin et al. 2000; Hedrick et al. 2002; Greenfield and Medlock 2007), anurans (Gerhardt 1978; Llusia et al. 2013), and fish

(Connaughton et al. 2000) have explicitly demonstrated the effect of temperature on the structure and rate of production of a sound signal (Ord and Stamps 2017). These changes in acoustic signals occur due to constraints posed on physiological and biochemical factors involved in muscle function which directly impact motor activities responsible for sound production (Greenfield and Medlock 2007; Llusia et al. 2013).

Crickets are nocturnal insects that produce sound by stridulating their modified forewing (as described in Chapter 1). It is expected that cricket calls will also vary with temperature, as neuromuscular system which is involved in sound production gets affected by the variation in temperature (Martin et al. 2000; Walker and Cade 2003). According to Dolbear's law, there is a relation between the air temperature and chirp rate of the crickets. Dolbear discovered that with increase in temperature, chirp rate also increases (Dolbear 1897). In trilling field cricket, it has been shown that pulse rate increases with temperature in a linear fashion (Walker 2000). Other calling properties also get affected due to faster closing of wings. For instance, calling frequency of song also gets affected (Metrani and Balakrishnan 2005). It is mostly known and well-studied in tree crickets (Oecanthinae) that frequency increases with an increase in temperature, often by several kilohertz (Walker 1962a; Metrani and Balakrishnan 2005). In *Oecanthus henryi*, peak frequency changes by 1 kHz when temperature range from 18 to 28°C. However, in field crickets, the effects of temperature on frequency are still unclear as various studies give different conclusions (Doherty 1985; Pires and Hoy 1992; Van Wyk and Ferguson 1995). Therefore, in the case of field crickets, the effect of temperature on calling features, particularly concerning peak frequency of field cricket in tropics, still needs to be investigated. Further, the impact of immediate ambient temperature and the rearing temperature may be independent and it is important to examine both independently but also in synergy.

Most studies examining the effect of temperature on animals are lab-based, however, for multivoltine animals that emerge multiple times in the year, the adults are likely to experience different climatic conditions depending on when they emerge. A laboratory experiment does not fully explore the ecological consequences of increasing temperature faced by animals in their natural habitat. Information about the temperatures that animals naturally experience and their activity patterns across temperatures, is, therefore, critical in understanding the relationship between temperature and behaviour.

This study aims at understanding the effect of temperature on life-history traits, development and the calling behaviour of a multivoltine field cricket, *Acanthogryllus asiaticus.* I examined the following objectives:

1) Investigating the impact of temperature on life-history traits, such as hatchability, survival to adulthood, developmental time, adult lifespan, total lifespan and body morphometry.

2) Understanding the seasonal differences in the temporal and spectral feature of calls of the species.

3) Examining the influence of immediate ambient temperature on the call features.

4) Determining the impact of developmental temperature on the call features.

6.2 Materials and methods

6.2.1 Life history experiment

Breeding: Adult males and females from lab culture were set for mating at 25°C. The two rounds of mating were conducted on October 7, 2018, with 6 mating pairs and February 26, 2019, with 4 mating pairs. Each mating pair had one male and one female each. Eggs

from each set were segregated on October 23, 2018, and March 16, 2018, respectively and equally divided into three parts. The segregated eggs from the first round were exposed to 25°C, 30°C and 35°C, and the eggs from the second round were exposed to 20°C, 25°C and 30°C on the same day of segregation. All the eggs were kept in constant temperature cabinets and room at a relative humidity ranging from 40-70% and a daily 12L:12D light cycle.

Rearing: These eggs were placed on wet cotton pads in petri-dish which was kept in plastic containers (15 X 12 X 10 cm) having lids with a 10 X 5 cm hole covered with mosquito screening mesh to allow air circulation. On the arrival of nymphs, they were transferred to other plastic containers (35 X 25 X 12 cm) having lids with a 10 X 10 cm hole covered with mosquito screening mesh to allow air circulation. The bottom of each of this container was lined with egg cartons and two Petri-dishes filled with dogfood powder and wet cotton pads. Observation for growth and developmental stages was carried out daily during initial days and later every third day. Periodic morphometric measurements were also carried out for nymphs at different temperature 20°C, 25°C and 30°C on 45th, 65th, 95th, 120th, 155th, 220th, 280th and 315th day by randomly selecting three individuals from the breeding containers for each temperature for the measurement. After the final moult, individuals were kept in a separate box of diameter 12 cm and height 5 cm with a mesh (9 X 8 cm) lid and provided with wet cotton and dog food.

Morphometric analyses: Body morphometry for adults was carried out by considering the following morphometric parameters: body length, pronotum length, pronotum width, wing length and ovipositor length for females. Periodic morphometric measurements for nymphs was carried out using body length as a parameter. All the morphometry measurements were done using a digital camera (Leica MC120HD, Leica Microsystems GmbH, Wetzlar,

Germany) connected with Leica Stereo Zoom Microscope (M 205C, Leica Microsystems GmbH, Wetzlar, Germany). Bodyweight for male and females was measured using a weighing balance (Sartorius analytical balance: BSA224S-W, Sartorius AG, Goettingen, Germany). Developmental time, adult lifespan and total lifespan were also measured. Developmental time is defined as the total number of days taken by nymphs to reach adulthood. Adult lifespan is the total number of days an individual survived after reaching adulthood. Total life span is the sum of developmental time and adult lifespan.

6.2.2 Seasonal variation in calling behaviour

Field recordings were done for calling individuals during winter (February) and summer (March-May) seasons in 2017 and 2018 between 1900-2200 h. During each recording, weather parameters such as humidity and temperature were measured just above the ground using a pocket weather meter (Kestrel 4000, Nielsen-Kellerman, Chester, U.S.A.). For the winter season, recordings collected at temperatures ranging between 15°C to 19°C were selected, while for summer, recordings collected at temperatures ranging between 24 °C to 30°C were selected.

6.2.3 Effect of immediate ambient temperature on call features

Adult males were collected from the field in April 2016 from IISER campus and brought back to the lab. Individuals were kept separately in a plastic container (diameter - 12 cm and height - 6 cm) covered with cloth mesh in a climatic chamber (Memmert GmbH+Co.KG, Germany) maintained at 24°C, 40 - 70 % humidity, 12L:12D light cycle. *Ad libitum* food and water were provided. After a week, individuals were recorded at 22°C, 24°C, 26°C, 28°C and 30°C. Prior recording, animals were kept for at least 5 hours in the recording temperature. This was also carried on lab-bred individuals bred at 25°C and 30°C and recorded at 25°C and 30°C.

6.2.4 Effect of developmental temperature on call features

Adult males bred at 25°C and 30°C were recorded at their respective breeding temperatures and then same individuals were recorded at the other temperature (30°C and 25°C). Animals exposed to non-breeding temperatures were kept for at least 5 hours in these temperatures before the recordings were made. All the recordings were done in a dark silent room (ambient noise at 15 dB at 5 kHz).

All audio recordings were made as 16-bit WAV files at a sampling rate of 44.1 kHz using Tascam, Linear PCM Recorder (DR-07 Mk II, TEAC Professional, USA). All recordings were digitised in Raven Pro1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY). Temporal and spectral parameters, namely, chirp duration, chirp period, syllable duration, syllable period, number of syllables per chirp, and peak frequency, were analyzed.

6.2.5 Statistical analysis

Statistical tests were performed using Statistica 64 (Dell Inc.2015, Version 12) and R version 3.3.1. (R Core Team, 2016). Shapiro-Wilk test was used to check normality. A t-test was done to compare adult lifespan, total lifespan, weight, body morphometric traits: body length, wing size, pronotum length, pronotum width and ovipositor size for individuals bred at 25°C and 30°C. One-way ANOVA was carried out for nymph appearance duration and developmental life span. Calling behaviour during summer and winter was compared using a Mann-Whitney U test. Correlation of calling parameters with temperature and humidity was measured using Spearman rank-order correlations. Effect of immediate ambient temperature on calling behaviour in the laboratory environment was measured using a Kruskal-Wallis test followed by pairwise comparison for all the calling parameters. Variation in calling parameters at developmental temperature and exposed

(immediate ambient) temperature was compared using paired t-test while t-test was conducted for examining the effect of developmental temperature on calling parameters for individuals bred at 25°C and 30°C and recorded at 30°C.

6.3 Results

6.3.1 Life history experiment

The average egg length which was measured on the second week after mating was found to be 2.53 ± 0.15 mm (N = 5; 25°C) and average egg width to be 0.61 ± 0.08 mm (N = 5; 25°C). Developmental temperatures had a significant effect on the time taken to hatching, wherein nymphs hatched the soonest at 35°C (3 days after egg segregation) while it took longest at 20°C (One-way ANOVA, $F(3, 346) = 282.2$, $P < 0.05$; Figure 6.1; 6.2, Table E6.1). While the percentage of nymph hatched was the least at 35°C (Figure 6.3A); however, none of the nymph survived at 35°C and only 1 survived at 20°C (Figure 6.3B; 6.4). Around 23% of nymph hatched at 25°C and 30°C of which 20% and 18% survived at 25°C and 30°C, respectively. Hence, the remaining analyses were carried out only on the batches at 25°C and 30 °C.

Developmental temperatures had a significant effect on the time taken to reach to the adulthood, wherein it was highest at 20°C (284 days), while 30°C showed rapid development (96 days) (One-way ANOVA, $F(2, 52) = 69.73$, $P < 0.05$; Figure 6.5, Table E6.1). Since only one nymph survived as an adult at 20°C, adult lifespan and total lifespan was calculated only for individuals at 25°C and 30°C. Significant difference was found for adult lifespan between 25°C and 30°C (t-test, $t = 2.53$, $df = 52$, $P < 0.05$, Figure 6.6). Total lifespan was significantly different between 25 and 30°C, wherein for 25°C was (245 ±

18.35) while at 30°C it was 155 ± 24.8 days (t-test, t = 14.82, df = 52, P < 0.05; Figure 6.6, Table E6.1).

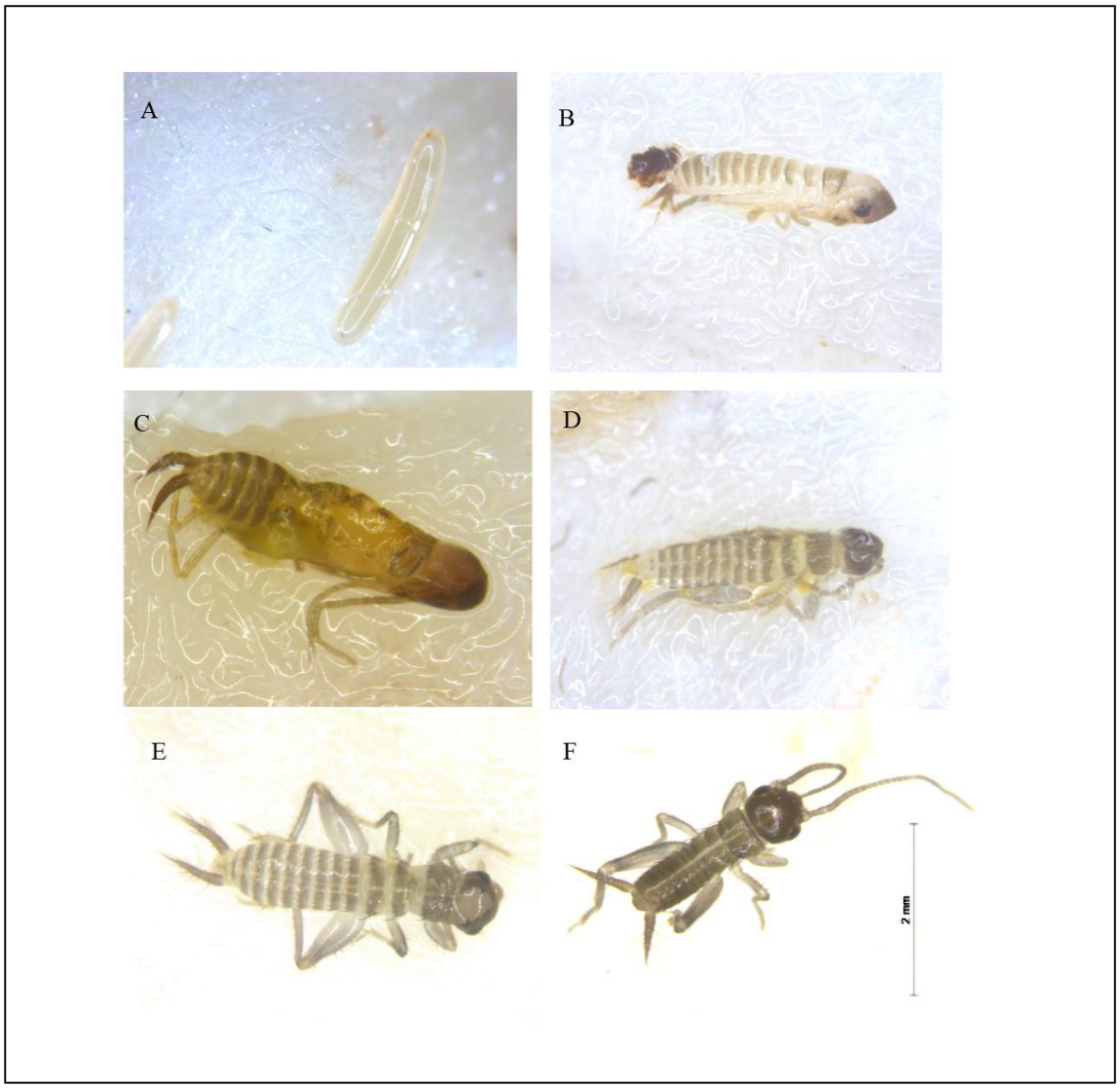

Figure 6.1. A. Egg of *A. asiaticus.* B to E. Developmental stages from an egg to a nymph showing appearance of eyespots, appendages and body segmentation. F. A newly hatched nymph.

