Examining calling behavior and the effects of age and social environment on intrasexual aggression in male *Acanthogryllus asiaticus*

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Certificate of Examination

This is to certify that the dissertation titled "Examining calling behavior and the effects of age and social environment on intrasexual aggression in male *Acanthogryllus asiaticus*" submitted by Ms. Lata Kalra (Reg. No. MS12092) for the partial fulfilment of BS-MS dual degree program of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. N.G. Prasad   Prof. Somdatta Sinha   Dr. Manjari Jain

(Thesis Supervisor)

Dated: April 21, 2017

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Manjari Jain at the 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 bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Lata Kalra

Dated: April 21, 2017

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

Dr. Manjari Jain

(Thesis Supervisor)

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Acronyms

LDMC: Long Distance Mating Call

VC: Victory Call

AC: Aggressive Call

RHP: Resource Holding Potential

SPL: Sound Pressure Level

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Chapter 1: General introduction on agonistic behaviour in animals

1.1 Intermale competition and the factors governing it

Individuals across a diverse range of species often compete for various reasons like food and territory (basic survival, governed by natural selection) and mate acquisition (reproductive advantage, governed by sexual selection) leading to prevalent exhibition of agonistic behaviours. Agonistic behaviour, as defined by Scott and Fredericson (1951), is a group of behavioral adjustments associated with fighting, which includes attack, escape, threat, defense and appeasement. Agonistic behaviour can be manifested either at intraspecific or interspecific levels (Lorenz 1966). At the interspecific level, agonistic behaviour is manifested in two forms- predatory aggression, the hunting behaviour of predator directed towards a prey which has been extensively observed and studied in systems like cats and mice (Adamec et al. 1980; Knutson & Hynan 1973) and anti-predator aggression which is exhibited as an attack from a prey to a predator as in the case of several grasshoppers that release toxic liquids on being threatened (Hingston 1927) , blood squirting by texas horned lizard (Middendorf et al. 1992) and mobbing behaviour in red colobus monkeys (Stanford 1995). At intraspecific level too agonistic behaviour can operate in different ways depending on the nature of interaction which could be male-male, male-female or female-female. Sexual coercion is a prevalent form of male-female aggression where males coerce female to mate with them (Parker & Clutton-Brock 1995) and is observed in diverse range of species such as wild orangutans (Mitani 1985), waterfowls (McKinney et al. 1983), white fronted bee eater (Emlen & Wrege 1986) and invertebrates such as water strider and panorpa scorpion flies (Thornhill 1980; Arnqvist 1989). Female-female aggression too can arise out of competition over food as in case of female collared lizard (Baird & Sloan 2003), grey seals (Boness et al. 1982) and soay sheep (Robinson & Kruuk 2007) and over territory as in case of common chimpanzee (Kahlenberg et al. 2008).

Among all the classes of intrasexual aggression, male-male aggression is most prevalent and widely observed. Different species may be under the influence of different selective forces that shape agonistic behaviours among conspecific males but irrespective of the operating selective forces the agonistic behaviours and outcomes of contests often follow similar patterns in terms of various displays and information content. In many species such contests are often limited to ritualized displays and threats without actually escalating to physical interaction and even when the contests involve physical they remain devoid of any serious injury or loss. Ritualized displays act as modes of mutual assessment for rival males whereby accessing the information conveyed through displays facilitates in preventing any serious injury or death due to involvement in an escalated fight with a more dominant male (Tinbergen 1951; Smith and Parker 1976; Smith and Price 1973). A wide range of acoustic and gestural displays have been observed in animals as a means to intimidate the opponent and exhibit physical strength like nest guarding behaviour in grey catbirds (Slack 1976), roaring contests in red deer (Brock and Albon 1979), shell rapping in hermit crabs (Mowles et al. 2010) and claw waving in male fiddler crabs (Morrell et al. 2005). On the other hand in many animals, extensive physical interactions form a part of ritualised fights in organisms like Bighorn sheep, Elks (Miller 2013), Black mambas (Fogden 2000) and Crickets (Alexander 1961). Several studies have dwelled on the functional and adaptive significance of these ‘limited war’ strategies (Smith and Price 1973). Different arguments suggest that the rarity of dangerous weapons among species is because of selective mechanisms such as group selection (Huxley 1956) and kin selection (Hamilton 1971). On the other hand many game theoretical models propose a frequency dependent selection mechanism acting on individual level in which conflict can be visualised as part of a game played against the other (Hamilton 1967) where the individuals can chose among a variety of strategies including displays, violent attack and retreat based on the fitness payoff of each strategy while continuously assessing the information from the strategy adopted by rival in order to maximise its own gains (Smith and Price 1974). In such contests, given a choice between ritualised or escalated fighting, ritualised fighting emerges as an evolutionarily stable strategy (Smith and Price 1973).

1.2 Outcomes of aggressive contests and role of different determining factors

Most contests are resolved with the emergence of a winner and a loser where the winner gets access to the resource of interest while the losers have to find other opportunities. Several intrinsic and extrinsic factors dictate the outcome of an aggressive contest (Landau 1951a, b). Intrinsic factors contribute to the inherent ability of an individual to win a fight and are collectively known as the Resource Holding Potential (RHP) or fighting ability (Parker 1974; Smith 1982). Extrinsic factors consist of external conditions or experiences that might influence the individual's ability to win a fight.

1) Intrinsic factors: Physical attributes like body size, weight, age and weaponry have been widely documented to contribute to the RHP of the individuals (Houpt et al. 1978; Knights 1987; Lott and Galland 1987) where it has been demonstrated that bigger and heavier individuals are dominant over smaller and lighter individuals. Besides size and weight, other features like bill size in birds (Shaw 1986) and genital papilla in fishes (Schwanck 1980) have also been found to be the predictors of dominance. Physiological traits like hormonal levels (Overli et al. 2004; Schjolden et al. 2005) and metabolic rates (Metcalfe et al. 1995; McCarthy 2001) have also been recognized as RHP traits. Some studies contradict this theory by claiming that it is the outcome of dominance conflicts that determine the hormonal states of the individuals and not the other way round (Eaton and Resko 1974; Sapolsky 1982; Trainor and Hofmann 2007). Some behavioural states like Resource Value (RV) or motivation (Enquist 1985; Barlow et al. 1986), described as the value of winning the resource for an individual and boldness or aggressiveness (Barlow et al. 1986; Smith and Harper 1988) also act as determinants of dominance in some species (Smith 1982; Sundstrom et al. 2004).
2) Extrinsic factors: These include the influence of the experience gained through either indulging in a fight in the past or being witness to a fight between individuals, one of who would be the focal male's subsequent rival. The first has been addressed as winner-loser effect while the latter is known as the bystander effect. In winner-loser effects, prior experience of winning or losing a contest increases the chances of winning or losing the subsequent contest. Studies in some organisms have meticulously documented the presence of winner (Bergman et al. 2003; Chase et al. 1994) as well as loser effects (Chase et al. 1994; Hsu et al. 2006). On the other hand, these effects are evidently non-existent in some species (Rutte et al. 2006).

Bystander effect is the manipulation of aggressive behaviour by an individual upon encounter with a rival it has already observed participating in another contest to which the individual was an audience. Bystander effects have been documented in species of fishes (Oliveira et al. 1998; Peake and McGregor 2004).

1.3 Role of social environment on aggressive displays

A simplistic communication scheme can be visualised as a dyadic system consisting of a signaller and a receiver. Animal communication systems in nature quite often have added complexities pertaining to the existing networks between individuals of the group. As a consequence, a signalling event often consists of added non intended receivers apart from the primary intended receivers called the 'bystanders' or 'audience'. Such a scenario is accompanied with fitness implications for both the signaller as well as the audience. The audience may employ the signal to extract information about the signaller and use this assessment on subsequent encounters with the signaller as in case of eavesdropping (Peake 2005). Both males and females act as audience to male-male interactions in nature for mate choice and assessment of rivals for subsequent agonistic interactions respectively (Oliveira et al. 1998; McGregor et al. 2001; Peake et al. 2001; Doutrelant & McGregor, 2000). On the other hand, the signaller may alter its signalling to manipulate the information broadcasted to the audience in order to maximize its own fitness (McGregor & Peake 2000) as in the case of 'audience effect' (Matos and Schlupp 2005). Females often act as an audience to aggressive and parental care events among males as a means to access the male quality and have been documented well in species like birds, fishes and primates (Doutrelant et al 2001; Matos & McGregor 2002; Hector et al. 1989; Baltz & Clark 1994). There is a dearth of studies examining the presence of audience effect among lower organism with relatively simple nervous system like insects. Some studies on crickets also have focused on investigating if the presence of an audience elicits any change in the aggressive displays of males engaged in agonistic interaction (Fitzsimmons and Bertram 2013, Montroy et al. 2016; Tachon et al. 1999; Judge et al. 2010) but if the lack of uniformity in the methodology of these experiments and contradictory results make the existence of audience effect in insects debatable.

1.4 The honesty of aggressive displays and information assessment during fights

For ritualized fights and displays to serve as modes of assessment of rival male's strength, they must involve a great deal of honest signalling and a way for the individuals to perceive the information displayed by the contender correctly. The issue of honest signalling poses a paradox here (Bond 1989)- If indeed displays are true indicators of individual motivation, shouldn't selection act against the display of a motivational level that is lower than the rival as certainly advertising a lower aggression in a game where the most aggressive one ultimately gets access to resources is not adaptive. In fact studies show that selection acts against the tendency of displays to manifest motivation information (Smith and Price 1974; Dawkins and Krebs 1978; Caryl 1979). If the reverse is true- aggressive displays are deceptive then they should've been evolved as a "single action pattern consistently produced at maximum intensity" (Smith 1984) but this is contradicted by the fact that aggressive displays exist as graded signals and showcase a lot of diversity as found in studies on dogs and geese (Lorenz 1966), stellar's jay (Brown 1964), lorikeets (Serpell 1984) and crickets (Alexander 1961). An alternate solution to this paradox can be achieved by considering displays as modes to achieve aggression rather than advertising it. In this scenario, displays are merely by-products of achieving aggressive motivational states and hence survive an opposition by selection because not performing aggression is more costly than the decision to not display it (Bond 1989).

Keeping the debate aside, even if aggressive displays do convey information about motivational levels the subsequent puzzle is how do individuals derive this information? Three models provide possible assessment rules for rival males during contests.

1) Sequential assessment model (Enquist and Leimar 1983) proposes that aggressive displays occur in distinct phases sequentially increasing in intensity or associated cost as well as reliability of information about the fighting ability. The phases of low intensity are bad indicators of fighting ability while the ones that are higher in intensity are more reliable. The thumb-rule is to assess yourself as well as the rival (mutual assessment) and give up when you realise the other is stronger. Hence in cases where a remarkable asymmetry exist between rival males' fighting ability, contests are settled at early stage where the displays needn't be highly reliable but as this asymmetry difference narrows down, the contest escalates to higher intensity displays that could provide more accurate information or assessment of fighting ability to resolve the conflict. Several studies have been done to check the validity of this model (Enquist & Jakobsson 1986; Englund & Olsson 1990; Enquist et al. 1990; Bridge et al. 2000).

2) Energetic war of attrition model (Mesterton-Gibbons et al. 1996; Payne & Pagel 1996) proposes a self-assessment strategy where the contest is primarily a game of endurance and the costs of display include time and energy expenditure. The thumb-rule here is to give up as soon as one's inner threshold for energy and time costs is crossed. This model has been tested for in dung flies (Parker and Thompson 1980).

3) Cumulative assessment model (Payne 1998) also proposes the assessment thumb-rule to give up as soon as an individual's threshold is reached but here the costs also include physical or physiological damage or injury inflicted by the rival during contest besides the energy and time expenditure.

1.5 Summary of literature review

1) Aggressive contest, analogous to a two player game, although comprise of a variety of aggressive displays and physical interactions leading to the evolution of male weaponry but mostly remain devoid of any serious injury or death.
2) Aggressive displays often are informative of an individual's fighting ability or RHP and aid in assessing the rival male.
3) Several intrinsic (internal physical, physiological and behavioural attributes) and extrinsic (prior experiences) factors act as determinants of dominance in conflicts and thus an asymmetry between rival males in the context of these factors can potentially predict the outcome of contest.
4) Aggressive signalling is often influenced by the social environment of the signaller whereby the presence of a non-intended receiver or audience my cause certain alterations in its signalling.

1.6 Crickets as a model system for studying aggression

1.6.1 Introduction to crickets:

Crickets are nocturnal insects that predominantly communicate by means of acoustic communication. They produce sound by the mechanism of stridulation that involves rubbing of wings together. A plectrum on the left forewing is swept across a row of files or teeth on the right wing (Sales and Pye 1974). As the plectrum progresses through each tooth a wave of signal is produced which is amplified by the surrounding veins that resonate at the same frequency as that of plectrum-tooth impact (Pierce 1948). The main amplifying structure is harp, a triangular area of wing cells (Bennet-Clark 1970; Nocke 1971;

Michelsen and Nocke 1974). Although both the wings have file as well as plectrum, since the right wing lies over the left one, only the files on the ventral surface of right wing can be struck against the plectrum of left wing and hence functionally the wings are asymmetric (Forrest, 1987).

