Signaling Vigour in *Gryllodes sigillatus*: Relationship with Age and Foreign Body Ingestion

By

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A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements of the degree of

Master's

in

Biology

Carleton University

Ottawa, Ontario

Abstract

Male *Gryllidae* acoustic signaling behaviour varies between and within species and may be influenced by a variety of factors including age, condition, and parasitism. I investigated variation within and among male *Gryllodes sigillatus'* mate attraction signaling behaviour and examined how variation in signaling vigour was influenced by age. I found extensive variation in male signaling between and within individuals; males differed in signaling vigour and quality, and their signaling changed over time. I found that smaller males signaled more consistently throughout their lives while larger males rarely called when younger and increased signaling vigour with age. As males have often been found to honestly signal their body condition (i.e., body mass), parasites may influence a male's perceived attractiveness to potential mates. I therefore also investigated how signaling vigour was influenced by the ingestion of the long barbed hastisetae from a typical pest species, dermestid (*Dermestes ater*), that plagues rearing facilities. These foreign bodies are regularly ingested during rearing. I found that males fed the highest concentration of dermestid hastisetae behaved differently than males in the control and high treatments. As individuals aged, smaller males greatly increased their signaling vigour and produced more attractive calls while larger males decreased their signaling vigour and produced less attractive calls. While further investigation into *Gryllodes sigillatus* signaling behaviour is needed, my results provide interesting insights into the factors shaping signaling variation in this species.

Acknowledgements

I am grateful to the Department of Biology at Carleton University for their assistance throughout my Master's education. I would like to personally thank Ed Bruggink and Dr. Genevieve Ferguson for helping with greenhouse maintenance and equipment repairs. I would also like to thank my committee members Drs. Gita Kolluru and Roslyn Dakin for providing invaluable feedback.

I would like to extend a huge thank you to my supervisor Dr. Sue Bertram. She has provided me with unending guidance and support over the last five years and has pushed me to be a better researcher, scientist and student. The countless hours of meetings, editing and educating that she has invested over the course of my education is invaluable. She never lost faith even when every single bad thing that could happen did. Without her belief in me, guidance and support I would never have made it to where I am now.

I would like to thank all members of the Bertram lab who have helped over the past few years. I would like to thank Mahir Awsaf, Emma McConnell and Olivia Gagnon who helped me immensely with cricket care, photographing and measuring. I would like to personally thank Matt Muzzatti for always being there to answer my questions, and for all his help on our joint dermestid research. Matt has taught me so much, especially (mostly against my will) about 'bugs'; I may never fully recover from our tarantula encounter. I would like to also thank my fellow Masters' students Jenna Watson and Trisha Skelton for always making me laugh when my research was crumbling around me. A huge thank you to our lab manager Michelle Leveillee for keeping our lab afloat and always making me laugh. Finally, I would like to thank former Bertram lab members Maria Doria and Dr. Ferguson for all their help

navigating the EARS, fixing the EARS and listening to me complain about the EARS. You all made my Master's experience unforgettable.

I would like to thank my partner Daniel Sawyer for his constant support and shoulder to cry on. He worked tirelessly to help me in any way that I needed including wrangling crickets when they escaped, caring for our six pets and always going on a snack run when I was working late. A huge thank you to my family, my parents Jenn & Duane Remus, my siblings Jackson, Justin, Zachary (Marissa), Tristan, Sheylannah, Carter & Zo-ee. You were all always there to lend a helping hand with my research and to give me any support that I needed throughout my marathon of an educational career. I would like to thank all my wonderful friends for always making me laugh and providing support when I most needed it. I would like to thank my best friends Linah Awadallah and Francesca Fox who were always there to buy me dinner and talk me down when I was ready to give up and for following me halfway around the world- for science. Finally, I would like to thank my dogs Molly & Ellie, cats Shadow & Sammy, and guinea pigs Chester & Butters for keeping me sane with their never-ending love and snuggles. I couldn't have done it without all of you.