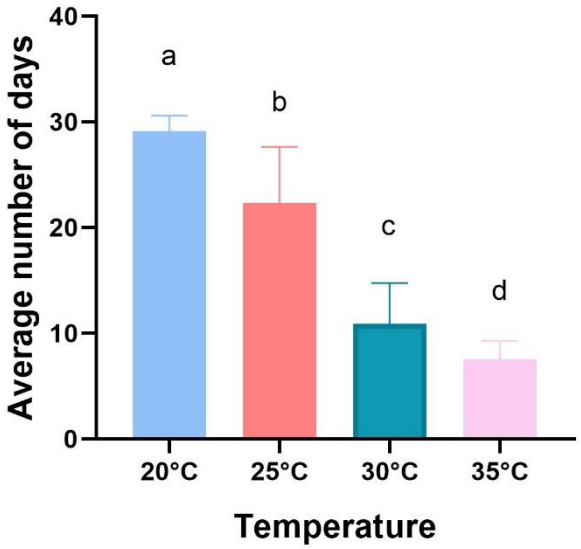

Figure 6.2. Average number of days taken by nymphs to appear in different temperature regimes. Different alphabets indicate significant difference ($P < 0.05$). N: 20°C = 59; 25°C = 135; 30°C = 138; 35°C = 18

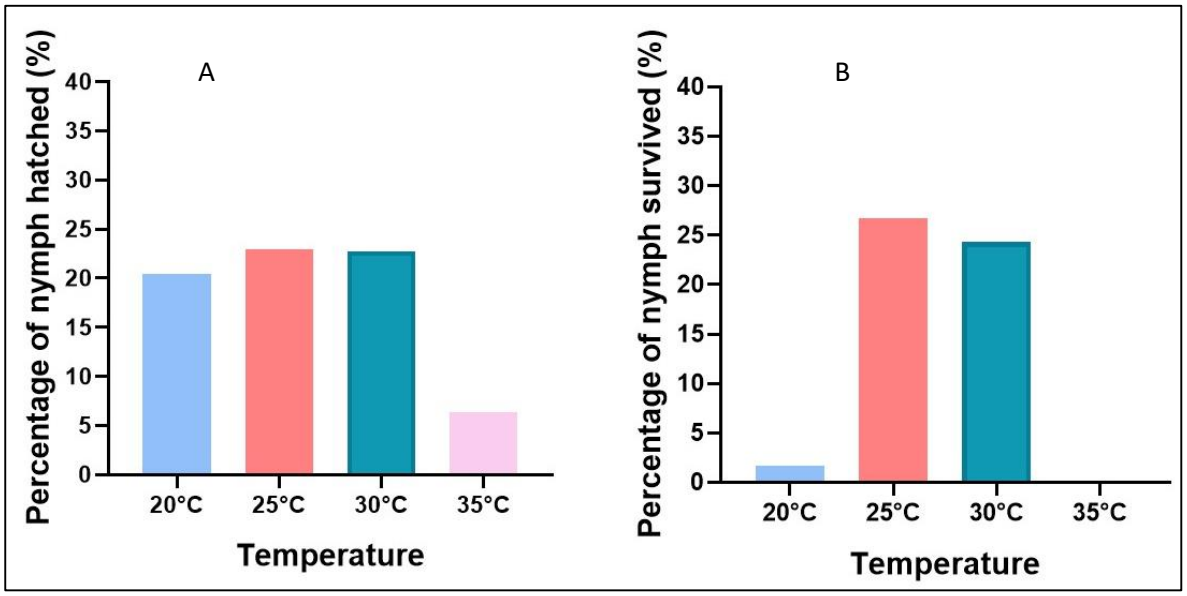

Figure 6.3. A. Percentage of nymph hatched at different temperature regimes. B. Percentage of nymph survived at different temperature regimes.

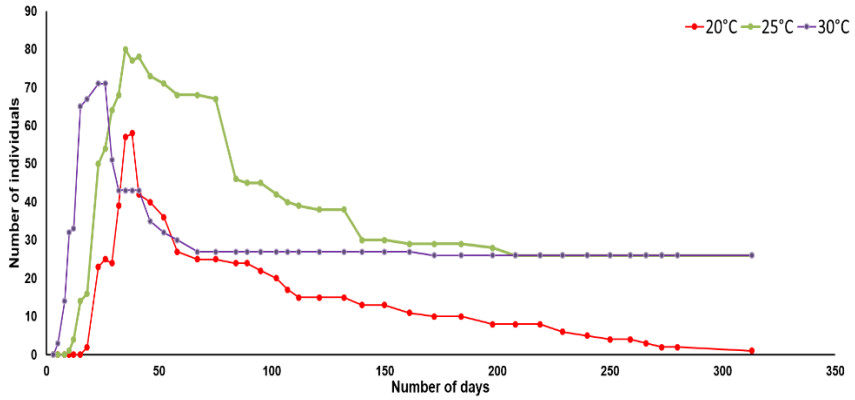

Figure 6.4. Frequency distribution of number of nymphs surviving to adulthood at different temperature regimes.

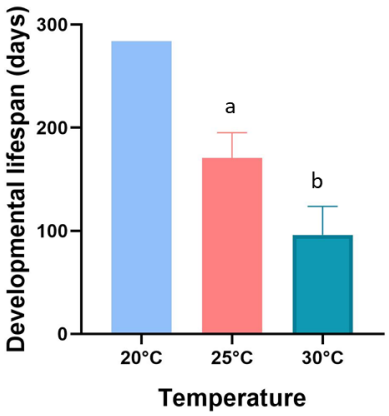

Figure 6.5. Developmental time of different individuals at different temperature regimes. Different alphabets indicate significant difference ($P < 0.05$). N: 20°C = 1; 25°C = 24; 30°C = 30.

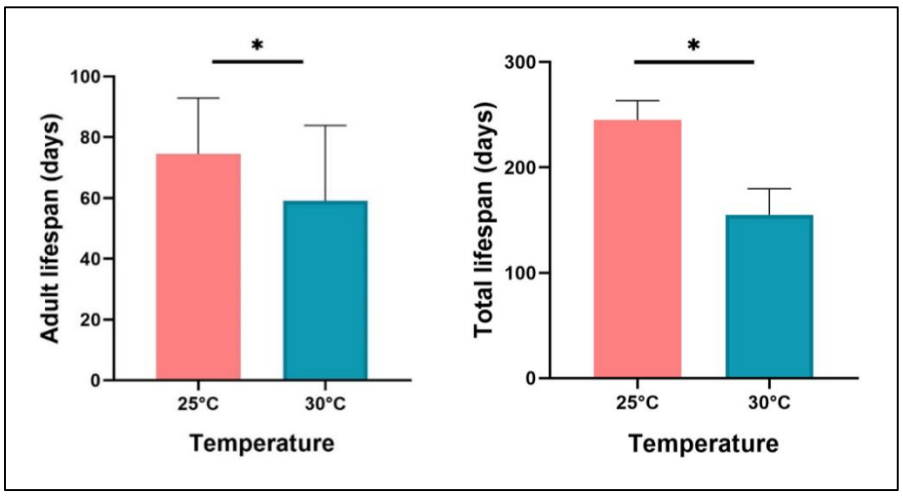

Figure 6.6. Adult lifespan and total lifespan at different temperature regimes. * indicates significant differences. N: 25°C = 24; 30°C = 30.

Body morphometry was measured for nymphs at different temperature 20°C, 25°C and 30°C on 45th, 65th, 95th, 120th, 155th, 220th, 280th and 315th day. 30°C showed rapid development, followed by 25°C and very slow development was observed in 20°C (Figure 6.7; 6.8). Morphometric analyses were carried out on different developmental stages from nymph to adulthood (Figure 6.9, TableE 6.2A).

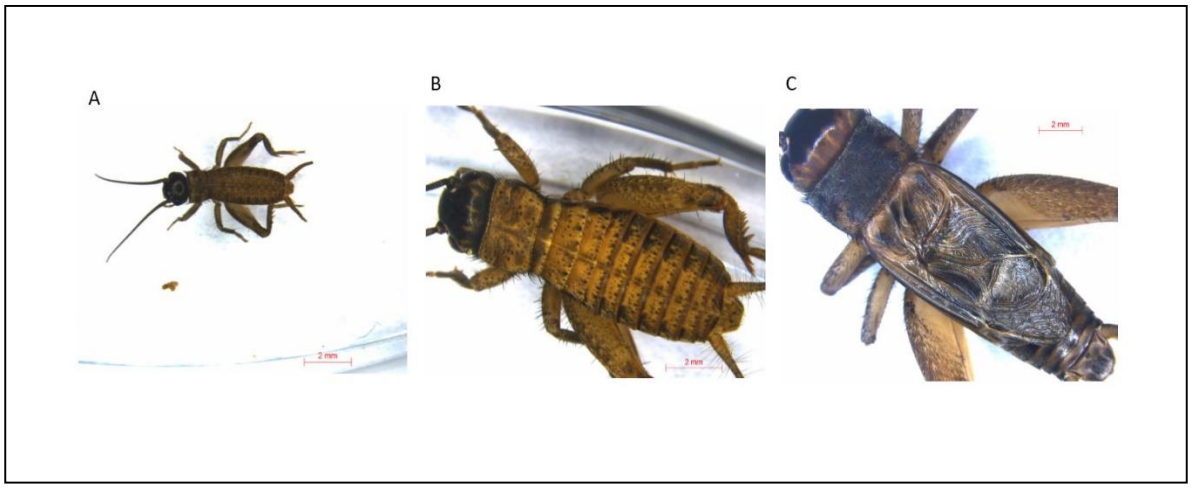

Figure 6.7. Variation in body sizes on 120th day from the egg segregation for individuals at A. 20°C, B. 25°C and C. 30°C on the scale of 2 mm.

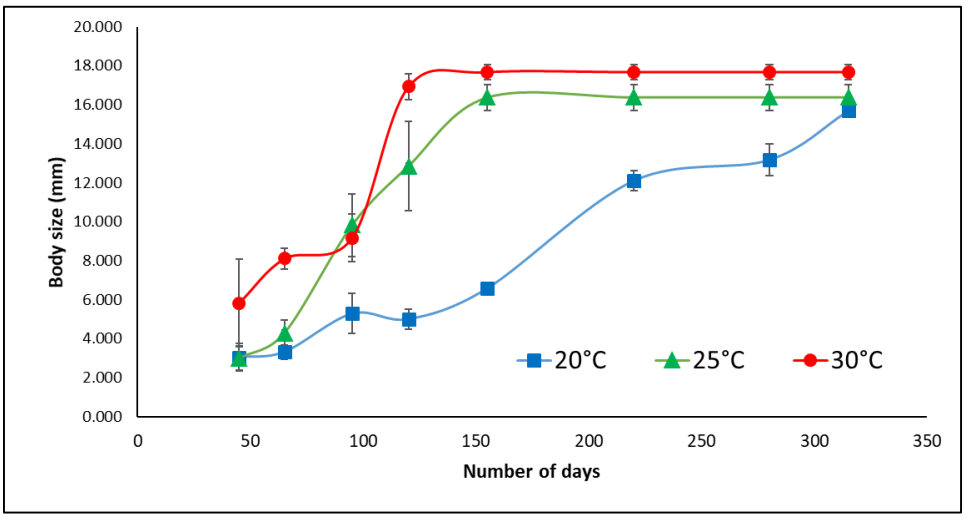

Figure 6.8. Variation in body morphometry from nymph to adult at different temperature regimes (Mean ± SD; N = 3 individuals at each temperature on the measurement day)

Figure 6.9. Different developmental stages from nymph (A-D) to adulthood (E-F) in *A. asiaticus*.