Pertaining to the nature of interaction different signals have been categorised in crickets:

1) Long Distance Mating Calls (Wagner 1995) – This song is produced by males in order to attract females who respond by phonotaxis. Female can chose mate either at the level of LDMC (Doherty & Hoy 1985) or at the stage of courtship (Brown & Gwynne, 1997).
2) Courtship call (Alexander 1962) – When the female is in vicinity of the male, it switches from the LDMC to produce a softer sound called the courtship call. These calls are considered essential for successful copulation as females have been observed to mate only with the males capable of producing courtship songs (Crankshaw 1979; Balakrishnan & Pollack 1996; Nelson & Nolen 1997; Boake 1983)
3) Post copulatory calls (Alexander 1962) – Produced by males after copulation.
4) Aggressive song (Alexander 1961) - Produced by males both during and after the conflict (also known as victory call in this case) that elicits agonistic behaviour and calling or retreat in the rival male.

1.6.2 Acoustic characteristics of cricket call:

The call of cricket can perceived as a collection of sound pulses, each pulse known as a chirp. Further a chirp is composed of several syllables. A structural characterization of the call can be achieved using different parameters.

Temporal parameters

i) Chirp duration: onset of one chirp to its offset.

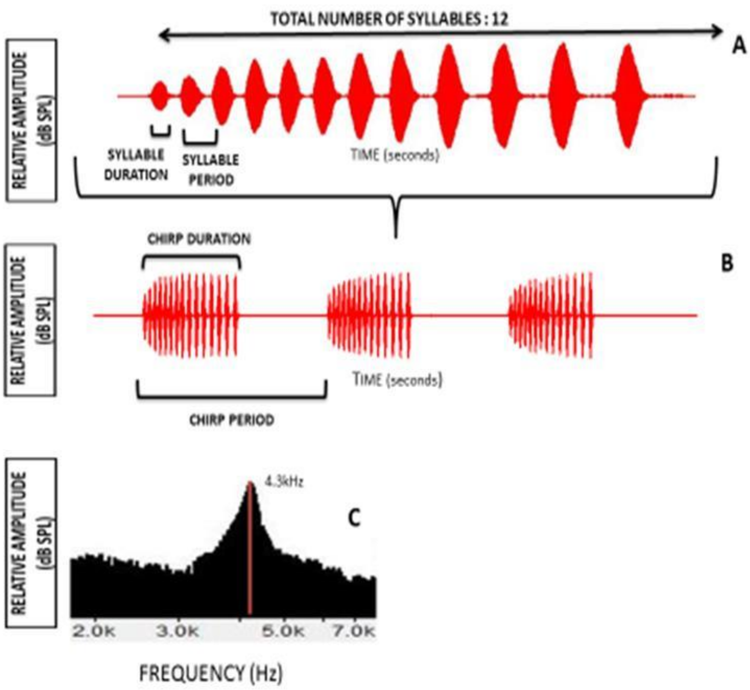

Figure 1.1. Acoustic characterization of a cricket's Long distance mating call – A and B (temporal features) and C (Spectral features).

ii) Chirp period: onset of one chirp to the onset of the subsequent chirp.

iii) Syllable duration: onset of one Syllable to its offset.

iv) Syllable period: onset of one Syllable to the onset of the subsequent Syllable.

v) Number of syllable per chirp

1) Spectral parameters: Includes peak frequency which is the frequency produced with maximum amplitude.
2) SPL (Sound pressure level): It is the loudness of the call measured in dB.

1.6.3 Crickets as model system of aggression

Conflict over food, territory and mates lead to frequent aggressive encounters among male crickets (Alexander 1961). This aggressive behaviour is not only highly prevalent across different cricket species but also has some remarkably interesting characteristics that make them especially suitable candidates for studying aggression-

1) Agonistic contests in crickets consist of a wide range of physical (Adamo and Hoy 1995; Hoffman and Schildberger 2001) as well as acoustic (Jang et al. 2008; Logue et al. 2010) interactions arranged in a highly stereotypic sequence where each behaviour escalates to a subsequent behaviour both higher in aggression intensity as well as energy expenditure (Alexander 1961; Hack 1977b) than itself. Contests are initiated by antennal contact or fencing that serves as a checkpoint for the decision to fight or flee (Katagiri 2008) and progress to higher intensity behaviours or aggression levels like mandible fencing and grappling (Alexander 1961; Hoffman and Schildberger 2001). The fight can terminate at any point (Alexander 1961) determining the

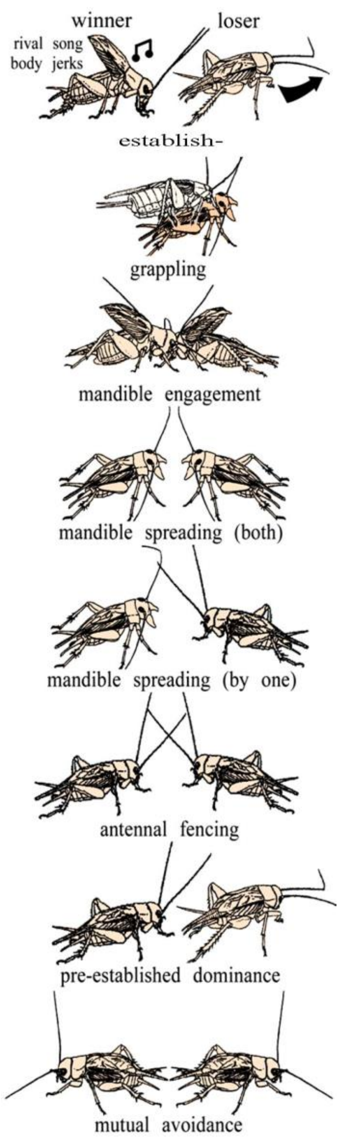

Figure 1.2: A schematic of rival male crickets engaged in an agonistic fight (Hofmann & Schildberger 2001)

maximal aggression level of that contest.

2) The aggressive behaviours are discrete, stereotypic and uniform across different cricket species making their categorization and scoring easy (Bertram et al. 2011).
3) The contests are resolved with the emergence of a clear winner and loser where the winner is characterized by rapid body jerks or rocking and the production of a characteristic 'victory song' (Hoffman and Schildberger 2001) while the loser retreats and refrains from initiating any aggressive interaction for some time (Hoffman and Stevenson 2000).

1.7 Objectives

In the light of the above discussion, I investigated different aspects of intrasexual aggression through the scope of four objectives:

1) Quantification and comparative analysis of aggressive call with victory and long distance mating call.
2) Examining the acoustic differences between the mating calls of winners and losers.
3) Investigating the role of age in determining the outcome of intermale agonistic contests.
4) Investigating the presence of 'audience effect' in intermale agonistic contests.

1.8 Study species

To approach the above research questions, I used *Acanthogryllus asiaticus*, a field cricket species. Some morphological characteristics of *A. asiaticus* (Gorochov 1990) are:

"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 ectoparameres."

A taxonomic characterization of *A. asiaticus* is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insects

Order: Orthoptera

Suborder: Ensifera

Superfamily: Grylloidea

Family: Gryllidae

Genus: Acanthogryllus

Species: *Acanthogryllus asiaticus*

Figure 1.3 *Acanthogryllus asiaticus*, on left- a stridulating male, to the right- a female (with a distinguishable ovipositor)

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Chapter 2: Quantification and comparative analysis of aggressive, victory and long distance mating calls

2.1 Background

Agonistic calls in crickets have received relatively less attention where some studies have focused on information content of 'pre-fight' agonistic calls (Brown 2006) and qualitative comparison between calling and courtship songs in house crickets (Nelson and Nolen 1997) and *Teleogryllus* (Balakrishnan and Pollack 1995) but no study so far has quantified the agonistic calls produced during the physical combat and compared it with post conflict calls and long distance calls. I hypothesize that due to differences in behavioural contexts, aggressive calls (the calls produced during the physical combat), victory and long distance calls would differ from each other. My first objective in this study is to describe the structure of aggressive calls and elucidate the differences between the three contextual calls of the species.

2.2 Methodology:

2.2.1 Collection and housing of crickets:

Adult and sub adult crickets were collected from IISER Mohali campus from August-November 2016 and kept in separate plastic box (ensuring no interaction with other males) with dog food and wet cotton balls for food and nourishment in an incubator (temp 24^0 C and humidity 40%). The adults were kept in lab conditions up to at least two days post their capture before using them for further experiment. The sub adults were kept until their eclosed into adults and the eclosion date was used as a reference point for calculating their age.

2.2.2 Acoustic recordings:

The recordings and SPL for aggressive calls (both during and post conflict) were acquired from the audio recording acquired during staging agonistic contests between rival males. For this, a hand held recorder TASCAM DR-07 MKII (TEAC Corp, US) fixed on tripod was used. To record the sound pressure level of different calls I used a Bruel and Kjaer handheld analyser type 2270 with microphone type 4189 (LAF values) which was placed at a distance of 50 cm from the base of the arena. For Long distance calls we recorded the calling male's calls and SPL in lab using the same protocol as aggressive call.

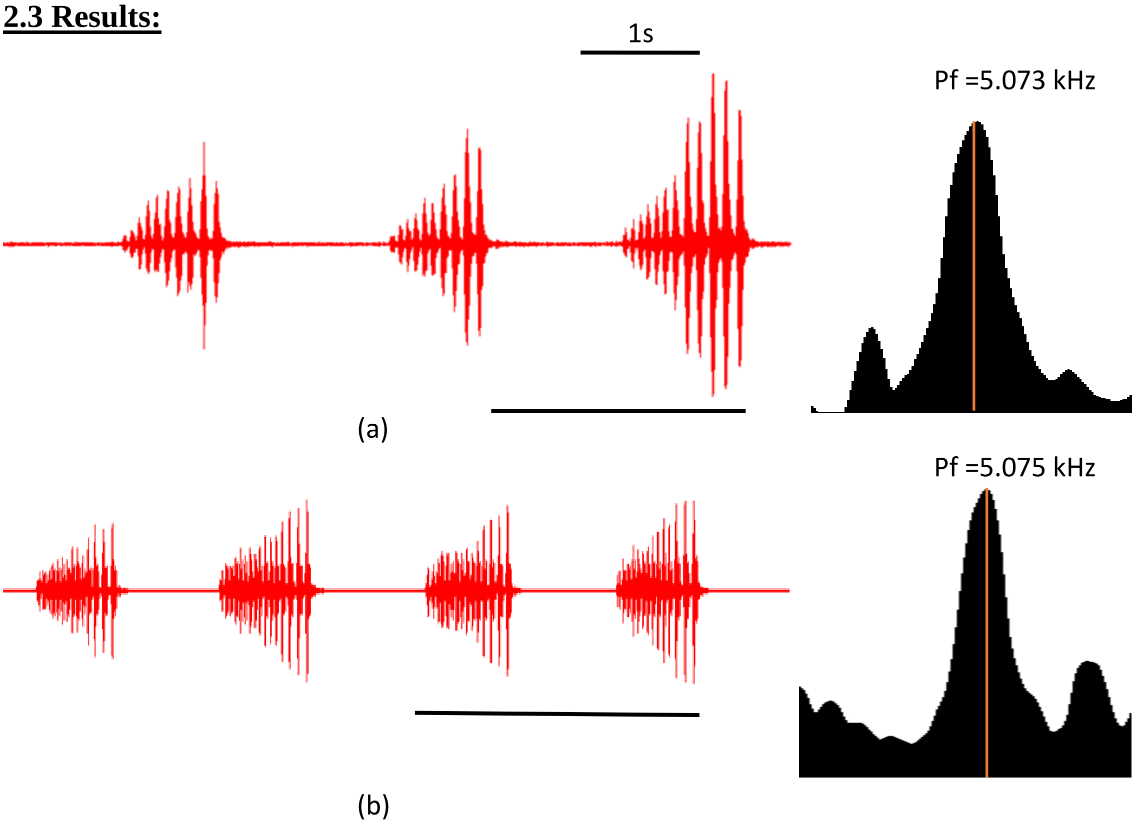
2.2.3 Data analysis:

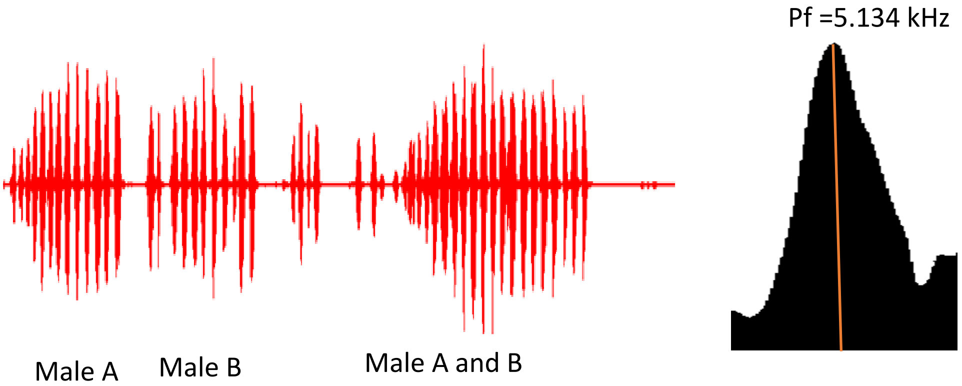
All the audio recordings were analysed (high pass filter: > 2000Hz) in Audacity 2.1.2 and Raven pro 1.4 for analysis of different spectral and temporal parameters of the calls. The parameters that we employed for the quantification and comparative analysis of the calls were peak frequency, chirp duration, chirp period, syllable duration, syllable period and number of syllable per chirp.