Funding for my research was provided through research grants awarded to my supervisor Dr. Sue Bertram, from the Natural Sciences and Engineering Research Council of Canada Discovery Grants, the Ontario Research Fund, Carleton University, and the Canadian Foundation for Innovation. I also received funding directly from the Department of Biology.

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Overview

Sexual selection is one of the main driving forces in evolution. Darwin coined the phrase sexual selection and defined it as a mode of natural selection that pertains to an individual's ability to obtain mate(s) (Darwin, 1871). Sexual selection occurs in two forms: intrasexual selection and intersexual selection. Intrasexual selection (male competition) occurs when members of the same sex compete for the opportunity to mate with member(s) of the opposite sex. Polar bear (*Ursus maritimus*) males, for example, will fight with each other for the opportunity to mate with available females (Ramsay & Stirling, 1986). Intrasexual selection has long been accepted by the scientific community, possibly because it can be easily observed and quantified. Conversely, intersexual selection (mate choice) occurs when one sex exhibits preference for traits or characteristics in the other sex. For example, female cichlids (*Haplochromis nyererei*) choose mates based on their colouration which is correlated with body size (Seehausen & van Alphen, 1998). Intersexual selection has historically been much more controversial than intrasexual selection, as intersexual selection was thought to be both a rarer and weaker selecting force. As a result, intersexual selection was not investigated intensely until the 1970s (Cronin, 1993; Jones & Ratterman, 2009). A contemporary definition of this evolutionary system removes sex and instead opts for a neutral approach. Courters and choosers exist in reproductive systems and these courters are expected to evolve toward more beautiful signals and healthier offspring for their choosers (Rosenthaal & Ryan, 2022). Choosers, conversely, evolve preferences in response to the costs imposed by courters (Rosenthaal & Ryan, 2022).

Since the 1970s empirical and theoretical researchers have shown that intersexual selection can easily evolve when the chooser receives direct benefits (e.g., nuptial food gifts

or parental care) from the courter. Female Northern cardinals (*Cardinalis cardinalis*), for example, choose males with brighter coloration due to their parental care (Linville et al., 1998). In *Tettigoniidae* (bushcrickets), females choose mates based on resource acquisition (Gwynne, 1984) and female grain beetles (*Tenebrio molitor*) choose their mates based on their ability to avoid parasites (Worden & Parker, 2005). However, when a chooser receives no direct benefits for their mate choice, the evolution of intersexual selection has been more controversial. Several indirect benefit models have been introduced to explain how mate choice could evolve. Here I focus on the condition-dependent indicator model, as it is the most widely accepted of the indirect benefit models. The condition-dependent indicator model postulates that courters develop elaborate ornaments or vigorous displays that are costly to produce and/or maintain (Hoikkala et al., 1998). As a result, only courters in good condition can afford to pay the associated costs (Smith, 1991). The degree of elaboration or vigour is thought to be proportional to the courter's condition; courters in good condition have more elaborate ornaments or more vigorous displays and, as a result, exhibit increased viability compared to courters in poor condition (Kokko, 1997). Choosers select courters based on these ornamental traits or vigorous displays, resulting in higher fitness for their offspring, provided the traits are heritable (Smith, 1991). Female *Phymata americana* (ambush bugs), for example, exhibit a high degree of preference for the coloration in males, coloration that is dependent upon diet and age (Punzalan et al., 2008). Intersexual selection is now considered to be one of the main driving forces behind the evolution of secondary sexual traits (Wagner, 1998). Choosers exert selective pressure on courters as they select for attractive traits, thus driving the evolution of secondary sexual traits. In fact, the congruency between chooser's

preference and courter's sexual traits can be determined by the strength of the mate preference function (Blankers et al., 2015).