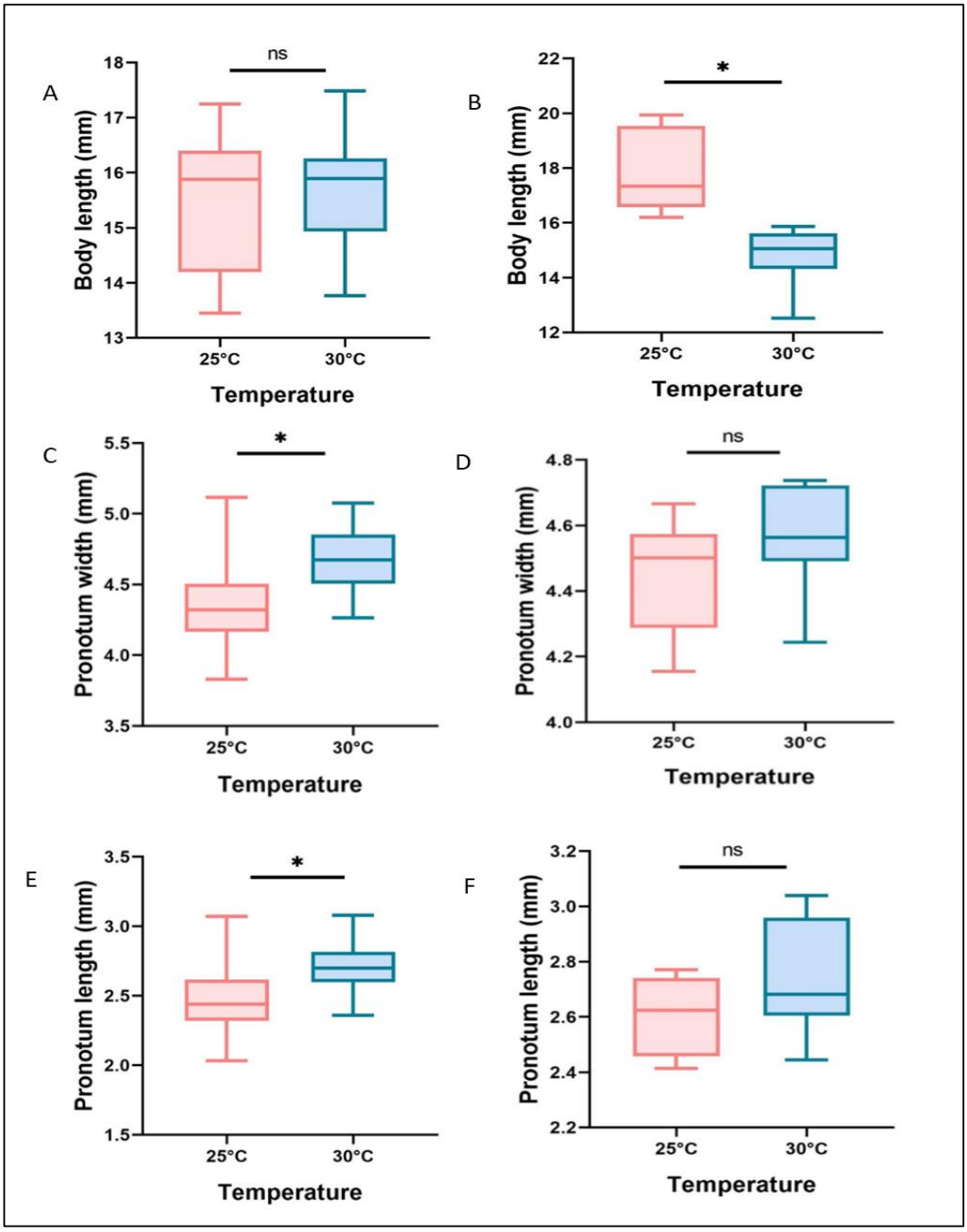

Figure 6.10. Variation in different body size parameters in adult males (A, C, E and G) and females (B, D, F, H and I) bred at 25 and 30°C. * indicates significant differences. N: Male = 40; Female = 17.

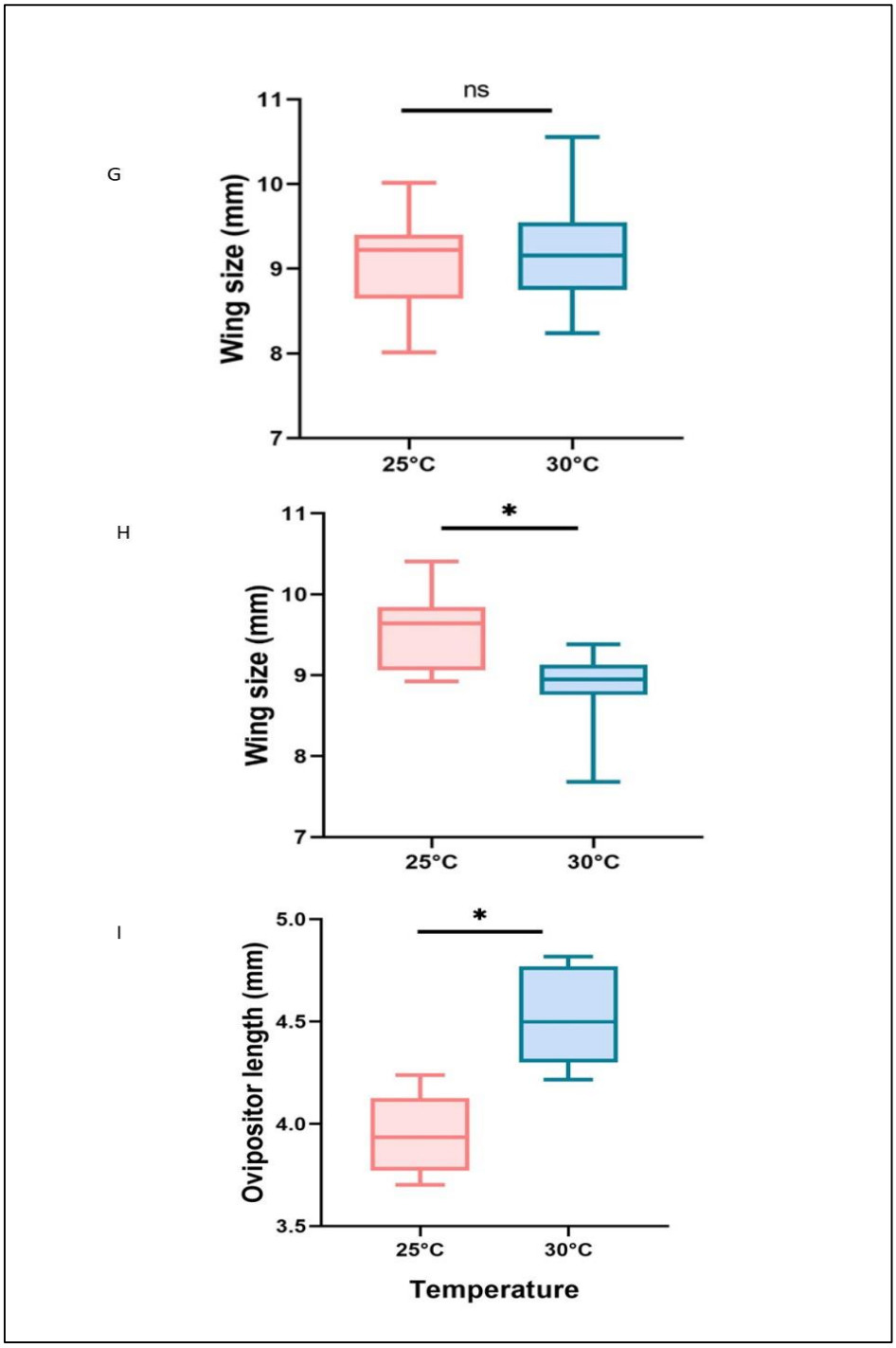

Figure 6.10. Variation in different body size parameters in adult males (A, C, E and G) and females (B, D, F, H and I) bred at 25 and 30°C. * indicates significant differences. N: Male = 40; Female = 17.

Females of 25°C were found to be having larger body length compared to males whereas males of 30°C were found to have larger body length compared to females (t-test, $P < 0.05$; Figure 6.10; Table E6.2B). Adult males at 30°C were found to have greater pronotum length and width compared to 25°C whereas no difference in pronotum length and width was observed in females (Figure 6.10; Table E6.2B). However, females have larger body length and wing size at 25°C compared to 30°C (Figure 6.10; Table E6.2B). Females at 25°C were heavier than males (t-test, $P < 0.05$; Figure 6.11; Table E6.2B), however, no difference in body weight was found between males and females at 30°C. Males at 30°C were found to be heavier than males at 25°C (Figure 6.11; Table E6.2B) but body weight of females at 30°C and 25°C showed no significant difference (Figure 6.11; Table E6.2B).

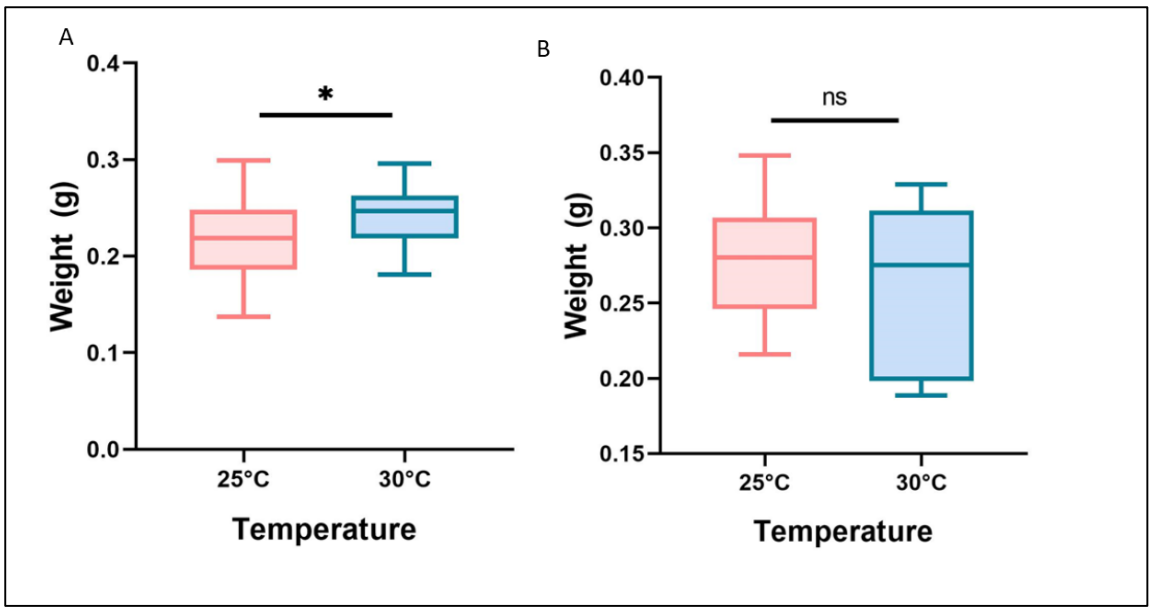

Figure 6.11. Variation in body weight in adult A. males and B. females bred at 25 and 30°C. * indicates significant differences. N: Males = 32; Female = 15.

6.3.2 Seasonal variation in call features

Chirp duration and chirp period found to be greater in winter (February) than summer (March-May) (Mann-Whitney U test, $P < 0.05$, Figure 6.12, Table E6.3). I found higher

chirp rates during summer compared to winter (Mann-Whitney U test, $P < 0.05$, Figure 6.12, Table E6.3). Syllable duration, syllable period and the number of syllables found to be higher during winters as compared to summer. I found peak frequency to be lower during winter as compared to summer (Mann-Whitney U test, $P < 0.05$, Figure 6.12, Table E6.3). I found a strong correlation of humidity and temperature with chirp duration, chirp period, the number of chirps/10 seconds and peak frequency (Spearman Rank Order Correlations, $P < 0.05$, Table 6.1). In addition, call features were found to be strongly correlated with each other (Spearman Rank Order Correlations, $P < 0.05$, Table 6.1).