2.2.4 Statistical analysis:

For each analysis normality tests (Shapero-wilk tests and Q-Q plots) and subsequent analysis were performed in Statistica version 12.

2.3 Results:



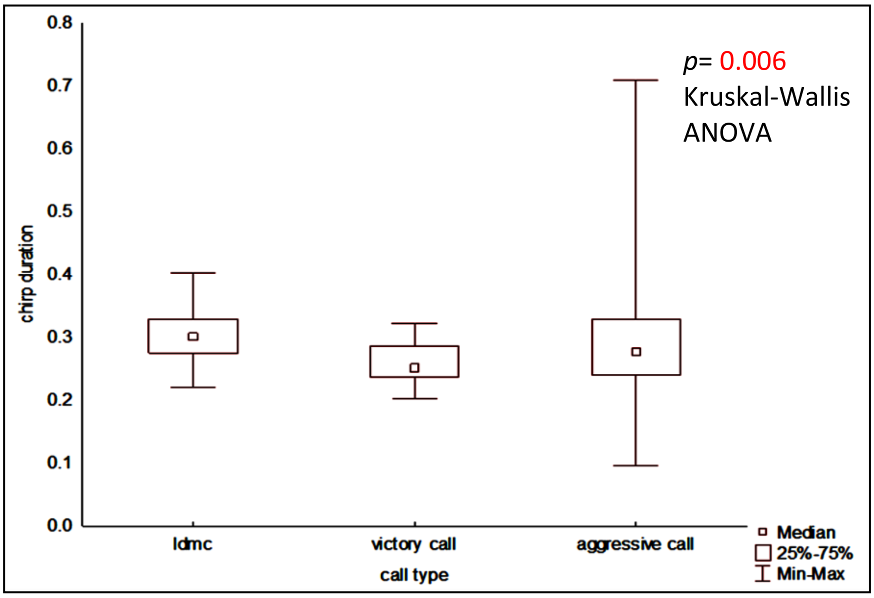


(c)

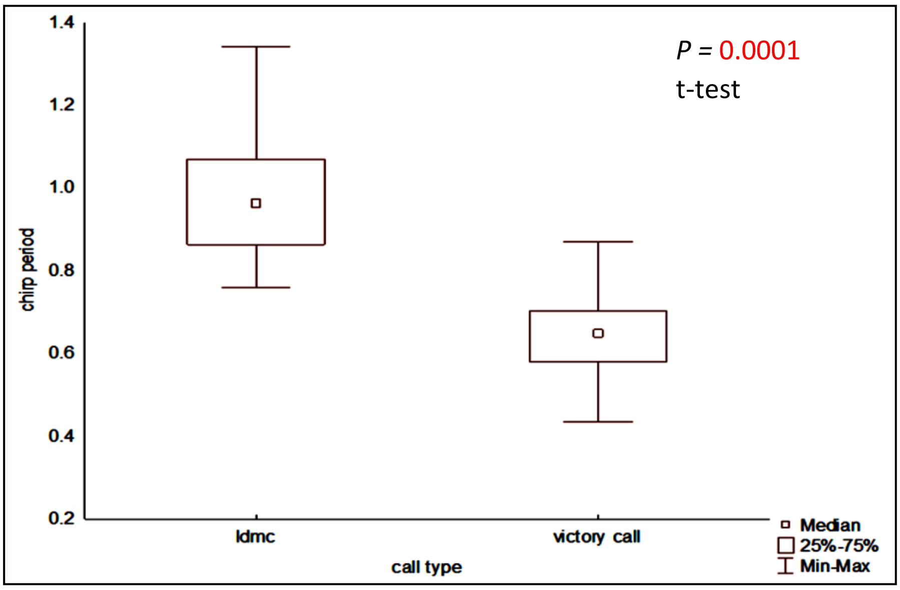
Figure 2.1. Oscillogram and power spectra of different cricket calls a) LDMC b) Victory call c) Aggressive call of two males engaged in agonistic contest. Oscillogram: x axis-time(s) and y-axis-amplitude (dB); Power spectrum: x-axis-frequency (Hz) and y-axis-amplitude (dB)

	Acoustic parameter	LDMC (n=30)	VC (n=20)	AC (n=18)	p value
1	Chirp duration (s)	0.30±0.04	0.26±0.03	0.31±0.13	0.006
2	Chirp period (s)	0.98±0.15	0.65±0.1	—	0.0001
3	Peak frequency (KHz)	5.073±0.31	5.075±0.23	5.134±0.29	0.74
4	No. of syllable/chirp	12.83±1.31	10.46±1.22	10.64±3.81	0.0004
5	Syllable duration (s)	0.013±0.001	0.013±0.001	0.014±0.002	0.052
6	Syllable period (s)	0.02±0.002	0.03±0.003	0.03±0.004	0.0001
7	SPL (dB)	69.66±4.82	71.2±6.32	71.9±5.48	0.0002

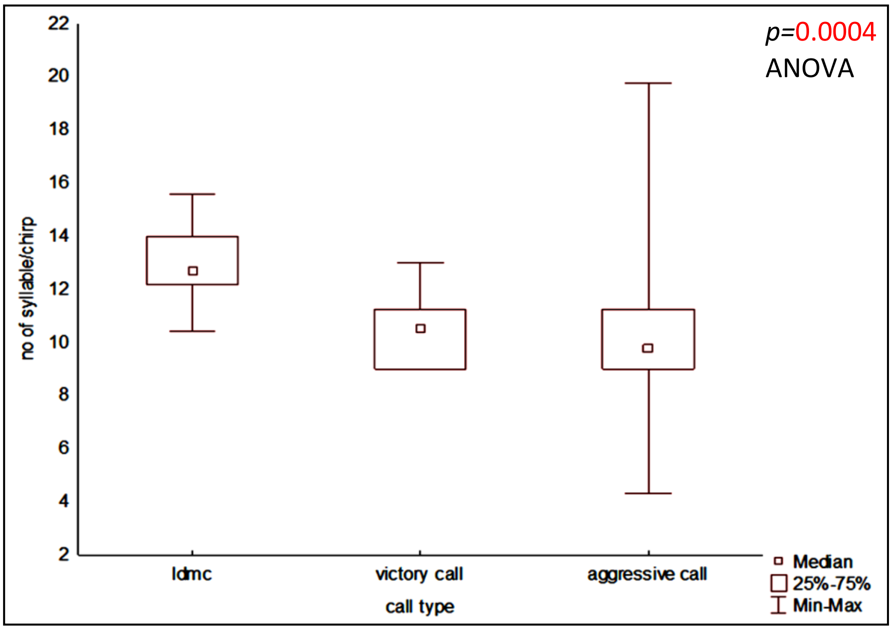
Table 2.1 Comparative analysis of LDMC, VC and AC for different temporal and spectral features


p= 0.006
Kruskal-Wallis
ANOVA
chirp duration
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ldmc
victory call
aggressive call
call type
Median
25%-75%
Min-Max

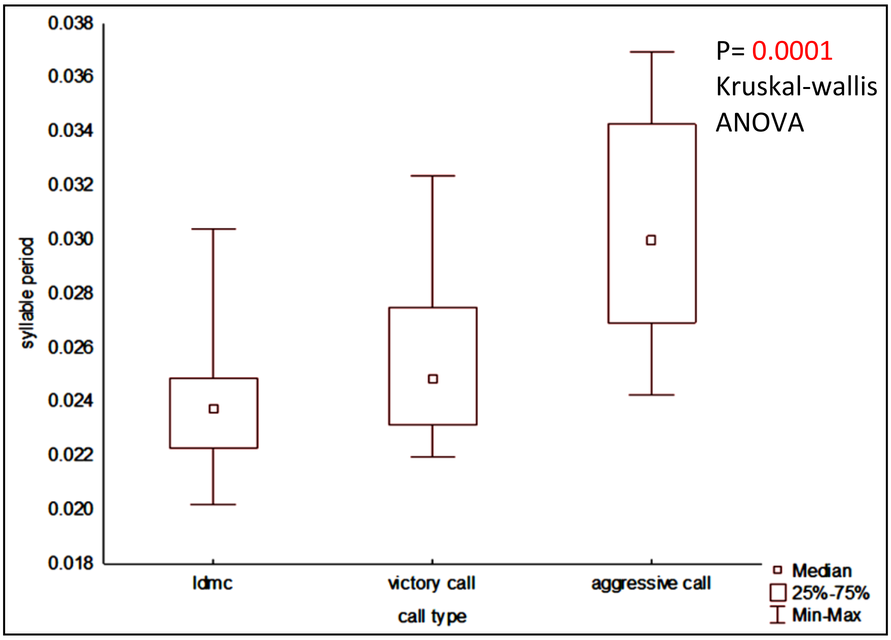
(a)


P = 0.0001
t-test
chirp period
1.4
1.2
1.0
0.8
0.6
0.4
0.2
ldmc
victory call
call type
Median
25%-75%
Min-Max

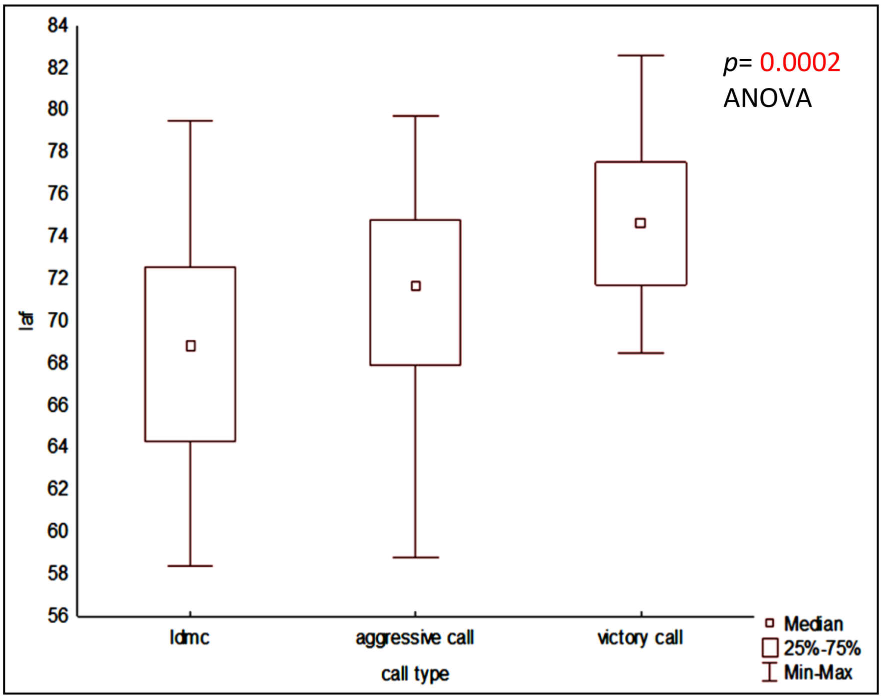
(b)


p=0.0004
ANOVA
no of syllable/chirp
22
20
18
16
14
12
10
8
6
4
2
ldmc
victory call
aggressive call
call type
Median
25%-75%
Min-Max

(c)


P= 0.0001
Kruskal-wallis
ANOVA
syllable period
0.038
0.036
0.034
0.032
0.030
0.028
0.026
0.024
0.022
0.020
0.018
ldmc
victory call
aggressive call
call type
Median
25%-75%
Min-Max

(d)



(e)

Fig 2.2: Box plots - a) Chirp duration versus call type, b) chirp period versus call type c) number of syllable/chirp versus call type d) syllable period versus call type e) SPL versus call type

2.4 Conclusion:

The three call types differ significantly for chirp duration, chirp period, number of syllables per chirp, syllable period and SPL.

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Chapter3: Examining acoustic differences between the mating calls of winners and losers

3.1 Background

Acoustic signals have been shown to contain information about the condition of signaller like its sex (Pfefferle et al. 2007), size (Vannoni and McElligot 2008), age and social rank (Fischer et al. 2003) and may serve as reliable indicators of male quality if their production is more costly for the individuals in poor condition compared to the ones in good condition (Zahavi 1975). Studies have been done in amphibians, birds and mammals investigating the information content of acoustic signals (Pfefferle et al. 2007; Davies and Halliday 1978; Clutton-Brock and Albon 1979; Templeton et al. 2005). Among crickets acoustic signal traits have been shown to be linked with immune system function (Fedorka and Mousseau 2006), male size and dominance in aggressive contests (Brown et al. 2006; Simmons 1988) providing evidence for the fact that some intraspecies variations in acoustic signals might be subject to selection in male-male competition as well as female choice (Simmons 1988; Hedrick 1986; Nandi and Balakrishnan 2013). To address these questions, in my second objective, I aim to investigate whether the information about the dominance of a male in an aggressive contest can be deciphered from the acoustic signals by comparing long distance calls, which are used to attract females, across a set of winners and losers of agonistic contests.