My MSc thesis investigated factors influencing intraspecific variation in male signaling (calling) vigour in the cricket species *Gryllodes sigillatus* (tropical house cricket). Crickets make an excellent model organism for studying sexual selection due to their ease of husbandry and proliferation (Horch et al., 2017), resulting in ample research having been conducted on cricket sexual selection (e.g., Garrison et al., 2020; Loranger & Bertram, 2016; Kortet & Hedrick, 2007). To complement and supplement this research, I quantified variation in male signaling vigour within and across individuals and how this variation was impacted by 1) age and 2) ingestion of a foreign body (*Dermestes ater* hastisetae) during development and into adulthood. I focused my research on the cricket species *Gryllodes sigillatus*, as little is known about factors influencing variation in signaling behaviour in this species. Below I provide details about cricket natural history, and I present my two research projects, one on how age and size impact variation in signaling quality and vigour (Chapter 1), and the other on how the hastisetae from a pest species, *Dermestes ater*, ingested in food, impact variation in signaling quality and vigour (Chapter 2).

Cricket Natural History

Male crickets typically fight for access to acoustic signaling territories (intrasexual selection; Leonard & Hedrick, 2009). Once males have access to signaling territories, they produce long-range acoustic calls to attract potential mates. Males signal acoustically by opening their wings and, during the closing stroke, rubbing the tegmen across the surface of the adjacent forewing (stridulation; Chapman et al., 2013). The tegmen has serrations on the

underside which rubs against the other wing, causing a pulse of sound. By repeatedly closing and then opening their wings, a male cricket groups together a series of pulses and silent segments into a chirp (Chapman et al., 2013). Males chirp (call or signal) vigorously to attract females, and once females arrive nearby, males switch to short range courtship calls.

Females can use different components of the male mate attraction call and courtship call to determine whether the signaling male is the correct species to mate with. Long distance male mate attraction calls vary in temporal parameters among species (Doherty & Storz, 1992; Figure 0.1). For example, *Gryllus pennsylvanicus* males produce mate attraction signals that include three pulses per chirp (Judge et al., 2010), whereas *Gryllus bimaculatus*, produce mate attraction signals that include four pulses per chirp (Verburgt et al., 2011). Conversely, *Gryllus assimilis* produce mate attraction signals that include eight pulses per chirp (Pacheco et al., 2013). *Gryllodes sigillatus* produce mate attraction signals that include three or four pulses per chirp (Champagnon & Cueva del Castillo, 2008).

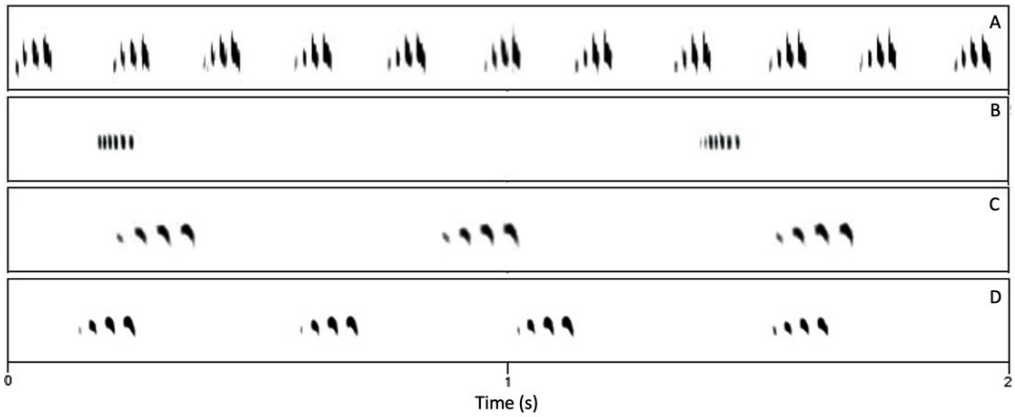

Figure 0.1. Spectrogram showing a two second excerpt of male acoustic mate attraction calling (signaling) in the following species: A.) *Gryllodes sigillatus*, B.) *Gryllus assimilis*, C.) *Gryllus pennsylvanicus* & D.) *Gryllus veletis*.