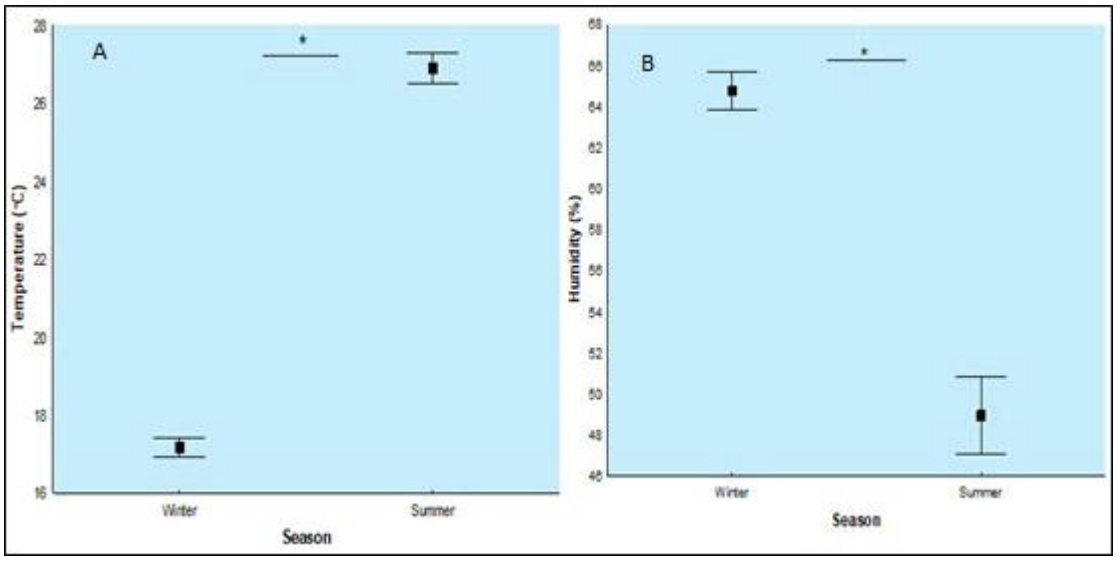

Figure 6.12. Seasonal variation in A. temperature, B. humidity and C to I. call properties. * represent $P < 0.05$; Mean ± 95% CI; N: Summer = 17 individuals; Winter = 12 individuals.

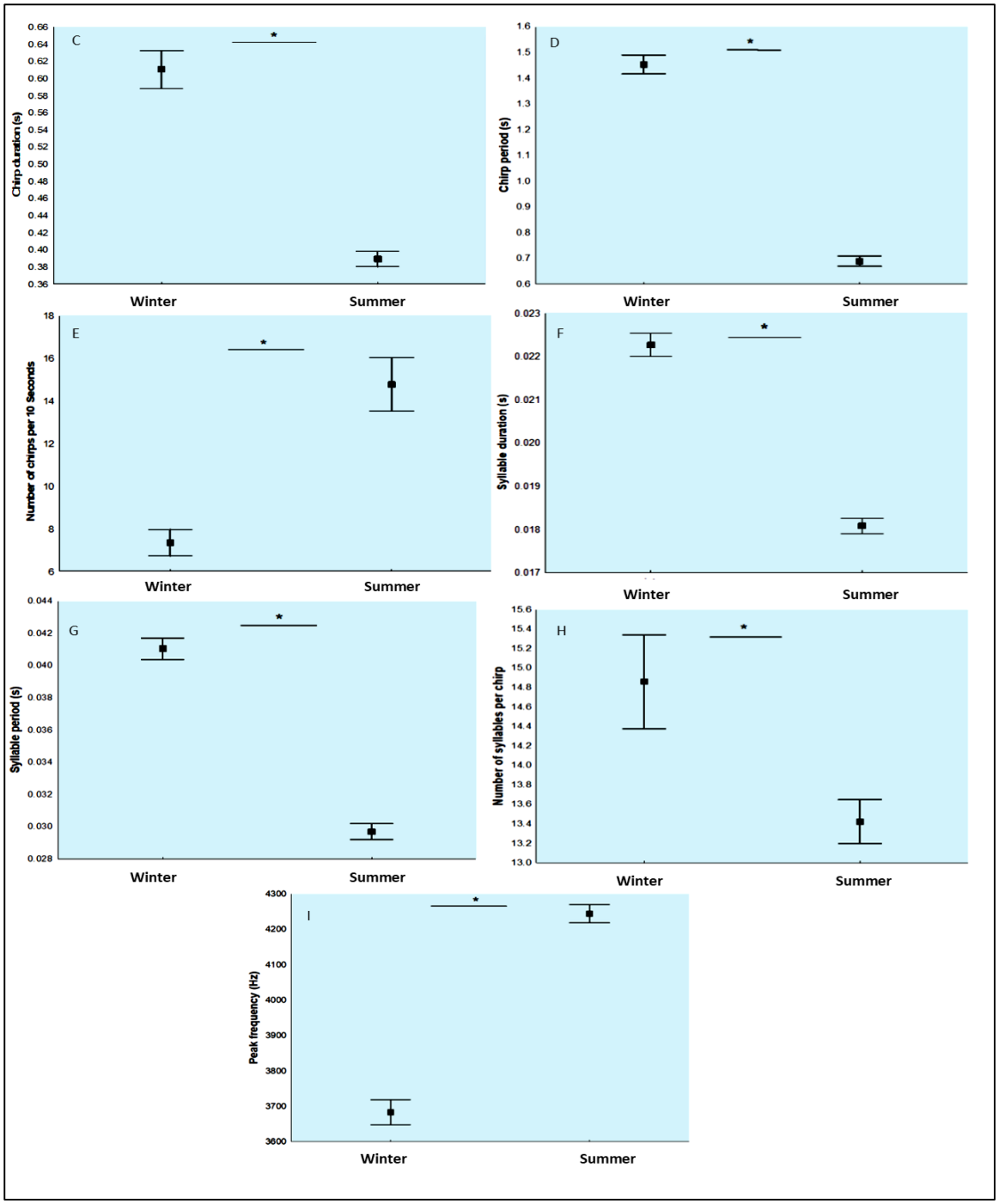

Figure 6.12. Seasonal variation in A. temperature, B. humidity and C to I. call properties. * represent $P < 0.05$; Mean ± 95% CI; N: Summer = 17 individuals; Winter = 12 individuals.

Table 6.1. Relationship of temperature and humidity with different call properties using Spearman rank-order correlations showing R value. Significant correlations are indicated in bold.

	Temperature	Humidity	Chirp duration (s)	Chirp period (s)	Peak frequency (Hz)	No. of syllables
Temperature						
Humidity	**-0.810**					
Chirp duration (s)	**-0.701**	**0.473**				
Chirp period (s)	**-0.854**	**0.608**	**0.775**			
Peak frequency (Hz)	**0.787**	**-0.554**	**-0.650**	**-0.776**		
No. of syllables/chirp	-0.115	0.034	**0.679**	0.233	-0.087	
No. of chirps/ 10 sec	**0.863**	**-0.863**	**-0.767**	**-0.834**	**0.662**	**-0.761**

6.3.3 Effect of immediate ambient temperature on call features

All temporal and spectral parameters found to be different when compared at different temperatures (Kruskal-Wallis test, Figure 6.13, Table E6.4). Chirp duration, chirp period, syllable duration, syllable period, number of syllables found to be decreasing with temperature, however, chirp rate and peak frequency found to be increasing with the temperature (Kruskal-Wallis test, Figure 6.13, Table E6.4). I did not find any linear trend, which could be the result of inter-individual variation since the same individual was not recorded for different temperatures.

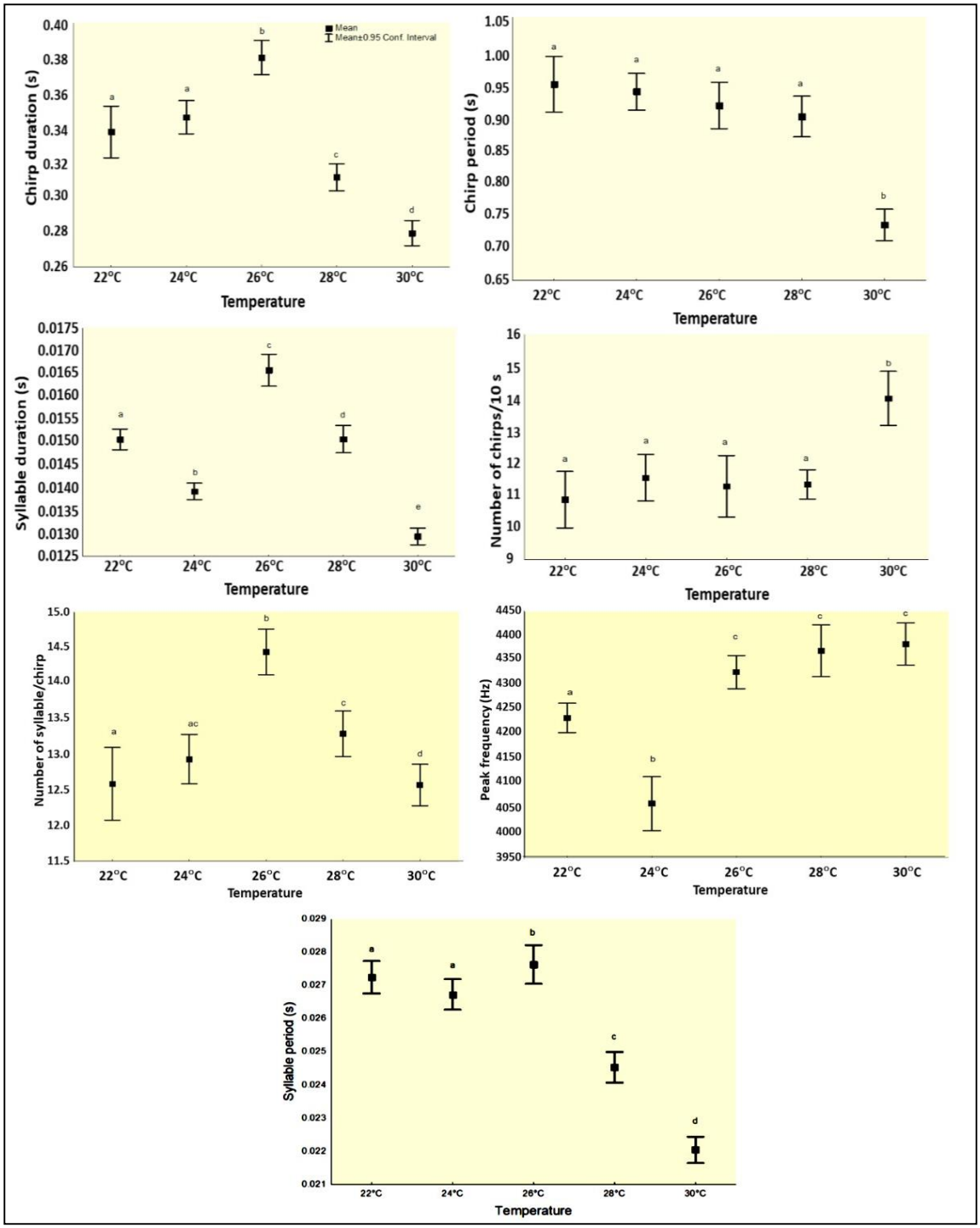

Figure 6.13. Effect of short term exposure of different temperature on different call properties. Different letters indicate significant difference $P < 0.05$. Mean ± 95% CI.

When recorded at their respective breeding temperature, I found that individuals bred at 25°C had higher chirp duration, chirp period, syllable duration, syllable period and number of syllable, however, chirp rate and peak frequency found to be higher at 30°C (Mann-Whitney U test, $P < 0.05$, Figure 6.14, Table E6.5). Individuals bred at 25°C when exposed to 30°C, decreased their chirp duration, chirp period, syllable period, number of syllables, while they increased the chirp rate (paired t-test, $P < 0.05$, Figure 6.15, Table E6.6). No change in peak frequency and syllable duration was observed (paired t-test, $P > 0.05$, Figure 6.15, Table E6.6). Individuals bred at 30°C, when exposed to 25°C, decreased chirp rates and peak frequency were observed. However, they increased their chirp duration, chirp period, syllable period, syllable duration and did not alter syllable number (paired t-test, $P < 0.05$, Figure 6.16, Table E6.6).