3.2 Methodology

3.2.1 Acoustic recordings:

To record the Long distance mating calls of individuals the same approach was followed as in chapter 2 methodology section.

3.2.2 Data analysis:

Five chirps were analysed per LDMC for different acoustic parameters. Average values for each LDMC were then analysed further.

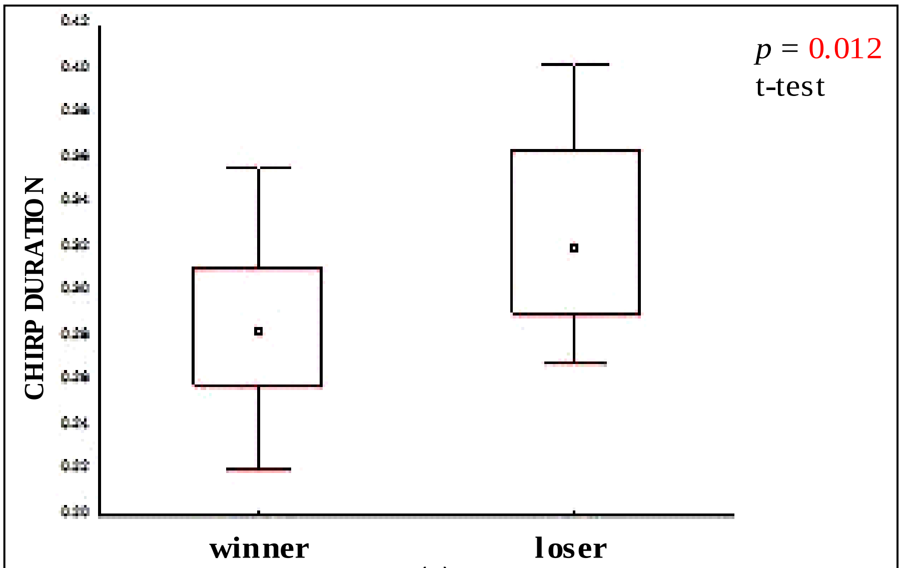
3.2.3 Statistical analysis:

For each analysis normality tests (Shapero-wilk tests and Q-Q plots) and subsequent analysis were performed in Statistica version 12.

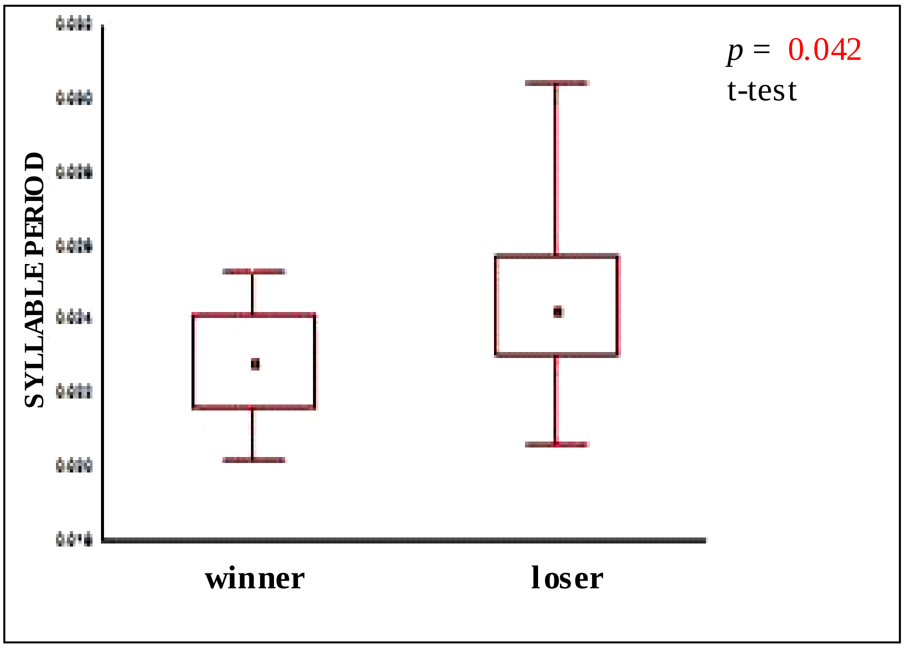
3.3 Results:

	Acoustic parameter	winner (n=15)	Loser (n=15)	p value
1	Chirp duration (s)	0.29±0.04	0.32±0.04	0.012
2	Chirp period (s)	0.96±0.16	1±0.14	0.49
3	Peak frequency (KHz)	5.08±0.26	5.06±0.37	0.88
4	Syllable duration (s)	0.0128±0.001	0.0127±0.0012	0.87
5	Syllable period (s)	0.023±0.0016	0.025±0.0023	0.042
6	No. of syllable/chirp	12.57±1.52	13.57±1.59	0.09
7	SPL for LDMC call (dB)*	70.54±4.26	67.09±5.28	0.03
	* n = 19			

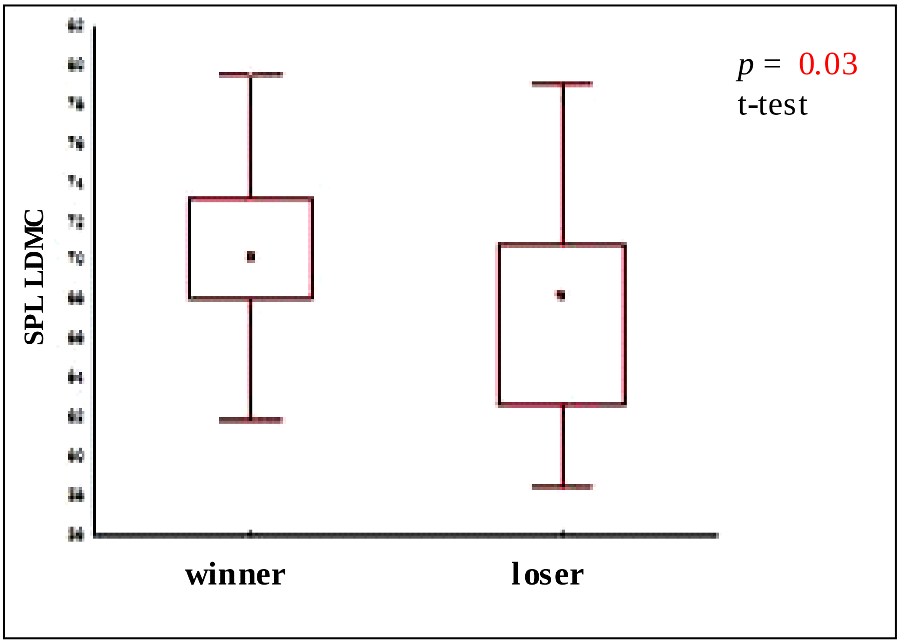
Table 3.1 Comparison of LDMC across winners and losers for different temporal and spectral features



(a)



(b)



(c)

Fig 3.1 Box plots for significant differences (Median □ 25%-75% ⊤ Min-Max)

a) Chirp duration versus male type b) syllable period versus male type c) SPL versus male type.

3.4 Conclusions

The results indicate that the mating calls of winners and losers differ significantly for chirp duration, syllable period and loudness (SPL).

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Chapter4: To investigate the role of age in determining the outcomes of agonistic contests.

4.1 Background:

Among the intrinsic determinants of dominance, the effect of body size on the outcome of contest has been widely studied (Houpt et al. 1978; Lott and Galland 1987; Nakano and Furukawa-Tanaka 1994) with relatively less attention on age except for some studies on social wasps, bees and ants (Strassmann and Meyer 1983; Michener 1974; Higashi et al. 1994). Among crickets, studies on *Gryllus integer* have investigated the role of age and body size as potential determinants of aggressive fight outcome (Dixon and Cade 1986). Findings from our preliminary experiments with *Acanthogryllus asiaticus* indicate no apparent effect of body size and weight on the fight outcome. As part of my third objective, I focus on investigating the role of age as a determining factor in the outcome of aggressive contests.

4.2 Methodology:

4.2.1 Staging agonistic contests:

Individuals were weighed and marked 7-8 hours prior to the experiment with acrylic colours on their thorax or hind leg to distinguish between the rival males unless obvious size or morphological differences were present. We did not size match the rival males as our preliminary experiments (performed by Saumya Gupta) with Acanthogryllus asiaticus indicated no apparent size effect on the outcome of the fight. The basic arena design was the same for both age effect and audience effect, consisting of a box having a removable partition in between to separate the males and control the initiation of the contest. A Sony cybershot™ DSC-HX-400V (Sony corp., Japan) was used for video recording. The individuals were kept on their respective sides of the contest arena for 2 minutes before removing the partition. Just before the start of contest the audio and video recorders were switched on simultaneously. At the initiation of fight the partition was removed and the rivals were allowed to interact. The fight was declared to be concluded when the loser retreated two times consecutively. Before each fight the arena was cleaned with 70% ethanol and the soil was shuffled to ensure the elimination of any pheromones.

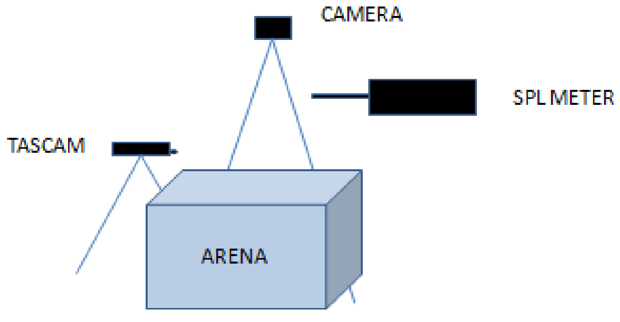

Figure 4.1. A schematic representation of contest experimental setup

Different experimental schemes were employed for checking the age-effect and audience effect.

4.2.2 Contest arena:

Contests were staged in a cardboard arena (15X20X14.5cm) with base covered with soil and the removable partition. Besides, a removable piece of cardboard was used to act as an escape route for the loser at the end of contest to avoid serious injury. In the contest old males were pitched against young ones. The young males were 7-10 days old. We maintained an age window of 15-30 days between the old and the young males and hence the old males were 22-40 days old.

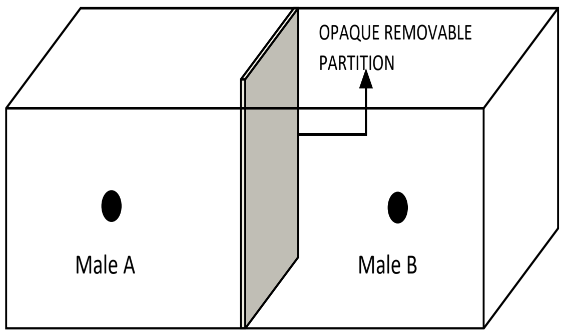

Figure 4.2. A schematic representation of the contest arena

4.2.3 Assigning winner and loser:

The individual who retreated twice and didn't approach the rival again was assigned the loser of the contest and the other rival male the winner.

4.3 Statistical analysis:

For each analysis normality tests (Shapero-wilk tests and Q-Q plots) and subsequent analysis were performed in Statistica version 12

4.3 Results:

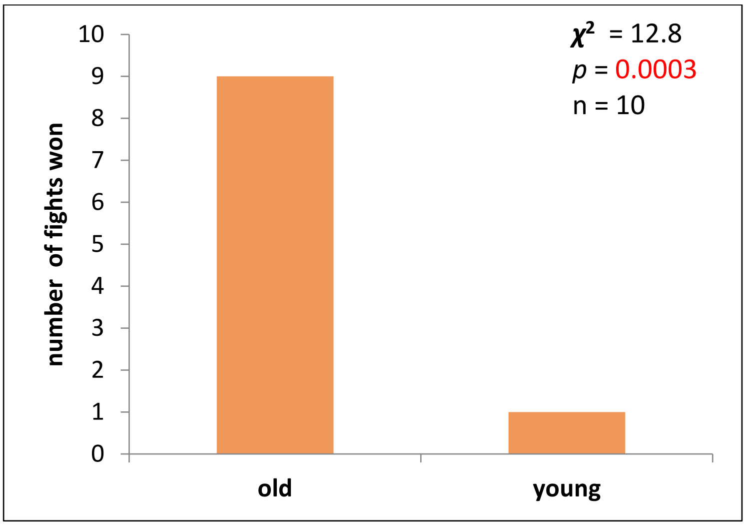

Figure 4.2. Effect of age on the outcome of agonistic contest

4.4 Conclusion:

Old and young males differ significantly in the number of fights won-significant effect of age on determining the outcome of agonistic contest.

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Chapter 5: To investigate the presence of 'audience effect' in intermale agonistic contests

5.1 Background:

Previous studies focusing on 'audience effects' in agonistic contests in crickets reveal contradictory results both supporting (Fitzsimmons and Bertram 2013, Montroy et al. 2016 and Tachon et al. 1999) as well as declining (Judge et al. 2010) it. Taking these investigations further, as a part of my fourth objective, I examine the presence of an audience effect on the aggressive behaviour in male crickets of our study species.

5.2 Methodology:

5.2.1 Staging agonistic contest:

Same approach for staging agonistic contest was followed as the one in chapter 4 for age-effect.