Females can also glean intraspecific information from male acoustic mate attraction signaling, as calls often broadcast different aspects of male condition to the female, such as his age (Judge et al., 2010), his body size (Harrison et al., 2013), and aspects of his overall health (Jacot & Brinkhof, 2004). Male long distance mate attraction signals vary intraspecifically in their carrier frequency, amplitude, pulses per chirp, pulse duration, pulse interval, chirp duration, and chirp interval (partially described by Figure 0.2). Further, each of these parameters can also vary within males as they age, and as their condition changes, allowing females a wide variety of components to select upon (Bertram et al., 2021). After males attract females from a distance and then court the female with courtship calls, the female will sometimes mount the male. If she does, the male transfers a spermatophore containing an ampulla and a spermatophylax. While females eat the spermatophylax, the ampulla is transferring the sperm to the female. When females finish eating the spermatophylax, they remove the ampulla, completing sperm transfer, regardless of whether there is still sperm left in the ampulla or not. Females often favour males that spend more time signaling (i.e., signal with more vigour) so males in better condition that live longer would be more likely to secure a mate, indicating that vigorous calls may be a fitness advantage (Tremblay, 2019; Bertram et al., 2021).

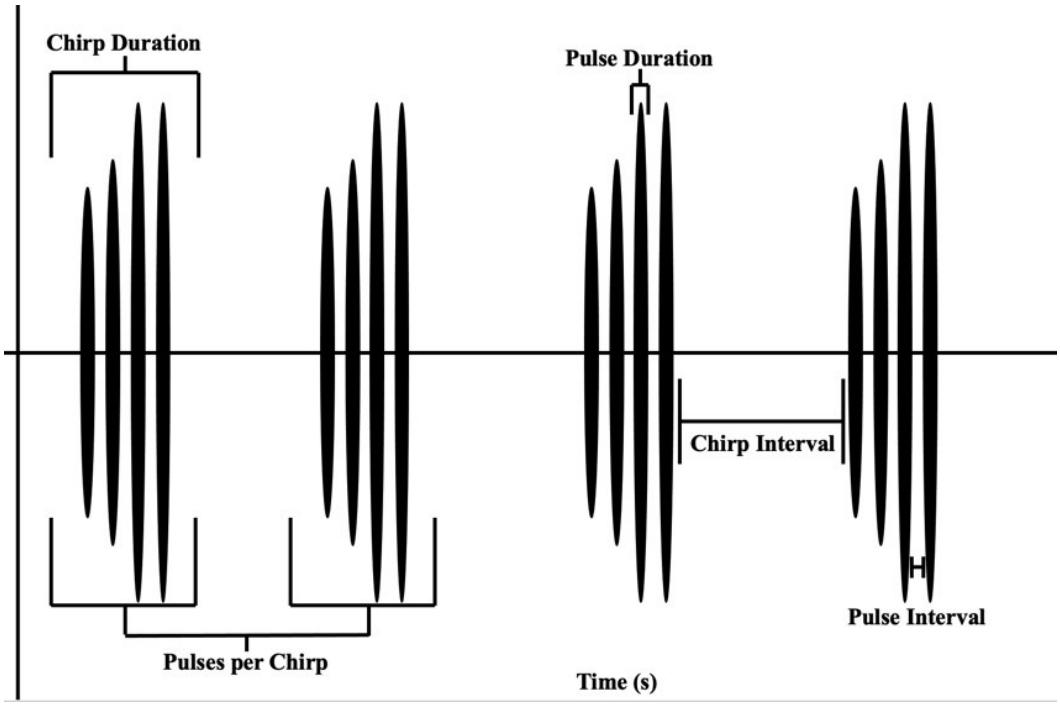

Figure 0. 2. Cricket acoustic mate attraction waveform, depicting male calling (signaling) parameters.