6.3.4 Effect of developmental temperature on calling behaviour

I found significant differences in the call features of individuals recorded in their respective developmental temperature and then exposed to immediate ambient temperature. Development temperature showed significant difference in various call feature of individuals bred at 25°C and 30°C and recorded at 25°C and 30°C. Pair-wise comparisons revealed that chirp period, chirp rate, syllable duration, syllable period and peak frequency were significantly different between individuals bred at 25°C and 30°C, and recorded at 30°C (t-test, $P < 0.05$, Fig. 4, Table E6.7). However, a significant difference was only observed in the chirp period and peak frequency when individuals bred at 25°C and 30°C were recorded at 25°C, (t-test, $P < 0.05$, Fig. 4, Table E6.7).

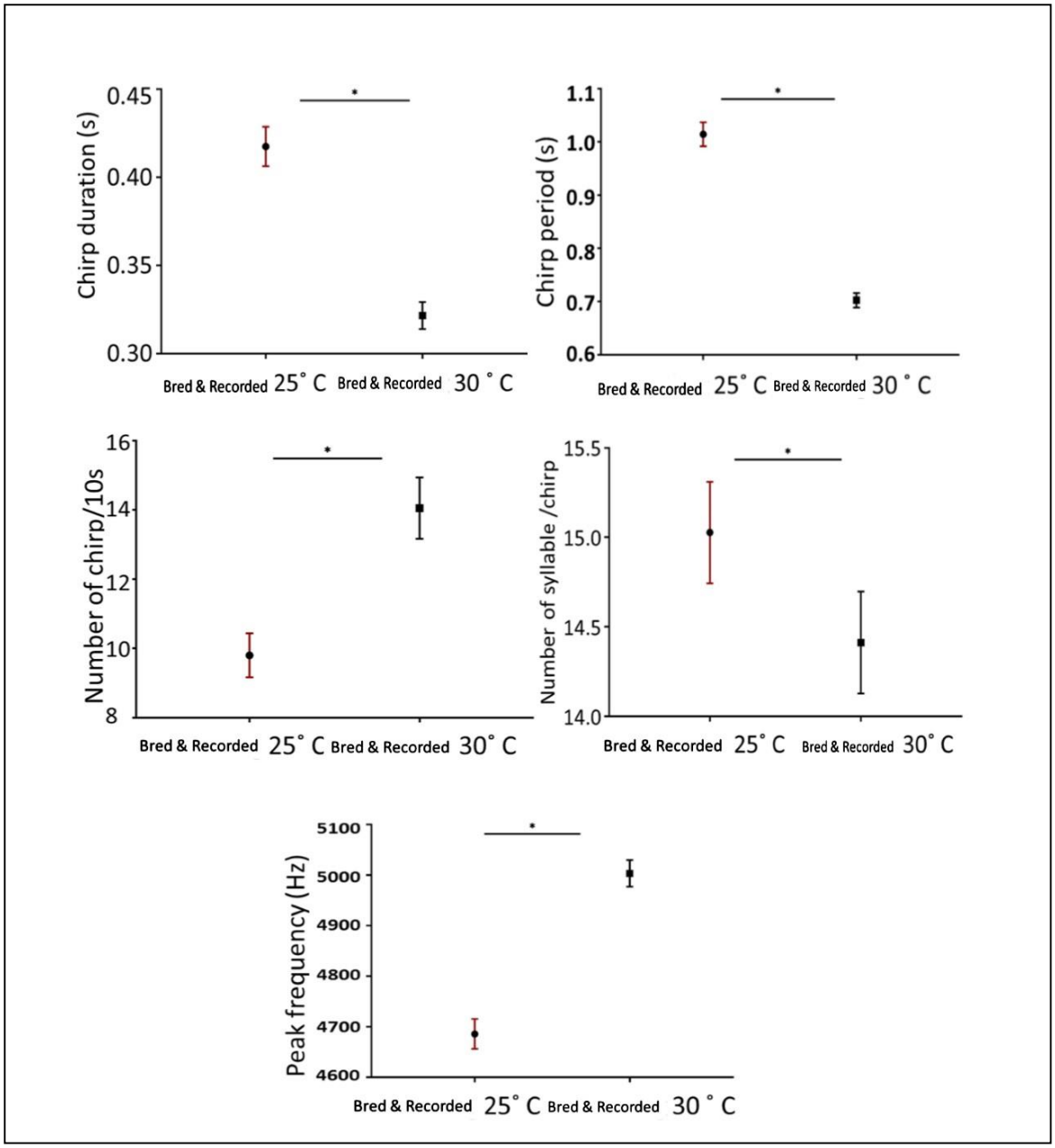

Figure 6.14. Variation in call properties for individuals bred at 25 and 30°C and recorded at their respective breeding temperature. * indicates significant differences. Mean ± 95% CI

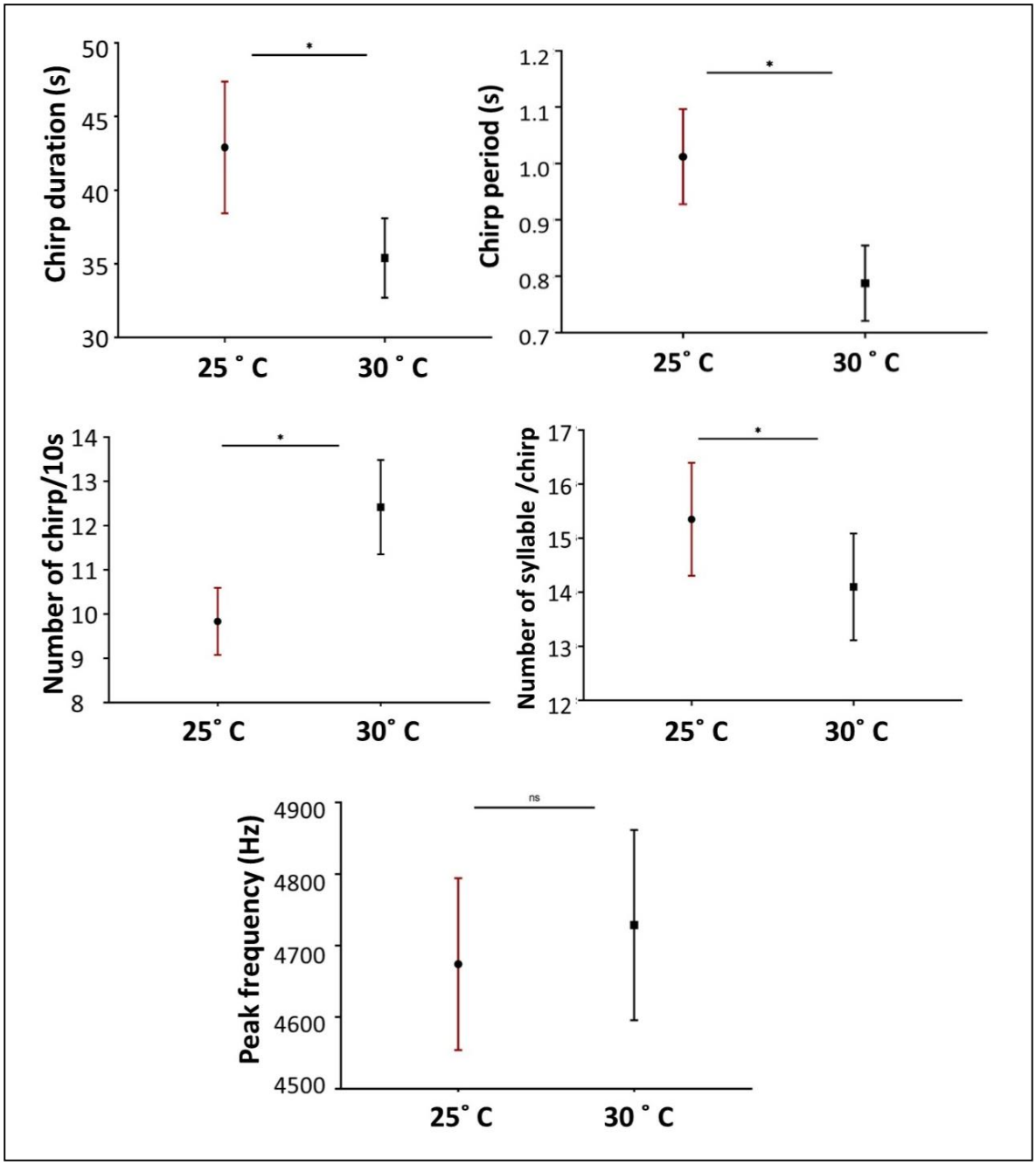

Figure 6.15. Variation in call properties for individuals bred at 25°C and recorded at 25°C and the same individuals recorded at 30°C. * indicates significant differences.

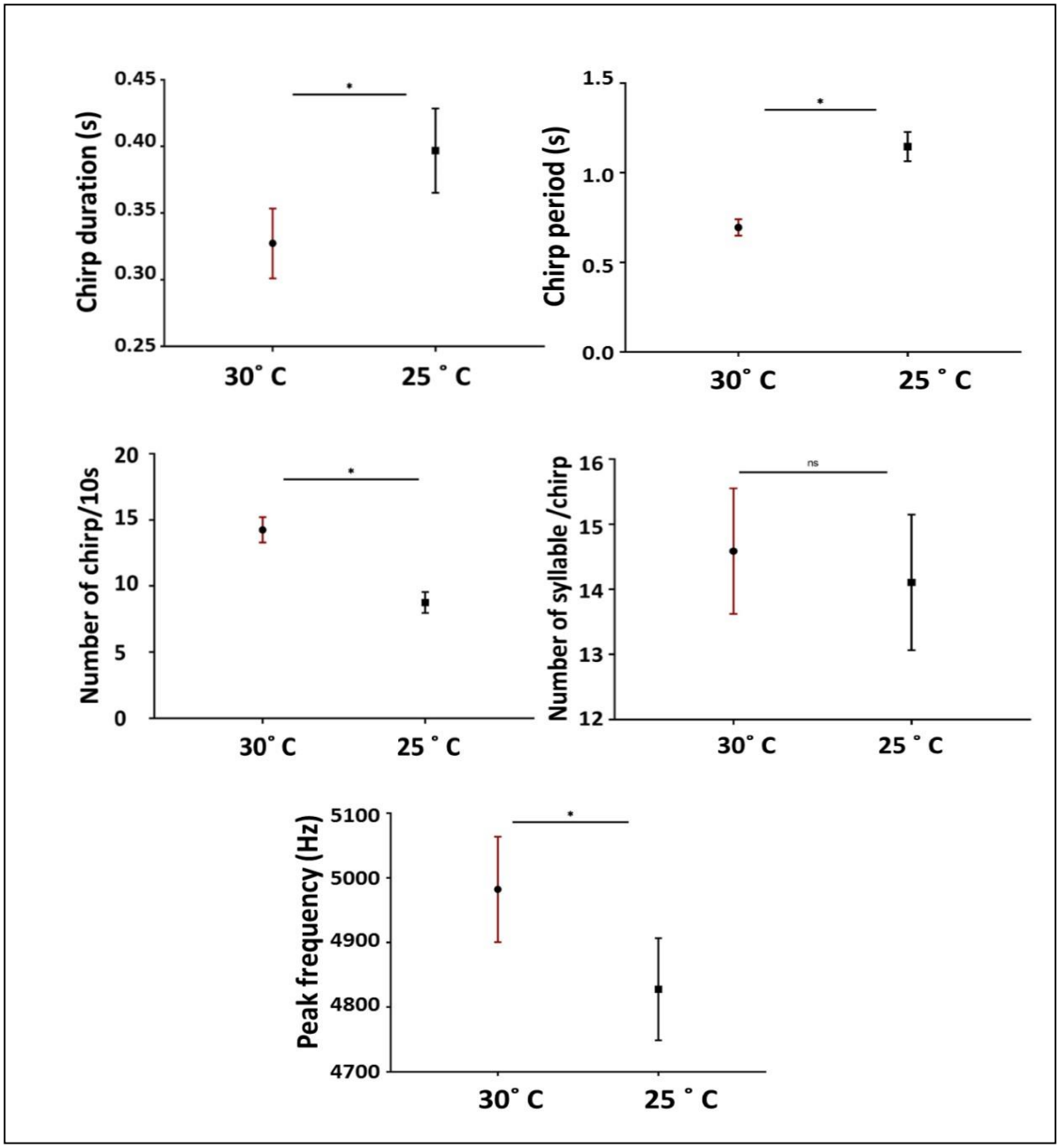

Figure 6.16. Variation in call properties for individuals bred at 30°C and recorded at 30°C and the same individuals recorded at 25°C. * indicates significant differences.