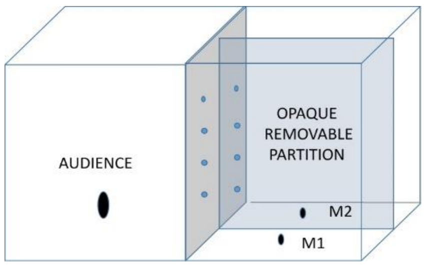


Figure 5.1: A schematic representation of the contest arena

5.2.2 Contest arena:

Contests were staged in a partitioned plexi glass arena (30X20X14.5cm) having an extra compartment for placing the audience. The wall separating the audience from the arena was having small holes to facilitate the transmission of sound and pheromones on both sides. First the audience was placed in its compartment and then the rival males were introduced into the arena on their respective sides of the partition. They were given 2 minutes before the partition was removed to provide the rival males with some time to sense the audience. We staged aggressive contest in three experimental sets – control (with no audience), male audience and female audience. Only naive males were used and no male was repeated in any other contest.

5.2.3 Video analysis

All videos were analysed in VLC media player (slowed down at 4X) and the audience effect was examined by comparing fight parameters like latency (time taken by males to initiate first act of physical interaction from the time of removal of the partition) and duration (total time for which contest occurs) as well as intensity of aggression of the fights. For quantifying the intensity of aggression, I followed the scheme of Bertram et. al 2011. I first analysed the videos to construct an ethogram of all the conspicuous aggressive behaviours, I then gave each behaviour an aggression score based on the order in which it occurs in a contest. So the behaviour occurring at the very beginning of contest was given the lowest score and the scores progressively increased for the subsequent behaviours. For each rival male in a given contest, I multiplied each behaviour with the duration (in seconds) for which it occurred in a contest and summed it across all the behaviours observed in the contest. I then summed the total scores of both the males in a contest and divided it by total fight duration to get the normalised score. The contest scores were then compared across the three experimental sets. Besides this, I also compared the intensity of aggression by comparing the amount and loudness of aggressive calls produced by rival males across the experimental sets.

5.3 Results:

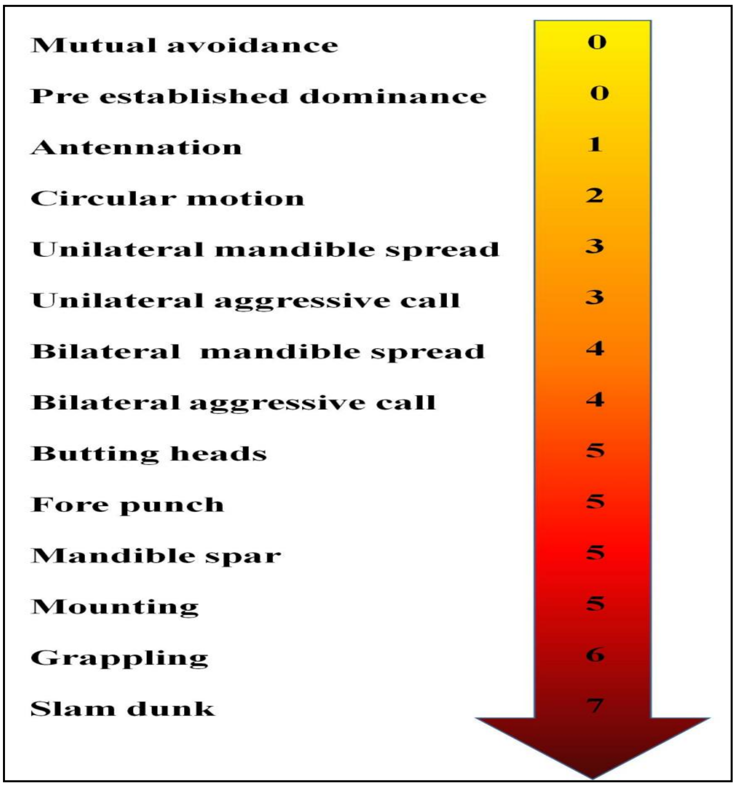
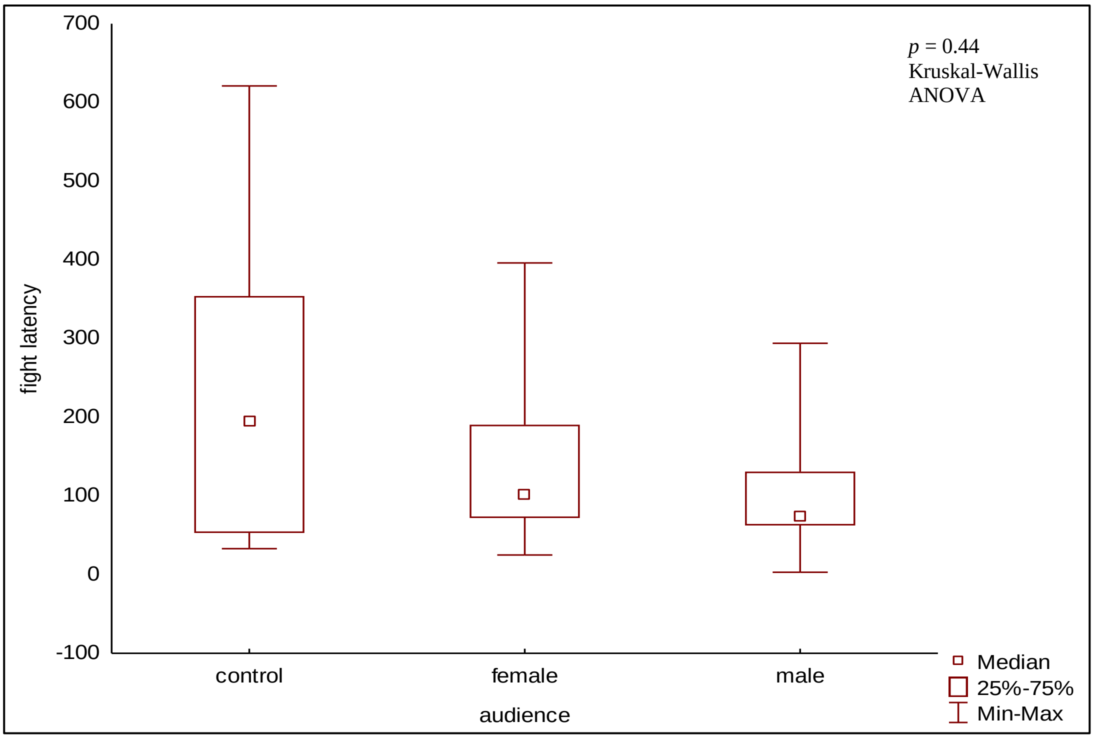
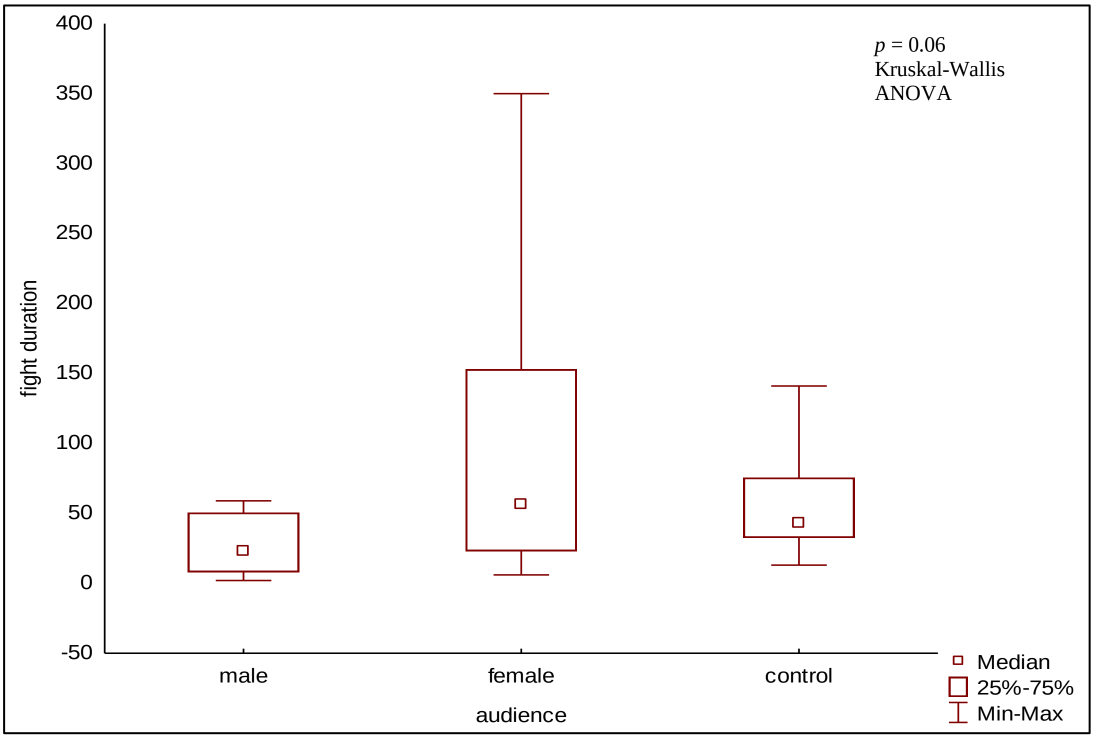


Fig 5.2 Ethogram of behaviours observed and their scoring



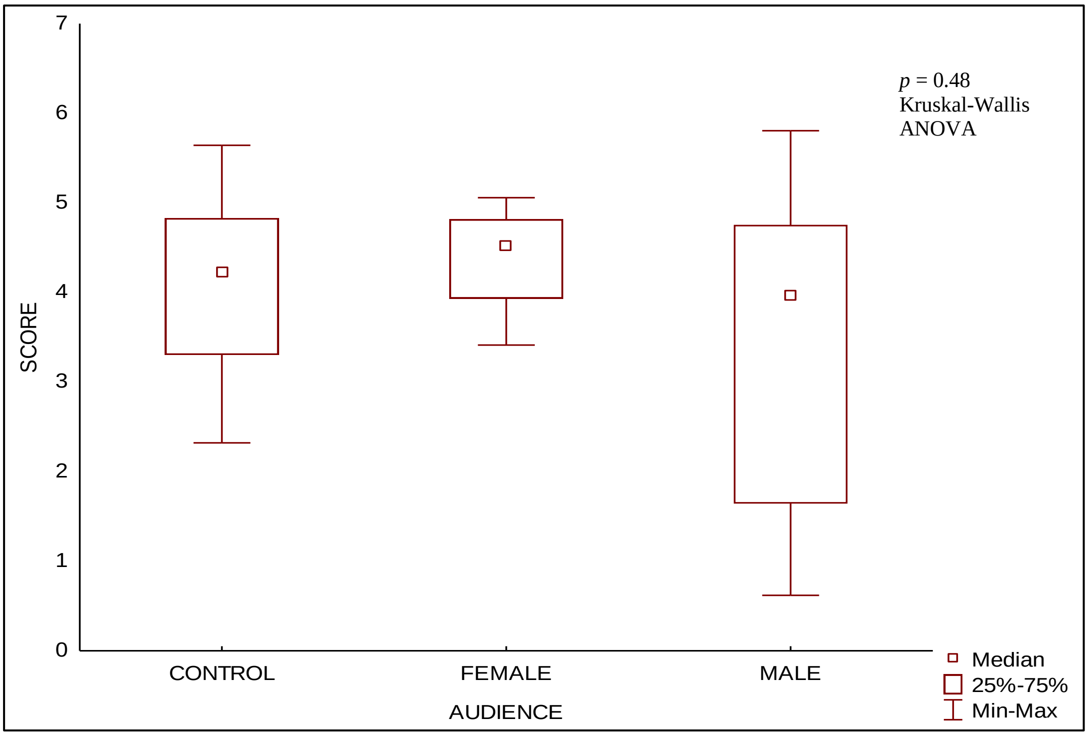
p = 0.44
Kruskal-Wallis
ANOVA
fight latency
700
600
500
400
300
200
100
0
-100
control
female
male
audience
Median
25%-75%
Min-Max

(a)