Mate attraction signaling is often correlated with body condition (e.g., body mass) and body size. For example, in a study on Jamaican field crickets (*Gryllus assimilis*), Whattam and Bertram (2011) revealed that interpulse duration, pulse rate and chirp duration were all found to be honest indicators of male juvenile condition (body mass and body size) and carrier frequency was found to honestly signal adult condition. Further, to test the condition-dependent handicap hypothesis using *Gryllus pennsylvanicus,* Tremblay (2019) manipulated adult diet and glued a weight to the pronotum of a subset of the males to see whether males that signaled more vigorously were better able to withstand the diet or weight stress and survive longer. Males that signaled louder and with a higher chirp rate (more vigorously) survived longer even if they were faced with a challenging diet or weight compared to males that chirped softer or at a lower chirp rate (Tremblay, 2019). In general, males that signaled

most often [high time spent calling (TSC)] lived longer. Males on lower quality diets died sooner and signaled less often (lower TSC), suggesting that males may be honestly signaling their condition (Tremblay, 2019). Further supporting Tremblay's (2019) findings that longer lived males signal more and are therefore in better condition, both *Gryllus veletis* and *Gryllodes sigillatus* males have been found to have a positive correlation between TSC and longevity (Bertram & Fitzsimmons, 2011; Houslay et al., 2015).

Chapter 1 – How Acoustic Mate Attraction Signaling Is Influenced by Age and Size

Intraspecific variation in mate attraction signaling is of great importance as choosers can distinguish among courters based in part on their mate attraction signals. Choosers are often most attracted to the signaling characteristics that appear to indicate a healthy courter. For example, choosers often prefer courters that signal for longer bouts, something that is correlated with different aspects of courter condition, such as age (Judge et al., 2010), body size (Harrison et al., 2013), and overall health (Jacot & Brinkhof, 2004). Choosers also prefer to mate with courters that signal using a higher chirp rate and longer chirp duration, possibly due to the nutritional dependence of these traits (Wagner & Hoback, 1999). Signaling bout duration, chirp rate, chirp duration, amplitude, and overall time spent calling are all indicators of a courter's overall signaling vigour, suggesting that choosers are most attracted to courters that signal most vigorously. A previous study of female preference in *Gryllodes sigillatus* revealed that females preferred males that signaled with faster chirp rates, a trait that is typically associated with larger males (Champagnon & Cueva del Castillo, 2008). It is unknown, however, whether older males also chirp at faster rates, and if so, whether females prefer older males, along with their preference for larger males. Overall, Bertram et al., (2021) suggests that intersexual selection favours males that signal vigorously, and only males in good condition should be able to signal consistently and with high vigour.

Why might mate attraction signaling change with the courter's age? Life history theory suggests that an individual's age should indicate their reproductive value, which is in part due to the terminal investment hypothesis. The terminal investment hypothesis suggests that when a courter ages or perceives a mortal threat, they should be selected to invest all their available resources into reproduction, prioritizing signaling over everything else (Duffield et al., 2017).

If courters are selected to prioritize signaling over everything else as they age, older courters should increase signaling. An increase in signaling as courters aged was observed in *Meloimorpha japonica* (bell cricket). This finding is similar to Bertram et al.'s (2021) findings on *G. assimilis*, as they found that the degree of change in time spent calling was positively correlated with courter body weight, indicating that older and better-quality males were better able to invest more resources into mating than younger or poor-quality males (Kuriwada & Kasuya, 2011). However, some studies suggest that older courters decrease the energetic investment in their mate attraction signals, as their syllable duration, calling bout duration and amplitude decrease, while the silent portions in their calls simultaneously increase in duration (Verburgt et al., 2011). These findings mirror a similar study that found male *G. veletis* decrease their time spent calling and amplitude and increase the silent portions within and between chirps as they age (Bertram & Fitzsimmons, 2011). These opposing results suggest it is important to understand how aging impacts signaling vigour in different courting species so that we can ascertain why some species align with the terminal investment hypothesis when others do not appear to do so. A recent review paper revealed that while all species exhibited age effects, the age effects were species dependent (Bertram et al., 2021). Specifically, male *Gryllus pennsylvanicus* signaling vigour peaked at 12 days post-imaginal eclosion; male *Gryllus assimilis* increased their signaling as they aged; conversely, *Gryllus veletis* decreased their signaling as they aged (Bertram et al., 2021). Like *Gryllus pennsylvanicus*, *Gryllus integer* males also exhibit a middle-aged peak in time spent calling (Bertram, 2000). Similar to Bertram et al.'s (2021) *G. veletis* findings, a 2011 study found that female *Gryllus bimaculatus* preferred the signals of younger males postulating that this was due to the degradation of stridulatory muscles in older males (Verburgt et al., 2011).