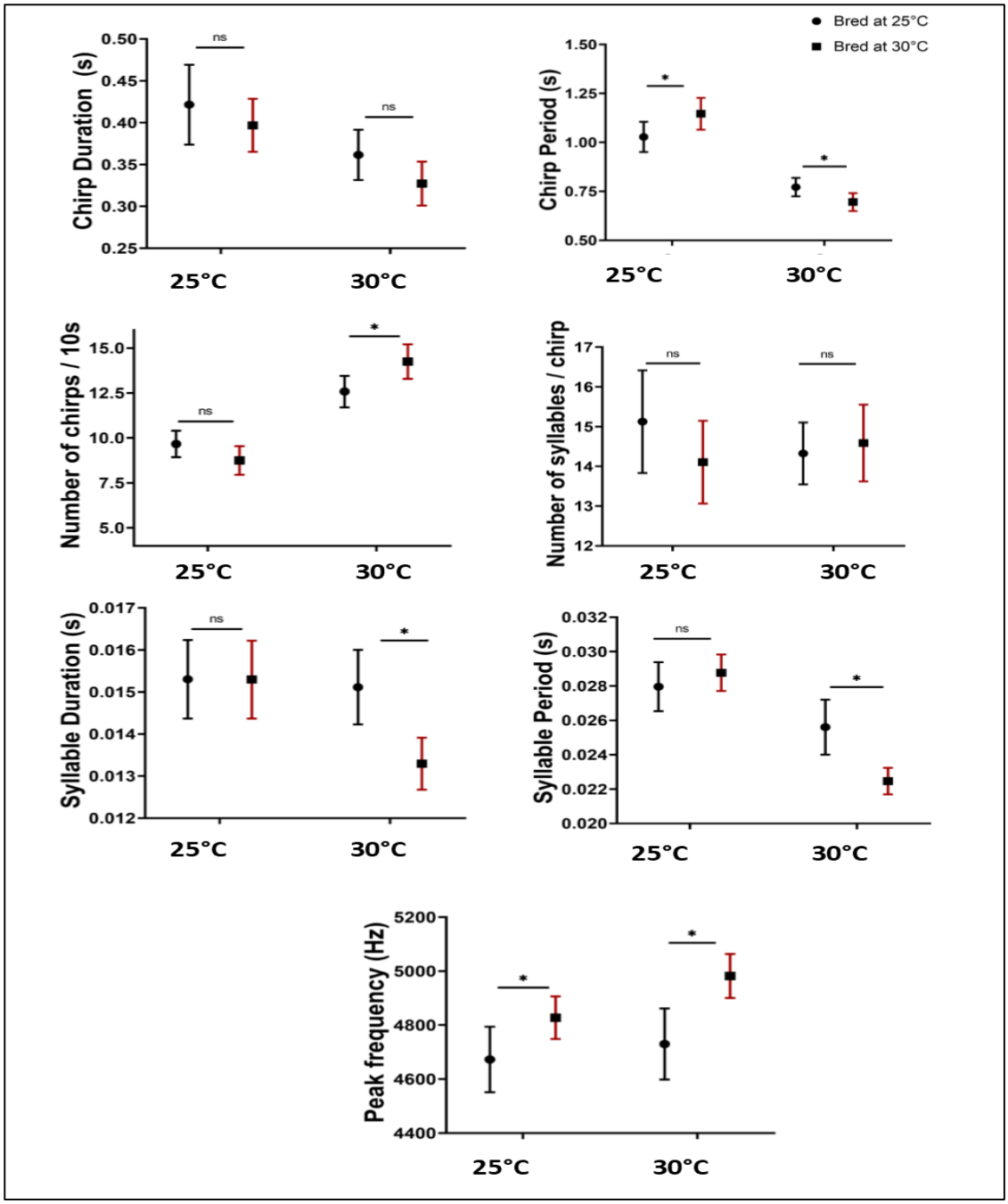

Figure 6.17. Variation in call properties for individuals bred at 30°C and 25°C and recorded at ambient temperatures: 25°C and 30°C. * indicates significant differences.

6.4 Discussion

The experimental results obtained in my study indicate that the developmental biology and calling behaviour of *A. asiaticus* is strongly affected by the increase in temperature.

6.4.1 Effect of temperature on life history

I found that the total time taken for the first nymph to appear was the least at higher temperatures (30°C and 35°C), while at a lower temperature (20°C), it took the longest to appear. However, the percentage of nymph hatched was the least at 35°C while an equal amount of nymphs hatched at 20, 25 and 30°C. I found that no nymph survived at 35°C and only 1% of nymph survived at 20°C. Similar results have been obtained in a study on *Teleogryllus emma*, where the effect of eight different temperatures (15, 18, 21, 25, 27, 29, 31 and 35°C) on life-history traits was examined (Kim et al. 2007). The highest survival rate was observed at 25°C – 31°C, while at lower temperatures (below 20°C), individuals did not survive at all (Kim et al. 2007). In that study, at 35°C, only 10% of individuals thrived while in my study, no nymph reached the adulthood stage. I found that both growth and development rates of *A. asiaticus* were faster at 30°C than 25°C and 20°C. I observed that crickets raised at 25°C grew slower and took longer to develop. In support of my finding, a similar trend was observed in house crickets *Acheta domesticus*, where newly enclosed crickets reared at 25°C took longer to develop than those at 28°C (Booth and Kiddell 2007). A similar trend was also observed in *T. emma* as individuals at 21°C to 29°C took longer time to develop than the one's at higher temperature of 35°C (Kim et al. 2007). Furthermore, in *Gryllus texensis*, development time decreased with increasing temperature (Adamo and Lovett 2011). Also, adult lifespan in *A. asiaticus* was found to be higher at 25°C than 30°C implying high temperature leads to a shorter lifespan. A similar trend was also noticed in *T. emma* (Kim et al. 2007) and *G. bimaculatus* (Behrens et al. 1983).

I also examined the relationship between temperature and body size. I found that at 25°C, females were larger than males, whereas at 30°C, I found the opposite. I found that the pronotum length and pronotum width of males at 30°C to be larger than males of 25°C. However, females bred at 25°C, found to have bigger body length as well as wing size than those raised at 30°C. Besides, the ovipositor of females at 30°C found to be bigger than that of 25°C. A study on *G. firmus* showed that at 24, 28 and 32 °C, there is a significant difference in body size but did not show any consistent pattern (Begin et al. 2004). I also found that males raised at 30°C were heavier than those grown at 25°C. Similarly, Roe et al. (1985) found that individuals of *A. domesticus* reared at 35°C to be heavier than those raised at 25°C. However, on the same species, weight at 25°C was found to be higher than those at 28°C (Booth and Kiddell 2007). My study indicates that a higher temperature is associated with a larger body which is converse to temperature-size rule.

My study shows that 25°C to 30°C is the ideal temperature range for the growth and development of nymphs belonging to *A. asiaticus*. In this study, I maintained the constant temperatures for breeding in contrast to the natural temperature regime. However, a study on *G. bimaculatus* using fluctuating temperatures showed similar result that number of offspring increased at higher temperature while lifespan was longest for the low temperature (Behrens et al. 1983).

Such developmental temperature does not only influence the life-history traits but also affects the expression of various behaviour. A study on *G. bimaculatus* showed that individuals raised at high temperatures were more explorative, indicating that different developmental temperatures give rise to varying amounts of behavioural stability in exploratory behaviour (Niemela et al. 2019).

6.4.2 Effect of temperature on call features

My findings confirm that calling song in *A. asiaticus* is affected by seasonal variation, immediate temperature and developmental temperature. My result provides evidence that experimental results obtained in a laboratory are relevant to those obtained in the field and determine how the environment shapes the behavioural development. I found that during winter where temperature varied between 14°C to 19°C, the population in the field produce calls with higher chirp and syllable period compared to calls produced by summer population where temperature varied between 24°C to 30°C. However, peak frequency and chirp rate were found to be maximum for the summer population as peak frequency increased by 500 Hz and chirp rate increased by 7 chirps. Along with the temperature, humidity also affected temporal and spectral call parameters, which establishes the strong impact of weather parameters on cricket calling behaviour. Walker (1962b) also found the influence of humidity on pulse rates in *Nemobius ambitiosus*, however, he also found that temperature had greater effects than the most extreme variations in humidity. Temporal call parameters were found to be significantly correlated with each other in the calls of *A. asiaticus*. Similarly, in *Plebeiogryllus guttiventris*, Nandi and Balakrishnan (2013) also found that most of the temporal call features were strongly correlated to each other.

I found the effect of immediate ambient temerature on temporal and spectral features of calls of field collected individuals which were maintained at 24°C in lab condition and were exposed to 22, 24, 26, 28 and 30°C for a short duration. While chirp duration, chirp period, syllable duration, syllable period, number of syllables were found to be lowest at 30°C, chirp rate and peak frequency were found to be maximum at 30°C. Similar trends in call properties with an increase in temperature have been found in various other field cricket species (Walker 1962b; Doherty 1985; Pires and Hoy 1992; Martin et al. 2000; Walker and

Cade 2003). One of the critical aspects of calling song is the number of pulses per unit song, which might also be affected due to an increase in temperature. It is shown in *G. integer* that the number of pulses per trill is an essential factor for female mate choice difference (Gray and Cade 1999). In *G. bimaculatus*, with variation in temperature, the number of pulses per chirp remains unchanged (Van Wyk and Ferguson 1995). Doherty (1985) showed that in *G. bimaculatus*, temperature influences temporal properties linearly between 15 and 24°C and showed no effect at higher temperatures (24-33°C). However, syllable duration and number of syllables per chirp remained unchanged with variation in temperature. In my study, I found that the number of pulses per chirp decreased with an increase in temperature. Studies on examining the effect of temperature on calling frequency in field crickets show different findings. For instance, in *G. integer*, variation in frequency was found to be about 400 Hz when temperature increased from 18 to 30°C (Martin et al. 2000). In *P. guttiventris*, with a temperature change of 16°C, carrier frequency showed a variation of 1.5 kHz (Mhatre and Balakrishnan 2006). Walker and Cade (2003) found a shift of about 300 Hz in the carrier frequency of *T. oceanicus* song from 23 to 30°C. I also found that with an increase in temperature from 23 to 30°C, peak frequency also increases by 300 Hz in *A. asiaticus*. Hedrick et al. (2002) showed that *G. integer* chose warmer sites to call from to increase the frequency and intensity of the calling songs. However, there are other studies which show no difference in peak frequency with an increase in temperature. For instance, in *G. firmus*, Pires and Hoy (1992) showed that temperature does not affect the frequency of the song. Similarly, in *G. bimaculatus*, no effect of temperature was found on calling frequency (Doherty 1985). In *G. rubens*, Walker (1962b) found that the effect of temperature on frequency was due to individual differences.

For lab-bred animals, I also examined the variation in temporal and spectral features of calls recorded in immediate ambient temperature and in their respective developmental

temperature. I found the effect of immediate ambient temperature on lab-bred individuals as individuals from both the population (bred at 25°C and 30°C) called with higher number of syllables, chirp duration, chirp period and lower chirp rate and peak frequency at 25°C and they decreased their chirp duration, chirp period, syllable period, number of syllables and increased their chirp rate at 30°C. I also tested the effect of developmental plasticity on the calling behaviour of field cricket. My results indicate that the environment in which a nymph develops can influence male calling song. When I compared individuals bred at 30°C and 25°C and recorded at 30°C, I found that at 30°C, chirp rates and peak frequency were higher by 2 chirps and 250 Hz while chirp period was less at 30°C by 0.09s compared to calls of individuals bred at 25°C. Similarly, variation in call parameters for individuals bred at 30°C and 25°C and recorded at 25°C was observerd but only for peak frequency and chirp period. This shows the effect of developmental temperature on call features. Olvido and Mousseau (1995), in striped ground crickets (*Allonemobius fasciatus*), tested the effect of two different developmental environments with different temperature and photoperiod (31°C, 15L:9D and 24°C, 11L:13D). They found that calling parameters like chirp rate, chirp duration, inter-chirp interval, pulse number, and carrier frequency were affected by temperature, implying that the developing environment influences the calling song. In Hawaiian cricket, *Laupala cerasina*, at two different environmental temperatures (20 and 25°C) also showed the effect of rearing environment on male signalling (Grace and Shaw 2004). In *G. rubens*, the individuals from the fall season called at a faster rate and higher peak frequency than those from the spring season (Beckers et al. 2019). However, in both of these studies, the call recordings were done at only one temperature; 20 and 24°C, respectively (Grace and Shaw 2004; Beckers et al. 2019). Both our study and study by Olvido and Mousseau (1995) suggest that the developmental effect on call parameters vary

with the immediate calling environment as different developmental environments can lead to inconsistent changes in call features in different immediate calling environments.