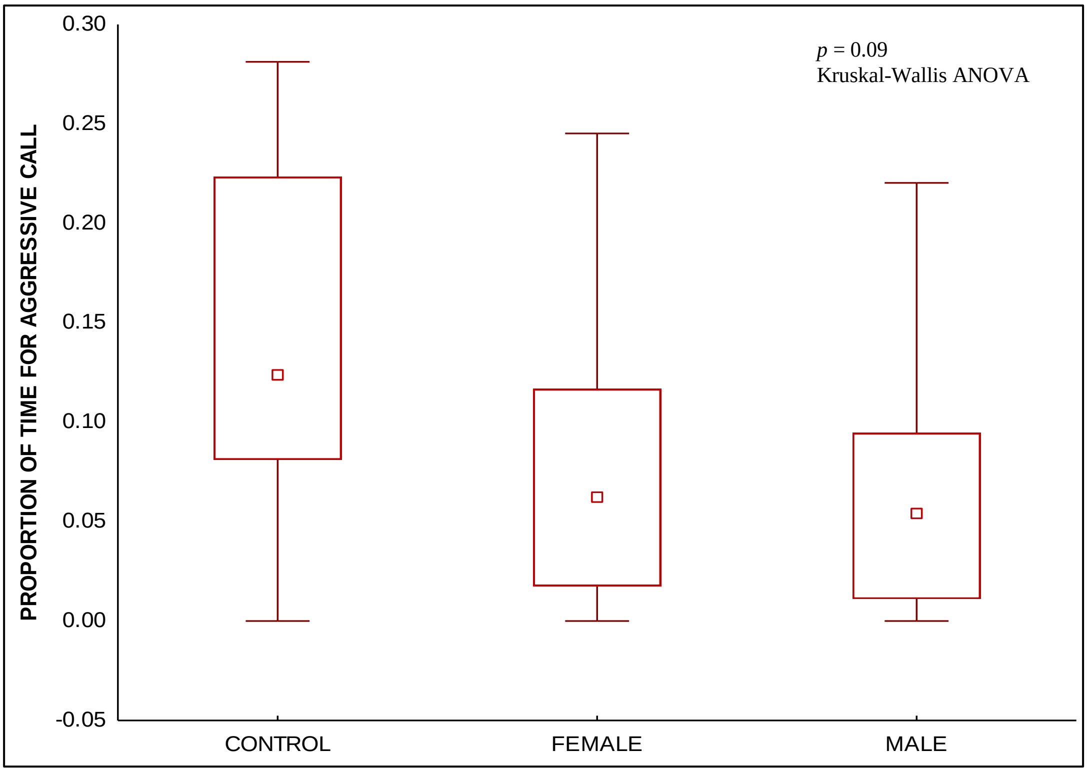


p = 0.06
Kruskal-Wallis
ANOVA
fight duration
400
350
300
250
200
150
100
50
0
-50
male
female
control
audience
Median
25%-75%
Min-Max

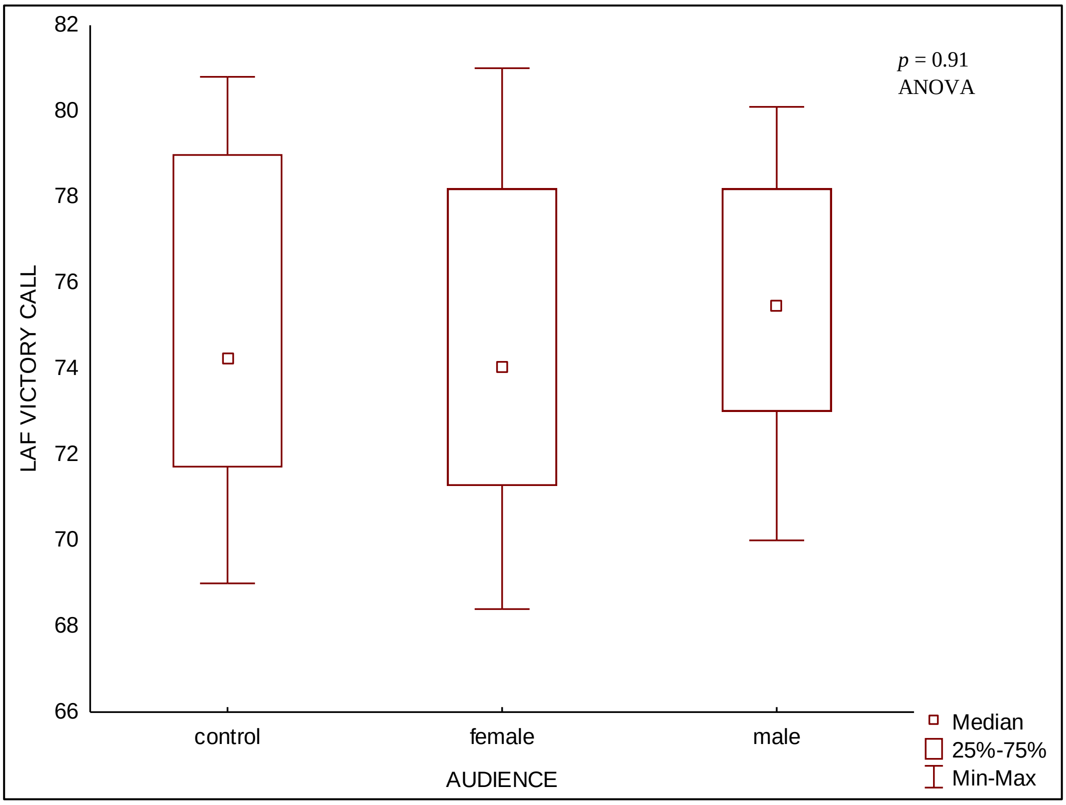
(b)


7
6
5
4
3
2
1
0
SCORE
p = 0.48
Kruskal-Wallis
ANOVA
CONTROL
FEMALE
MALE
AUDIENCE
Median
25%-75%
Min-Max

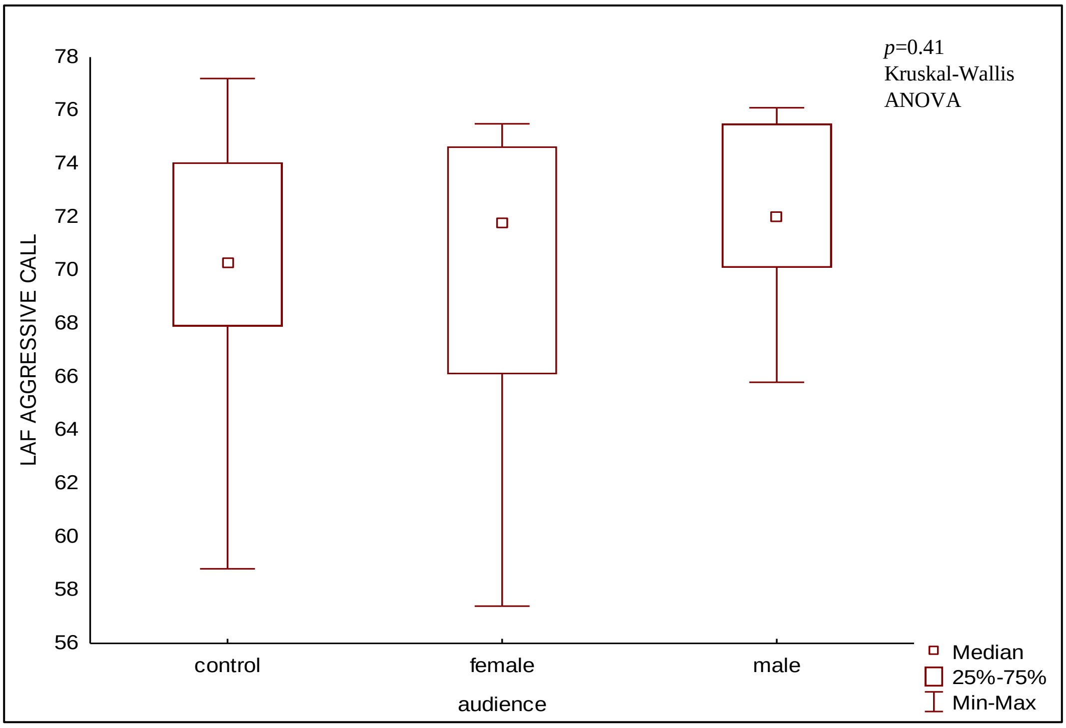
(c)


0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
PROPORTION OF TIME FOR AGGRESSIVE CALL
p = 0.09
Kruskal-Wallis ANOVA
CONTROL
FEMALE
MALE

(d)



(e)



(f)

Fig 5.3: Box plots – a) Fight duration versus audience b) fight latency versus audience c) proportion of aggressive call versus audience d) score versus audience e) SPL victory call versus audience f) SPL aggressive call versus audience

5.4 Conclusions:

There is no significant effect of the presence of audience on aggressive behaviour of rival males either in terms of fight parameters or in terms of intensity of aggressive behaviour.

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Chapter 6: Discussions

- **Objective 1**: A comparative analysis of three call types indicates that crickets have evolved call types differing by one or more acoustic traits to communicate in different behavioral scenarios. Interestingly, there is no significant difference between the calls for peak frequency which can be explained by the fact that members of species are fine-tuned to only specific range of frequencies ensuring conspecific recognition thus causing any major changes in the frequency highly unlikely. The remarkably high deviation in various acoustic traits in aggressive calls can be explained by the fact that these calls are produced during the contest where males vigorously engage in physical interactions with each other exposing them to varying levels of physical stress and energy expenditure.
- **Objective 2**: My findings indicate acoustic differences between winners and losers. While these distinctions may aid in establishing dominance between acoustically interacting rival males they might have additional fitness implications where females could be utilizing these cues during mate choice suggesting that acoustic signals are honest indicators of male strength. These speculations leave scope for future research investigating female choice thereby potentially linking the outcomes of the two modes of sexual selection - intermale competition and mate choice. Also, it would be worthy to note that since in another of my results (Objective 3), I have observed that older individuals are more likely to win a fight and since LDMC in objective 2 experiment has been taken for field caught (age-unknown) individuals, one can investigate whether these call differences are due to age or by the virtue of being a dominant-subordinate male. Experiments can be done recording calls at different stages of age to examine how call structure changes with age.
- **Objective3:** My results suggest individual's age as a potential determinant of the outcome of agonistic contests. While age could facilitate victory by increasing the chances of prior exposure and experience for agonistic contests for older

individuals, in our experiments, it is unlikely as the naivety of individuals was ensured. However, one possible rationale behind these observations could be that aged individuals may have better developed physical attributes and weaponry or different endocrinological and/or developmental states than the young ones who have recently eclosed. Future research can be done focusing on these aspects.

- **Objective 4:** My results indicate that the presence of any kind of audience illicit no significant changes in the aggressive and acoustic behavior of rival males as well as the temporal parameters of the contest. The explanation for these results lies in the social environment of the individuals. Habitat spacing studies on *A.asiaticus* in our lab indicate that the nearest neighbor distance for male-male as well as male-female pair is greater than 2 m (Singh & Jain, unpublished data) which implies less chances of physical interactions and the presence of an audience during aggressive contests in the field thus explaining the absence of a strong audience effect. Another possible explanation as described in Bond (1989) is that aggressive behaviour is merely a byproduct of achieving aggressive states and that it is not an active, conscious process and that might explain why the presence or absence of the audience shouldn't matter. Further, the utility of extracting information and storing it for subsequent retrieval by the audience and the implications of modulating behaviour in presence of an audience by signallers need to be examined.

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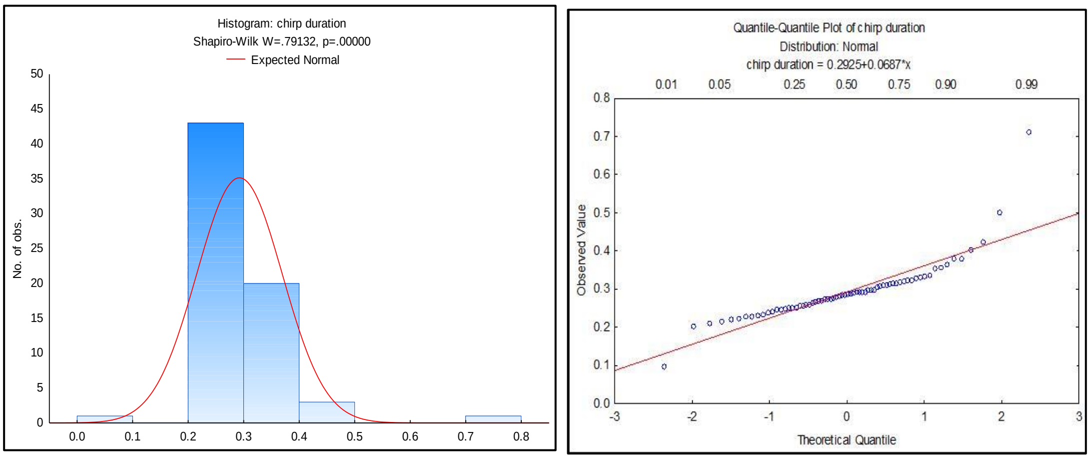
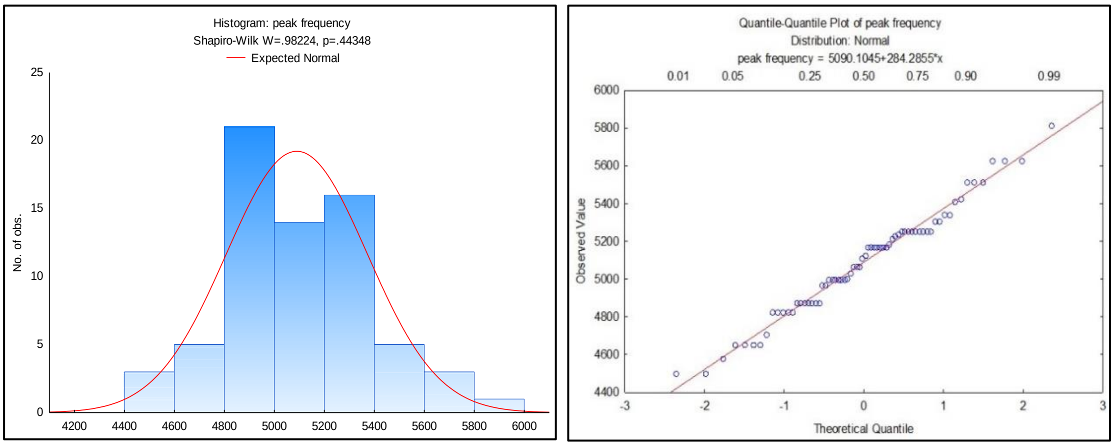
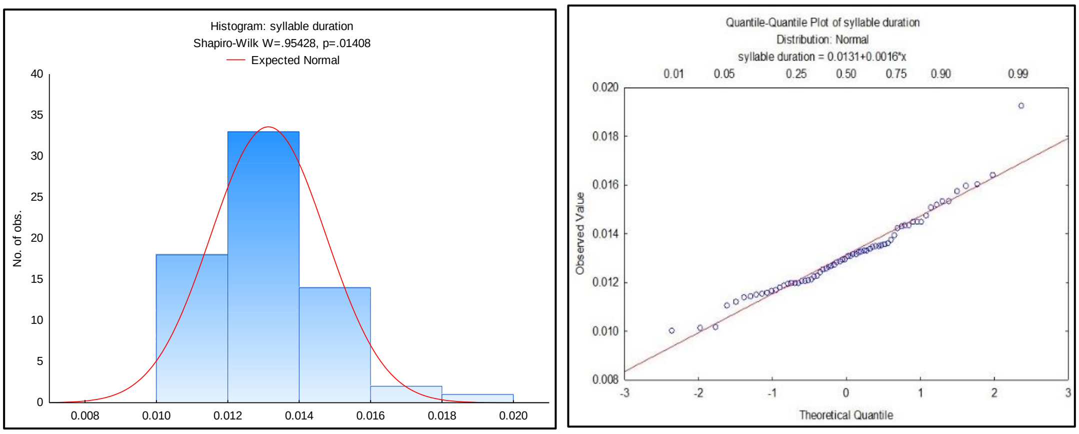
Appendix A