Archer et al., (2012) produced one of the few studies to date investigating how *Gryllodes sigillatus* mate attraction signaling changes with age. They investigated time spent calling (TSC) in 8 different inbred lines when males were 10, 20, and 30 days post imaginal moult and showed that time spent calling increased across those three different ages. Some inbred lines exhibited consistently higher signaling effort than other lines, suggesting the variation among males may be genetic in nature. Archer et al.'s (2012) study was a comprehensive one, comparing reproductive output in males and females and showing how reproductive output changed with age. They revealed that males lived longer and aged more slowly than their female counterparts, indicating divergent life-history strategies; this effect was further reinforced with their genetic analysis, as they found a positive correlation between early-life reproductive effort and aging rate (Archer et al., 2012). Overall, the Archer et al. (2012) study is a great start to understanding intraspecific variation in signaling vigour and how things change with age. To my knowledge, Houslay et al. (2015) produced the only other study to investigate how *Gryllodes sigillatus* male signaling behaviour changed with age, finding that *Gryllodes sigillatus* males increase signaling with age. The authors hypothesized that older males have increased reproductive output due to condition, mortality risk and lack of social feedback from females (Houslay et al., 2015). Unfortunately, the Archer et al. (2012) and Houslay et al. (2015) studies only investigated a small component of signaling vigour. They measured time spent calling and did so for only a few days. They did not assess how variation in other temporal or spectral qualities of the signal changed with age. As a result, my first study provides a comprehensive examination of how males vary their signaling vigour with age.

The individuals that I used came from a population of *Gryllodes sigillatus* that are reared at an Ontario farm. Crickets in the farm environment are very densely populated, with billions of individuals present at any one time. In addition to the high density, there is likely to be limited genetic variation as the farm population was started by a small population. Further, the farm kills all individuals at the 5-week mark, shortly after they achieve adulthood. Therefore, there is likely strong selective pressure on individuals to mate quickly and oviposit their eggs, with all individuals that have not yet done so at 5 weeks not adding their genotypes to the next generation. As a result of this artificial rearing environment, the cricket's calling behaviour may be very different than crickets in nature.

It was important for me to investigate variation in temporal signaling parameters beyond TSC, as a study on *Gryllus pennsylvanicus* revealed that as males aged, their pulse period and pulse duration decreased, while pulses per chirp increased (measures of an attractive call). Given females prefer to mate with males that signal with more pulses per chirp, females may prefer older males (Judge et al., 2010). Signal quality as a function of age has been studied in many different cricket species, with many studies showing a correlation between age and signaling behaviour (Archer et al., 2012 & Bertram et al., 2021). **I therefore investigated male signaling across the natural lifespan**. My approach enabled me to quantify the extent of natural variation in signaling vigour within and across males and explore how age and body size impacted this variation. I hypothesized that male signaling will change with age (H_1); as males age, they should invest more resources into signaling which would result in an increase in time spent calling. I predicted that calling vigour would increase with age. I also hypothesized that male signaling is condition dependent (H_2). I predicted that larger males would produce more attractive signals (i.e., signal more vigorously, louder chirps, lower

carrier frequency, etc.), and these differences would become more pronounced as individuals aged.

Methods

Crickets and eggs used were supplied by Entomo Farms in Norwood, Ontario, delivered to Carleton University during Spring 2022. Eggs were hatched in incubators at Carleton University. Pinheads were reared in communal bins (L x W x H = 64 x 40 x 42 cm) with shelter (egg cartons), and *ad libitum* food (Earth's Harvest Organic Cricket Grower) and water. When males reached their penultimate moult, they were separated from the colony and housed in individual containers (540 mL) with *ad libitum* food and water, under a 16:8-hour light cycle, at 30 ± 2 °C. When males achieved their imaginal moult, I weighed each male (OHAUS Pioneer Analytical Balance Model: Adventurer SL AS64; SE = 0.0001g), and then quantified their acoustic mate attraction signaling behaviour (N = 38).