Females are likely to adjust with such changes in a male signal due to temperature by showing 'temperature coupling' (Gerhardt 1978). There are evidences of the presence of song-temperature coupling in female crickets (Doherty 1985; Pires and Hoy 1992). Mhatre et al. (2011) reported that females of *O. henryi* show equal response to frequencies produced within the naturally occurring range of temperatures i.e. 18 to 27°C. Whether *A. asiaticus* female show temperature coupling to the difference of 300-500 Hz is still needed to be examined. Also how developmental temperature influences the temperature coupling in female is understudied (but see Grace and Shaw 2004; Beckers et al. 2019). Examining the same in *A. asiaticus* will shed more light to this phenomenon.

6.5 References

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6.6 Appendix E

Table E6.1. Comparison of different life history traits of *Acanthogryllus asiaticus* at 25°C and 30°C using t-test. Significant values are indicated in bold**.**

	Mean ± SD	Mean ± SD	t-value	df	P	N	N
(days)	25℃	30℃				25℃	30℃
Developmental time	170.5 ± 24.67	95.87 ± 27.85	10.29	52	<0.01	24	30
Adult lifespan	74.5 ± 18.36	59.07 ± 24.81	2.54	52	<0.01	24	30
Total lifespan	245 ± 18.36	154 ± 24	14.82	52	<0.01	24	30

Table E6.2. A. Average body length of nymph to adult at 20, 25 and 30°C (Mean ± SD; N = 3). B. Comparison of body morphometry of male and female bred at 25 and 30°C using t-test. Significant values are indicated in bold.

A			
	Average body length from nymph to adult		
Days	20°C	25°C	30°C
45	3.05 ± 0.69	3.00 ± 0.63	5.83 ± 2.24
65	3.32 ± 0.36	4.29 ± 0.66	8.10 ± 0.52
95	5.28 ±1.03	9.81 ± 1.59	9.16 ±1.23
120	5.00 ± 0.51	12.85 ± 2.29	16.93 ± 0.67
155	6.59 ± 0.05	16.37 ± 0.65	17.68 ± 0.39
220	12.11 ± 0.51	16.37 ± 0.65	17.68 ± 0.39
280	13.18 ± 0.82	16.37 ± 0.65	17.68 ± 0.39
315	15.69	16.37 ± 0.65	17.68 ± 0.39

B	Mean ± SD	Mean ± SD	t-value	df	P
Female	25°C	30°C			
Weight (g)	0.28 ± 0.04	0.26 ± 0.05	0.709	13	0.491
Body length (mm)	17.82 ± 1.46	14.82 ± 1.03	5.116	16	**<0.01**
Pronotum length (mm)	2.60 ± 0.15	2.74 ± 0.20	-1.669	16	0.114
Pronotum width (mm)	4.45 ± 0.17	4.57 ± 0.16	-1.485	16	0.157
Wing length (mm)	9.58 ± 0.49	8.85 ± 0.48	3.157	16	**<0.01**
Ovipositor length (mm)	5.31 ± 0.28	5.76 ± 0.20	3.981	16	**<0.01**

Male					
Weight (g)	0.21 ± 0.04	0.24±0.03	-2.292	32	**0.027**
Body length (mm)	15.43 ± 1.23	15.72 ± 0.96	-0.842	38	0.405
Pronotum length (mm)	2.45 ± 0.26	2.69 ± 0.17	-3.488	38	**<0.01**
Pronotum width (mm)	4.38 ± 0.31	4.69 ± 0.25	-3.384	38	**<0.01**
Wing length (mm)	9.06 ± 0.58	9.21 ± 0.58	-0.825	38	0.415

Table E6.3. Seasonal variation in different call properties compared using Mann-Whitney U test. Significant values are indicated in bold.

Call properties	Mean ± SD (Winter)	Mean ± SD (Summer)	U	P	N (Winter)	N (Summer)
Chirp Duration (s)	0.61 ± 0.12	0.39 ± 0.06	558.5	<0.01	120	170
Chirp Period (s)	1.45 ± 0.20	0.69 ± 0.13	14	<0.01	120	170
Syllable Duration (s)	0.022 ± 0.004	0.018 ± 0.003	178676.5	<0.01	833	1130
Syllable Period (s)	0.041 ± 0.009	0.030 ± 0.006	135524.5	<0.01	782	1047
Peak Frequency (Hz)	3683 ±194	4244 ± 171	457	<0.01	120	170
No of Syllables	14.86 ± 2.67	13.42 ±1.48	6964	<0.01	120	170
No of chirps per 10s	7.33 ± 0.98	14.76 ± 2.44	0	<0.01	12	17

Table E6.4. A. Comparison of call properties when of individuals monitored at 25℃ and recorded at different temperature (22, 24, 26, 28 and 30 ℃) during short term exposure using Kruskal-Walis ANOVA. Significant differences indicated in bold. B. Descriptive statistics for all the call features recorded at different temperature.

A	Call properties	H	df	N	P
	Chirp Duration (s)	152.856	4	362	**<0.01**
	Chirp Period (s)	111.367	4	347	**<0.01**
	Syllable Duration (s)	394.718	4	4644	**<0.01**
	Syllable Period (s)	607.075	4	4283	**<0.01**
	Peak Frequency (Hz)	76.346	4	422	**<0.01**
	No of Syllables	52.129	4	362	**<0.01**
	No of chirps per 10s	26.900	4	71	**<0.01**

B	Mean ± SD 22°C	Mean ± SD 24°C	Mean ± SD 26°C	Mean ± SD 28°C	Mean ± SD 30°C
Chirp Duration (s)	0.34 ± 0.06	0.35 ± 0.04	0.38 ± 0.03	0.31 ± 0.03	0.28 ± 0.04
Chirp Period (s)	0.95 ± 0.17	0.94 ± 0.12	0.92 ± 0.12	0.90 ± 0.13	0.73 ± 0.12
Peak Frequency (Hz)	4234 ± 126	4059 ± 262	4327 ± 163	4370 ± 221	4384 ± 216
Syllable Duration(s)	0.015 ± 0.003	0.014 ± 0.003	0.017 ± 0.005	0.015 ± 0.005	0.013 ± 0.003
Syllable Period (s)	0.027 ± 0.007	0.027 ± 0.007	0.028 ± 0.007	0.025 ± 0.007	0.022 ± 0.007
No of syllable	12.59 ± 2.14	12.93 ± 1.51	14.44 ± 1.11	13.29 ± 1.33	12.57 ± 1.47
No of chirps per 10s	10.86 ± 1.51	11.54 ± 1.20	11.27 ± 1.42	11.33 ± 0.82	14.00 ± 1.68

C. Comparison	Chirp duration (s)	Chirp period (s)	Syllable duration (s)	Syllable period (s)	Peak frequency (Hz)	No of syllables per chirps	No of chirps per 10 s
22°C vs 24°C	ns	ns	**<0.01**	**<0.01**	**<0.01**	ns	ns
22°C vs 26°C	**<0.01**	ns	**<0.01**	ns	**<0.01**	**<0.01**	ns
22°C vs 28°C	**<0.01**	ns	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns
22°C vs 30°C	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns	**<0.01**
24°C vs 26°C	**<0.01**	ns	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns
24°C vs 28°C	**<0.01**	ns	**<0.01**	**<0.01**	**<0.01**	ns	ns
24°C vs 30°C	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns	**<0.01**
26°C vs 28°C	**<0.01**	ns	**<0.01**	**<0.01**	ns	**<0.01**	ns
26°C vs 30°C	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns	**<0.01**	**<0.01**
28°C vs 30°C	**<0.01**	**<0.01**	**<0.01**	**<0.01**	ns	**<0.01**	**<0.01**

Table E6.5. Comparison of call properties of individuals bred at 25°C and 30°C and recorded at their respective temperatures using Mann-Whitney U Test. Significant differences indicated in bold.

Call properties	Mean ± SD 25°C	Mean ± SD 30°C	N (25°C)	N (30°C)	U	P
Chirp Duration (s)	0.42 ± 0.07	0.32 ± 0.05	150	180	3404	**<0.01**
Chirp Period (s)	1.01 ± 0.14	0.70 ± 0.09	150	180	506	**<0.01**
Syllable Duration (s)	0.015 ± 0.003	0.013 ± 0.003	672	777	157841.5	**<0.01**
Syllable Period (s)	0.028 ± 0.006	0.022 ± 0.006	627	725	110358.5	**<0.01**
Peak Frequency (Hz)	4685 ± 184	5003 ± 178	150	180	3144	**<0.01**
No of Syllables	15.03 ± 1.76	14.41 ± 1.94	150	180	11429.5	**0.016**
No of chirps per 10s	9.80 ± 1.15	14.05 ± 1.84	15	19	5	**<0.01**

Table E6.6. Comparison of call properties of individuals A. bred at 25°C and recorded at 25 and 30°C, B bred at 30°C and recorded at 25 and 30°C. using Paired t-test. Significant differences indicated in bold.

A Call properties	Mean ± SD Recording 25°C	Mean ± SD Recording 30°C	t	df	P
Chirp Duration (s)	0.42 ± 0.07	0.36 ± 0.05	2.375	11	**<0.01**
Chirp Period (s)	1.03 ± 0.12	0.77 ± 0.07	8.027	11	**<0.01**
Syllable Duration (s)	0.015 ± 0.001	0.015 ± 0.001	0.413	11	0.175
Syllable Period (s)	0.028 ±0.002	0.026 ± 0.002	2.82	11	**<0.01**
Peak Frequency (Hz)	4672 ± 190	4730 ± 207	-1.89	11	0.104
No of Syllables	15.13 ± 2.03	14.33 ± 1.23	1.44	11	**0.023**
No of chirps per 10s	9.67 ± 1.15	12.58 ± 1.38	-6.2359	11	**<0.01**
B Call properties	Mean ± SD Recording 30°C	Mean ± SD Recording 25°C	t	df	P
Chirp Duration (s)	0.33 ± 0.05	0.40 ± 0.06	-4.176	15	**<0.01**
Chirp Period (s)	0.70 ± 0.09	1.15 ± 0.15	-8.924	15	**<0.01**
Syllable Duration (s)	0.013 ± 0.001	0.015 ± 0.002	-4.766	15	**<0.01**

Syllable Period (s)	0.022 ± 0.001	0.029 ± 0.002	-11.249	15	<**0.01**
Peak Frequency (Hz)	4982 ± 153	4828 ± 148	5.566	15	<**0.01**
No of Syllables	14.59 ± 1.81	14.11 ± 1.95	1.088	15	0.294
No of chirps per 10s	14.25 ± 1.81	8.75 ±1.48	8.788	15	<**0.01**

Table E6.7. Comparison of call properties of individuals bred at 30°C and 25°C and recorded at A. 30°C and B. 25°C using t-test. Significant differences indicated in bold.

A Call parameters	Mean ± SD 25°C	Mean ± SD 30°C	t-value	df	P
Chirp duration (s)	0.36 ± 0.05	0.33 ± 0.05	1.855	26	0.08
Chirp period (s)	0.77 ± 0.07	0.70 ± 0.08	2.468	26	**0.02**
Number of chirps/10 s	12.58 ±1.4	14.25 ± 1.81	-2.661	26	**0.01**
Syllable duration (s)	0.015 ± 0.001	0.013 ± 0.001	3.769	26	<**0.01**
Syllable period (s)	0.026 ± 0.003	0.022 ± 0.001	4.168	26	<**0.01**
Number of syllables/chirp	14.33 ± 1.23	14.59 ± 1.81	-0.432	26	0.67
Peak frequency (Hz)	4730 ± 207	4982 ±152	-3.707	26	<**0.01**

B Call parameters	Mean ± SD 25°C	Mean ± SD 30°C	t-value	df	P
Chirp duration (s)	0.42 ± 0.07	0.40 ± 0.06	0.971	26	0.340
Chirp period (s)	1.03 ± 0.12	1.15 ± 0.15	-2.200	26	**0.037**
Number of chirps/10 s	9.67 ± 1.15	8.75 ± 1.48	1.773	26	0.088
Syllable duration (s)	0.015 ± 0.001	0.015 ± 0.001	0.012	26	0.991
Syllable period (s)	0.028 ± 0.002	0.029 ± 0.002	-1.012	26	0.321
Number of syllables/chirp	15.13 ± 2.03	14.11 ± 1.95	1.343	26	0.191
Peak frequency (Hz)	4672 ± 191	4828 ± 148	-2.425	26	**0.023**

Chapter 7

Conclusions and Future Directions

Illustration credit: Karthik T

7.1 Thesis conclusions

This study elucidates the nature and extent of alteration the external environmental conditions can cause to the biology and behaviour of a nocturnal insect. Specifically, it shows that increase in ambient temperature, light and noise significantly impact its calling behaviour, thereby providing evidence of the ecological consequences of sensory pollution. Major findings from all the five work chapters are discussed below:

7.1.1 Chapter 2: Calling activity patterns, intersexual call types and call producing structures in a field cricket, Acanthogryllus asiaticus

This chapter of the thesis serves as an introduction to the study species. In this chapter, I examined the temporal variation in calling activity of *A. asiaticus* on a diel and seasonal scale. Further, I compared the acoustic parameters of calls produced during intersexual interactions, namely, long-distance mating call (LDMC), courtship call (CC) and post-copulatory call (PCC). Finally, I assessed the relationship of sound-producing structures with body morphometry and tested whether peak frequency of LDMC was an indicator of male body size.