(a)



(b)



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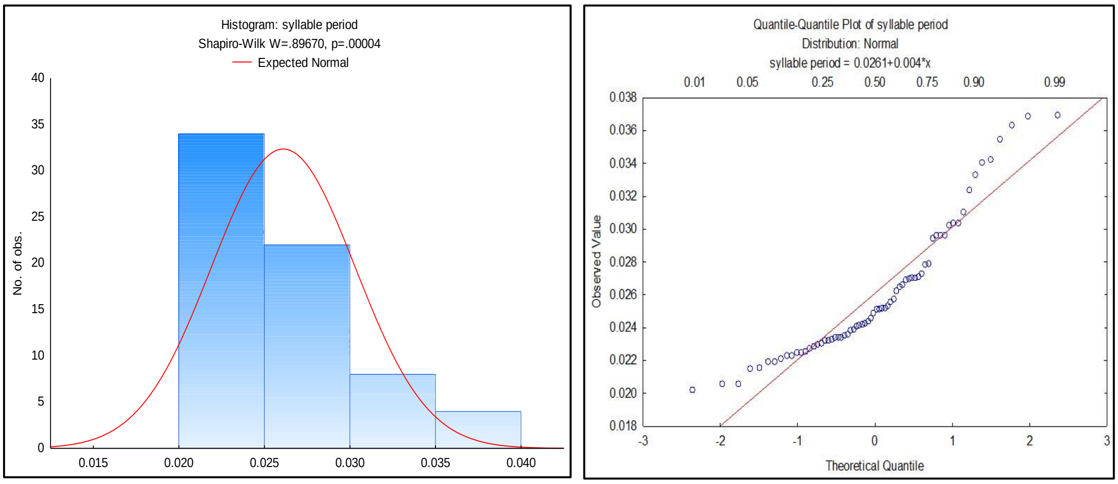

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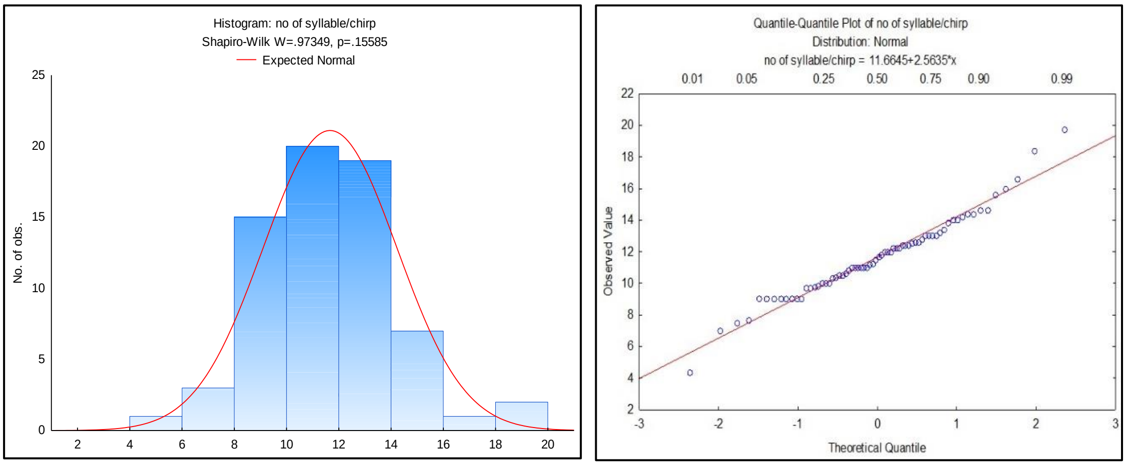

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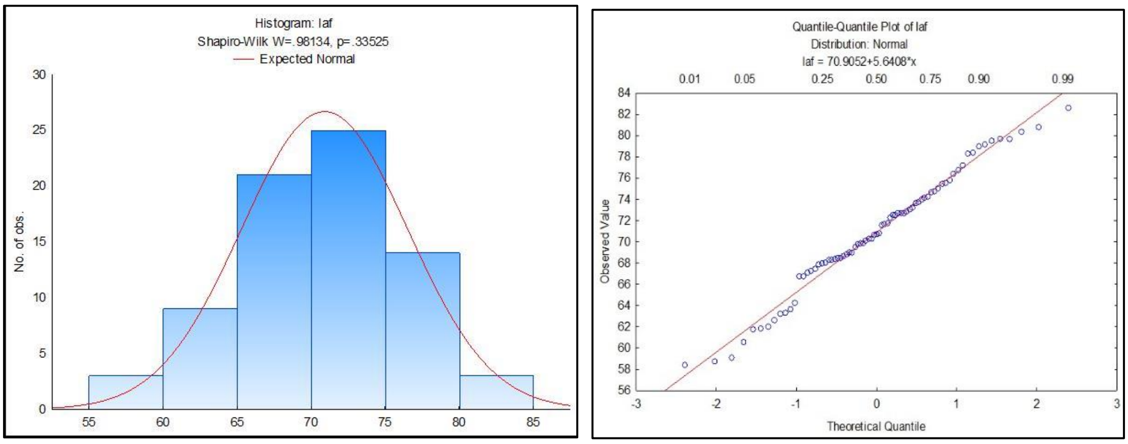
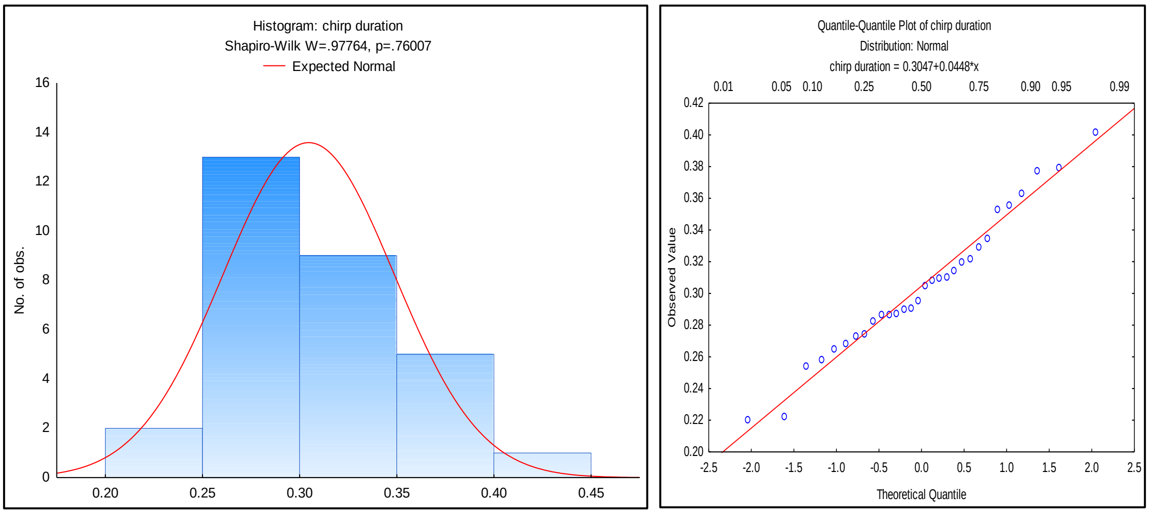


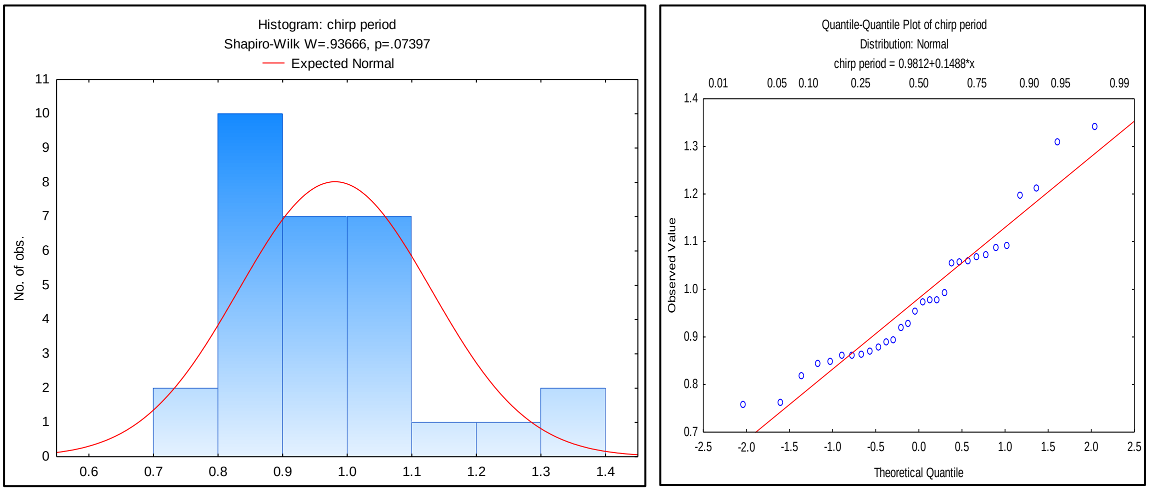
(f)

Figure1. Shapero Wilk test and Q-Q plots for acoustic parameters for LDMC, victory and Aggressive call- a) chirp duration b) peak frequency c) syllable duration d) syllable period e) number of syllable/chirp f) SPL (laf)



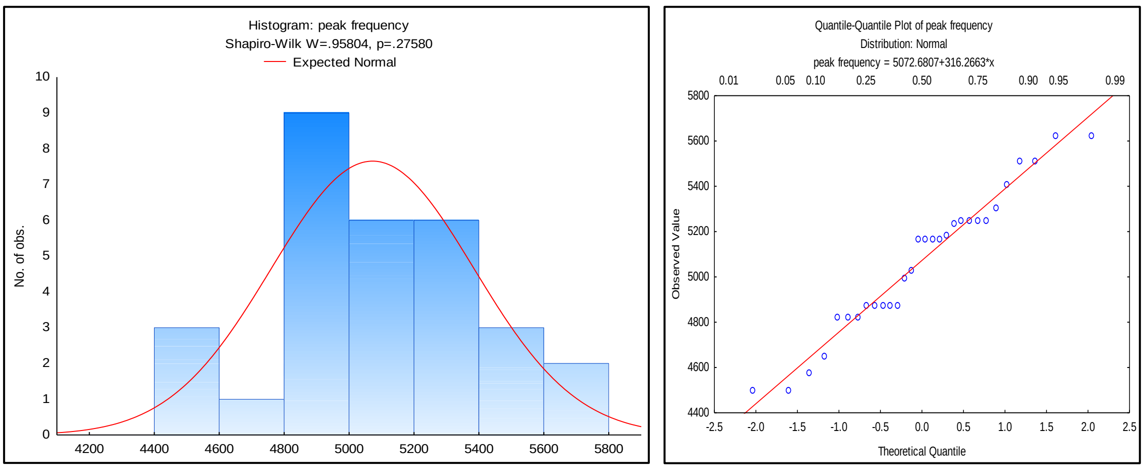
Histogram: chirp duration
Shapiro-Wilk W=.97764, p=.76007
Expected Normal
No. of obs.
Quantile-Quantile Plot of chirp duration
Distribution: Normal
chirp duration = 0.3047+0.0448*x
Observed Value
Theoretical Quantile

(a)