Male Mate Attraction Signaling

To quantify acoustic mate attraction signaling behaviour, I placed each male (N = 38) into its own chamber within the Electronic Acoustic Recording System (EARs; designed by Cambridge Electronic Design, Cambridge, UK) from the day of their imaginal moult up to 46 days post imaginal moult. The system consisted of 64 individually recording microphones with an LED light that provided individuals with the same light cycle (16:8) as their environmental chamber. Each microphone was housed in an acoustically isolated enclosure (Styrofoam 2-inch-thick cooler box, lined with 1-inch-thick acoustic foam) to reduce background noise contamination. The EARs recorded each male's acoustic mate signaling

behaviour with CricketSong Software (Cambridge Electronic Design Ltd, Cambridge, U.K.). The CricketSong Software continuously analyzed cricket signaling and calculated hourly and daily mean pulse duration, mean interpulse duration, mean number of pulses, mean chirp duration, mean interchirp duration, mean amplitude, mean frequency of call, mean time spent calling per night and mean call duration (Whattam & Bertram, 2011). If individuals died while in the EARs, they were removed and frozen. Any individuals still alive at the end of the monitoring period were cold euthanized by placing them in the freezer. The following morphological measurements were made using photographs (Carl Zeiss, Stereo Microscope Stemi 305 KMAT) of the dorsal view with ImageJ: head width and pronotum length and width (Figure 1.1).

Figure 1.1. Male *Gryllodes sigillatus*; the left photograph shows an example measurement of pronotum length; the right photograph shows an example measurement of pronotum width and head width.

I analyzed the EARS files using Spike2 (designed by Cambridge Electronic Design, Cambridge, UK); this program parsed each data file into useable data. These data were then analyzed in JMP 16.0.0.

Statistical Analysis

I first quantified the population level averages and variance for time spent calling (TSC) and all fine scale temporal and spectral signaling traits. I also calculated the probability that a male signaled or not (Call/ Not) each night to determine his overall probability of signaling. I then examined the factors explaining variation within and across individuals in their signaling behaviour. To quantify the cause of variation in vigour, I used a general linear mixed model. The model contained the following fixed effect predictors: age, body size (principal component analysis of adult mass, pronotum length and pronotum width), and the interaction between age and size. I statistically controlled for within-individual variation in signaling by including individual (ID) as a random effect. I log-transformed time spent calling data to meet Gaussian model fit assumptions.

Results

Population Level Analysis of *Gryllodes sigillatus* Signaling Behaviour

Male *G. sigillatus* spent an average of 26 minutes calling per 24 hours (time spent calling (TSC): $\overline{x} \pm SE = 25.6 \pm 2.8$) and the average call duration was 22 minutes ($\overline{x} \pm SE = 22.3 \pm 1.5$; Table 1.1; Figure 1.2). Average pulse duration was 8 ms ($\overline{x} \pm SE = 7.85 \pm 0.04$) with 7 ms between pulses (interpulse duration; $\overline{x} \pm SE = 7.13 \pm 0.09$). Calls had ~3 pulses per chirp ($\overline{x} \pm SE = 2.65 \pm 0.02$), with the average chirp lasting ~29 ms ($\overline{x} \pm SE = 29.2 \pm 0.28$). Chirps had an average carrier frequency of 6460 Hz ($\overline{x} \pm SE = 6460 \pm 21.7$) at 36 dB (amplitude; $\overline{x} \pm SE = 36.3 \pm 0.39$). The probability that a male signaled in a 24-hour period was 27%; most 24-hour periods did not contain signaling (Figure 1.3).

It is important to note, that males exhibited extensive inter-individual variation in signaling behaviour, with some males rarely signaling, others signaling often. Further, the fine scale temporal and spectral components also exhibited significant inter-individual variation. Therefore, below I explore how differences in body size and age (and their interactions) explains this variation.

Table 1.1. Mean and standard error for call components in *Gryllodes sigillatus*.