- My findings suggest that calling activity of *A. asiaticus* peak during summer season (March – May)
- The results also suggest that on a diel scale *A. asiaticus* partition calling activity which peak between 21:00 – 24:00 h.
- The comparative analyses of three calls produced in the context of intersexual interaction: long-distance mating call (LDMC), courtship call (CC) and post-copulatory call (PCC) indicate that these calls are structurally different from each other.

- The results of wing morphometry show that both inter-tooth distance and teeth width vary along the file length, however, number of teeth do not correlate with file length.
- I found that area of sound-producing structures, i.e. harp and mirror, are highly correlated with each other and harp area shows significant correlations with various proxies of body size.
- My findings also show that peak frequency of LDMC is significantly negatively correlated with harp area, which reflects that peak frequency can potentially be used as an indicator of male body size in this species.

7.1.2 Chapter 3: Effect of natural and artificial light at night (ALAN) on the calling behaviour of A. asiaticus

Crickets are nocturnal insects and it is expected that the absence of light plays an important role in regulating their behaviour. Increased illumination during full moon can impact their behaviour ('lunar phobia' hypothesis). In addition, the recent growth in ALAN has altered nocturnal landscape for various organisms. Therefore, in this chapter, I examined the effect of natural and artificial light on the calling behaviour of *A asiaticus*.

- My findings suggest that artificially-lit areas have 500 times higher light intensities than naturally dark areas during new moon. Such bright lighting significantly reduces cricket calling activity in artificially-lit areas compared to dark areas.

- The result of monitoring calling behaviour during different moon phases indicates that calling activity of *A. asiaticus* is not under the influence of moon phases, hence, not lending support to the 'lunar phobia' hypothesis.

- Laboratory experiments provide evidence that constant illumination (LL treatment) impacts the rhythmicity of calling behaviour.

- My results show that artificial supplementation of melatonin restores calling rhythm in cricket that show a free-running rhythm with respect to calling, indicating that melatonin might play an essential role in regulating calling behaviour in crickets.

7.1.3 Chapter 4: Spatial distribution, masking interference and acoustic interaction in males of A. asiaticus

Acoustically communicating animals experience masking interference when multiple signallers with similar call characteristics call at the same time and space. I examined the problem of conspecific acoustic masking interference in male field crickets and investigated the strategies they use to solve it.

- I found that males of *A. asiaticus* are spatially aggregated during calling and form active choruses.

- Given the natural spacing of males in the field, signallers do face the problem of acoustic masking interference in these choruses as acoustic spaces of focal males significantly overlap with masking neighbours.

- Chorus reconstruction results based on signal attenuation profiles and male spacing suggest that a calling male, on an average, has two maskers, of which one is within the hearing range of the focal calling male.

- Field and lab playback experiments elucidate that males solve the masking problem with their nearest audible neighbour by alternating their chirps.

- Acoustic analyses indicate that alternation is achieved by males by resetting various temporal features of their calls with respect to the masker in order to escape masking interference.

- My results also reveal that on an average, Effective Acoustic Overlap faced by signallers in conspecific choruses is no more than 0.17. In other words, despite the aggregated calling, overlap in time of signalling and call features, *A. asiaticus* males, on an average only have a 17% chance of being masked by another conspecific. This, is incredibly low value, keeping in mind that conspecific masking is expected to be rather severe in choruses.

- My findings also suggest that the calling louder is not a strategy used in this species in response to a masking neighbour. This is not surprising if the males which are already calling at maximum amplitude.

7.1.4 Chapter 5: Effect of traffic noise on cricket calling behaviour

Road traffic noise is one of the most pervasive forms of noise pollution and has the potential to affect acoustic communication of various species inhabiting in areas prone to traffic. I examined the effect of exposure to road traffic noise on the acoustic signals of *A. asiaticus* over long and short-term.

- My findings suggest that ambient noise profiles of four quiet and five traffic-prone areas are significantly different from each other, with traffic-prone areas being 20 dB louder than quiet areas.

- My work examining population level differences in the SPL of calls of *A. asiaticus,* show that on average, males in traffic-prone areas are 5 dB louder than those from quiet areas.

- Further, I also found that calls of males in traffic-prone areas also differ with respect to temporal features (chirp period and chirp rate) from males in quiet habitats unlike in case of spectral features.

- My findings also reveal that short-term noise exposure affects neither the calling behaviour nor the calls of individuals wherein males did not make any immediate adjustment in their signals to avoid masking from traffic noise.

7.1.5 Chapter 6: Effect of temperature on life history traits and calling behaviour of Acanthogryllus asiaticus

Crickets are ectotherms and their behaviour and biology are expected to be affected by the change in ambient temperature. In this chapter, I tried to investigate the effect of ambient temperature on life-history traits in *A. asiaticus*. I also examined how temporal and spectral features of calls vary with seasonal variation in temperature. I also tested the effect of immediate temperature on call properties. Finally, I examined the effect of developmental and immediate ambient temperature on call features of individuals that were raised in same or different temperature regimes.

- My findings suggest that higher temperature results in faster growth and development rates as adult stage was reached earlier at 30°C compared to 20°C. However, lifespan was shorter in high temperature as compared to low temperature.

- Effect of temperature on life-history traits provides evidence that 25°C to 30°C is the ideal temperature range for the growth and development of nymphs of *A. asiaticus.* The results also reveal that various proxies of body size are temperature-dependent for both males and females.

- Calls produced by the population of winter season (14°C to 19°C) are temporally and spectrally different compared to calls produced by the population of summer season (24°C to 30°C).

- My results also indicate that immediate change in ambient temperature impacts both temporal and spectral features of calls.

- My findings show that the developmental temperature and immediate temperature have an independent effect on the calls of *A. asiaticus* as differential variation in calling parameters were observed for individuals raised in different environment and exposed to different ambient immediate temperature.

7.2 Overall summary

This study reveals that altered profiles of ambient light, noise and temperature significantly impact the behaviour and biology of nocturnal ectotherm by altering its signalling behaviour and various life-history traits. My findings on the effects of environmental factors on signalling behaviour are based on both field and lab-based observations and experiments (Chapter 3, 4, 5 and 6). Results obtained in a laboratory were corroborated by those obtained in the field (Chapter 4 and 6).

My research is novel in the following ways: it is the first study on the behavioural ecology of the species, *A. asiaticus.* It provides the first description of three different intersexual call types of *A. asiaticus*. This is the first study which gives a quantitative description of post-copulatory calls in a field cricket. It also demonstrates, for the first time, that artificial light disrupts calling activity in an insect, both in the field and lab conditions. In addition, it provides evidence that traffic noise has an effect on male calling behaviour at the

population level. Furthermore, it indicates the impact of developmental and immediate ambient temperature on the signals of this species.

7.3 Future directions

While this study contributes to the understanding of the effects of changing environment on the biology and behaviour of animals, it also opens avenues for future studies to assess ecological and potential evolutionary consequences of sensory pollution on organisms.

There are several important directions for future research which are listed as follows:

- Long-distance mating call, courtship call and post-copulatory call are acoustically different in this species but whether they provide reliable information about male quality such as body size, to females is yet to be tested. We also know that peak frequency can potentially act as an indicator of the male body size, but whether females utilize this information in mate choice decisions needs to be investigated.

- We currently know that in *A. asiaticus*, calling activity is affected by ALAN. Whether this translates to consequences on mating and reproductive success needs to be explicitly tested. Insect decline in relation to the pervasive problem of ALAN has been reported globally for various species of fireflies. While ALAN disrupts communication in fireflies because they themselves use light for communication, the absence of ambient light is essential for all nocturnal and even diurnal animals. Whether other nocturnal insects, such as crickets, katydids and moths are also declining due to the rapid spread of ALAN needs to be examined. Appropriate actions on the urban planning and design and use of night light need to be taken urgently.

- I found that melatonin is likely to play a role in regulating the circadian rhythm of calling activity. However, it is imperative to investigate how internal melatonin levels in crickets vary over day and night and in artificially-lit and dark areas. It is likely that not just melatonin but an entire host of metabolites are affected by ALAN and other sensory pollutants. It is worth examining the nature and extent of such proximate changes.

- Given that males of *A. asiaticus* form active choruses with significant overlap of signalling space with neighbouring conspecific males, this makes mate-sampling by females an ecological possibility. Thus, it will be interesting to examine the same and if it exists, one could test what mate sampling strategy is used by females of this species. For this, it will be useful to investigate the female spacing in this species in relation to calling males to explore the potential for mate sampling.

- Another future direction would be to examine female strategies to deal with masking interference in noisy choruses of multiple signalling males. This can be done at the mechanistic, neural and/or behavioural level.

- Anthropogenic noise affects signalling in crickets. Measuring their metabolic stress in such a noisy environment will reveal the impact on their physiology. Investigating how chronic traffic noise exposure during development affects calling behaviour can be another interesting line of investigation. It will be interesting to understand how mating, reproductive success and aggressive behaviour are affected in noise prone areas. It will be worthwhile to study noise-dependent hearing loss or differences in males and females of this species in populations exposed to chronic noise.

- Rearing temperature affects male signalling in this species, but if females exhibit temperature-coupling phenomenon in this species is still need to be tested. In addition, how rearing temperature affects other behaviours such as mating and aggression in field crickets is also need to be examined.

List of Awards

Conference Awards

Third place in 3MT (3-minute thesis) competition - Behaviour 2019, University of Illinois, Chicago.

Best Oral Presentation Award - Talks get hotter: Effect of temperature on the song parameters of the field cricket, *Acanthogryllus asiaticus.* National conference on Behavioural Ecology, Gujarat, 2017.

Best Oral Presentation Award - Spacing pattern and acoustic interactions in a field cricket species. Young Ecologists Talk and Interact, Assam, 2017.

Best Poster Presentation Award - Acoustic monitoring of ensiferan diversity of India. Conference on Insect Biodiversity Studies, Kerala, 2016

Best Poster Presentation Award - Acoustic communication and male spacing in field cricket species from north-western India, National Symposium on Behavioural Ecology, Varanasi, 2019

Travel grants

IBAC Travel grant award, University of Sussex. UK, 2019	550 GBP
ASAB Diversity travel grant award, University of Konstanz, Germany, 2019	1500 Euro
ASAB Conference attendance grant, 2019	500 GBP
ABS Diversity travel grant Behaviour-2019, University of Illinois, Chicago, 2019	115 $
DST International travel grant, Government of India, 2019	1.04 lac

Media coverage

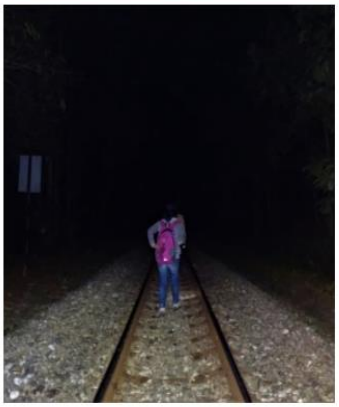
More to cricket than sleep spoiler, thrillers, The Times of India, Chandigarh, Feb 21, 2020.

On wings of night song, Hindustan Times, Chandigarh and Ludhiana, March 15, 2020.









The poetry of earth is ceasing never:
On a lone winter evening, when the frost
Has wrought a silence, from the stove there shrills
The Cricket's song, in warmth increasing ever,
And seems to one in drowsiness half lost,
The Grasshopper's among some grassy hills.
- John Keats (On the Grasshopper and Cricket)