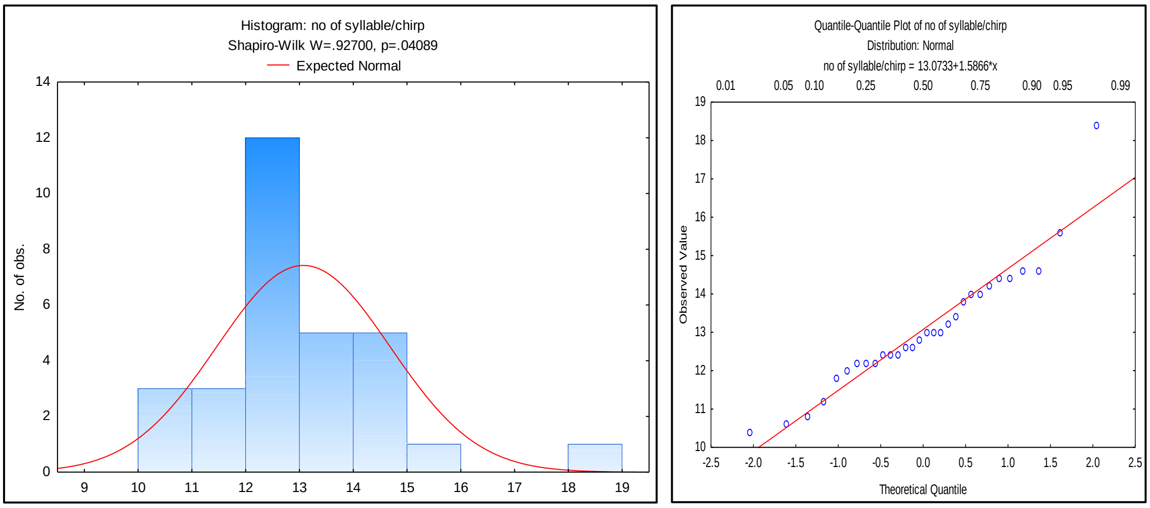
Histogram: chirp period
Shapiro-Wilk W=.93666, p=.07397
Expected Normal
No. of obs.
Quantile-Quantile Plot of chirp period
Distribution: Normal
chirp period = 0.9812+0.1488*x
Observed Value
Theoretical Quantile

(b)

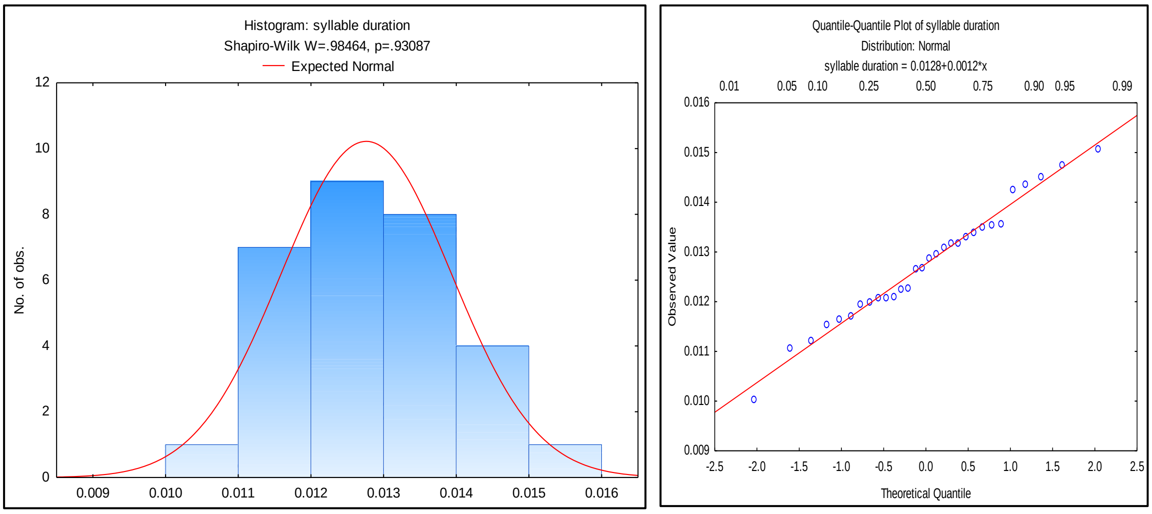


Histogram: peak frequency
Shapiro-Wilk W=.95804, p=.27580
Expected Normal
No. of obs.
Quantile-Quantile Plot of peak frequency
Distribution: Normal
peak frequency = 5072.6807+316.2663*x
Observed Value
Theoretical Quantile

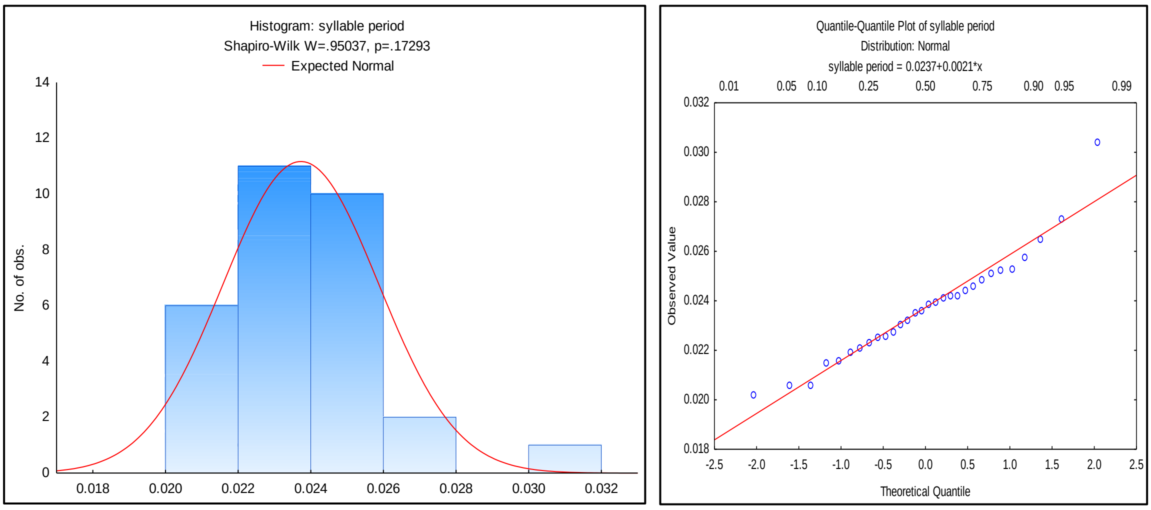
(c)


Histogram: no of syllable/chirp
Shapiro-Wilk W=.92700, p=.04089
Expected Normal
No. of obs.
Quantile-Quantile Plot of no of syllable/chirp
Distribution: Normal
no of syllable/chirp = 13.0733+1.5866*x
Observed Value
Theoretical Quantile

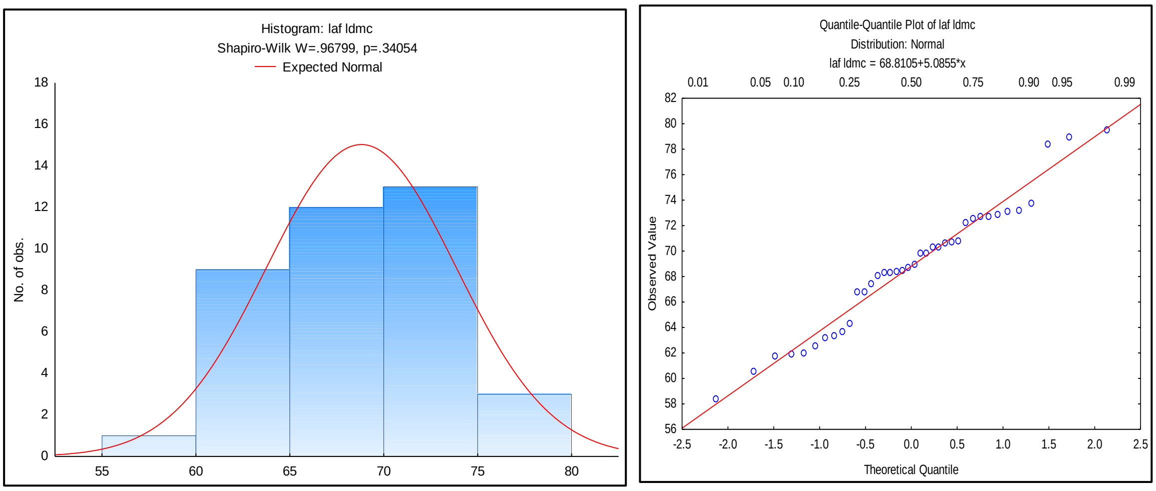
(d)


Histogram: syllable duration
Shapiro-Wilk W=.98464, p=.93087
Expected Normal
No. of obs.
Quantile-Quantile Plot of syllable duration
Distribution: Normal
syllable duration = 0.0128+0.0012*x
Observed Value
Theoretical Quantile

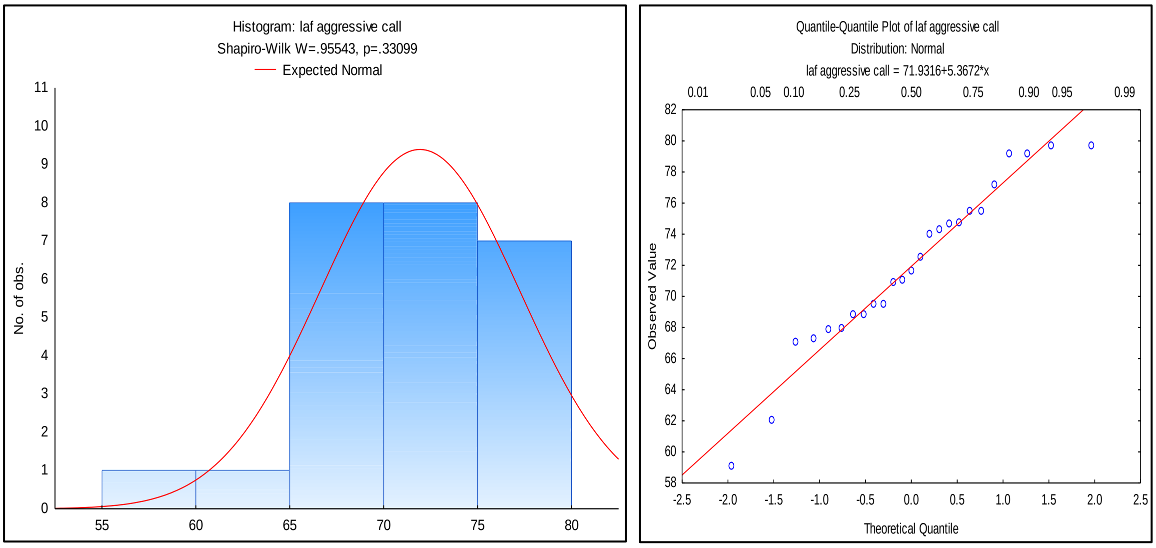
(e)


Histogram: syllable period
Shapiro-Wilk W=.95037, p=.17293
Expected Normal
No. of obs.
Quantile-Quantile Plot of syllable period
Distribution: Normal
syllable period = 0.0237+0.0021*x
Observed Value
Theoretical Quantile

(f)



(g)



(h)

Figure 2: Shapero Wilk test and Q-Q plots for acoustic parameters for winners and losers - a) chirp duration b) chirp period c) peak frequency d) number of syllable/chirp e) syllable duration f) syllable period g) SPL (laf) LDMC h) SPL (laf) aggressive call.

Appendix B

Depend.: chirp duration	Multiple Comparisons p values (2-tailed); chirp duration (compaartive_analysis_calls_lata) Independent (grouping) variable: call type Kruskal-Wallis test: H (2, N= 68) =10.30008 p =.0058		
	ldmc R:41.983	victory call R:23.675	aggressive call R:34.056
ldmc		0.004018	0.536125
victory call	0.004018		0.318403
aggressive call	0.536125	0.318403	

(a)

Depend.: syllable period	Multiple Comparisons p values (2-tailed); syllable period (compaartive_analysis_calls_lata) Independent (grouping) variable: call type Kruskal-Wallis test: H (2, N= 68) =26.95078 p =.0000		
	ldmc R:23.500	victory call R:33.400	aggressive call R:54.056
ldmc		0.248567	0.000001
victory call	0.248567		0.003911
aggressive call	0.000001	0.003911	

(b)

call type	Unequal N HSD; Variable: no of syllable/chirp (compaartive_analysis_calls_lata) Marked differences are significant at p < .05000		
	{1} M=10.558	{2} M=10.545	{3} M=12.890
victory call {1}		0.999851	0.003740
aggressive call {2}	0.999851		0.005868
ldmc {3}	0.003740	0.005868	

(c)

call type	Unequal N HSD; Variable: laf (compaartive_analysis_calls_lata) Marked differences are significant at p < .05000		
	{1} M=68.811	{2} M=70.939	{3} M=74.856
ldmc {1}		0.442248	0.001035
aggressive call {2}	0.442248		0.068892
victory call {3}	0.001035	0.068892	

(d)

Figure 1: Post hoc analysis for acoustic parameters of different call types- a) chirp duration b) syllable period c) no of syllable/chirp d) SPL (laf)