Trait	Mean	Standard Error
time spent calling	25.63	2.79
pulse duration	7.849	0.04
interpulse duration	7.132	0.09
pulses per chirp	2.647	0.02
chirp duration	29.24	0.28
interchirp duration	62.55	0.82
carrier frequency	6466	21.7
amplitude	36.32	0.39
call duration	22.27	1.49

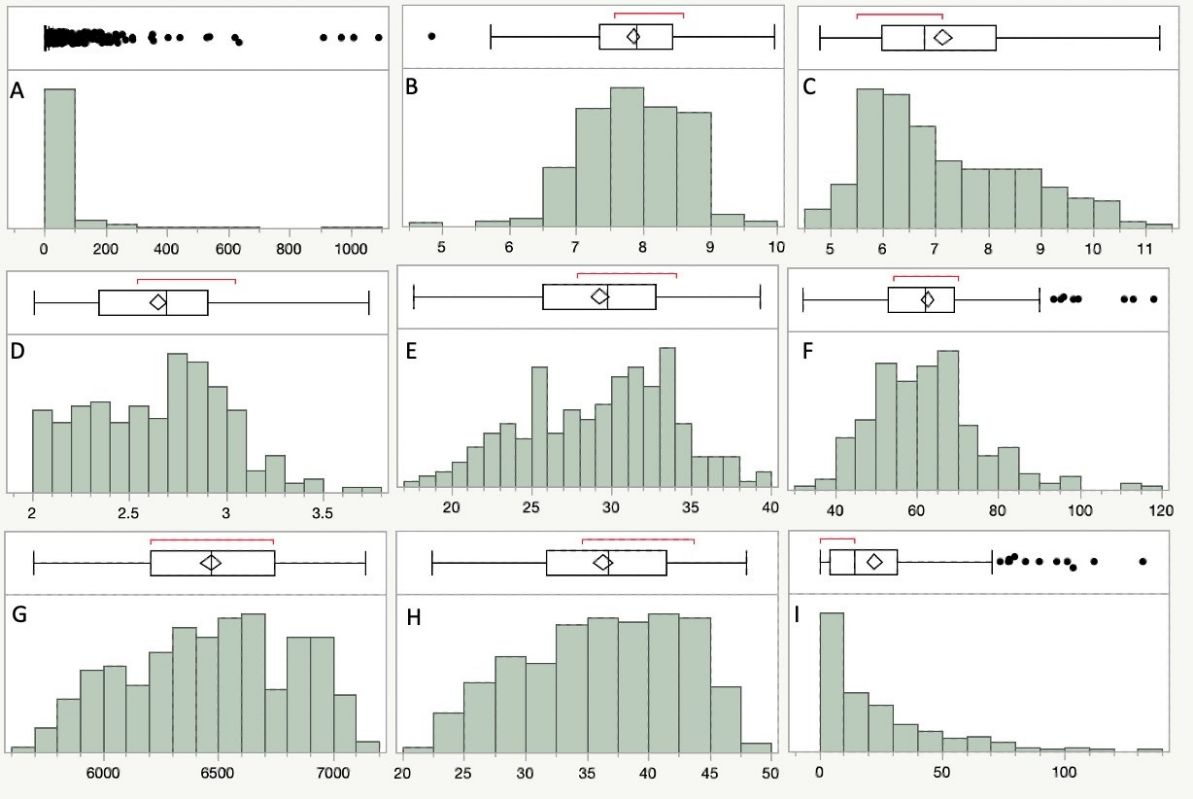

Figure 1.2. Distribution of call characteristics across individuals. A.) Time Spent Calling (mins), B.) Pulse Duration (ms), C.) Interpulse Duration (ms), D.) Number of pulses per chirp, E.) Chirp Duration (ms), F.) Interchirp duration (ms), G.) Carrier Frequency (Hz), H.) Amplitude (dB) & I.) Call Duration (mins). The horizontal line represents the median, the confidence diamond contains the mean and the upper and lower 95% of the mean, the ends of the box represent the 25th and 75th quantiles respectively and the red bracket represents the shortest half.

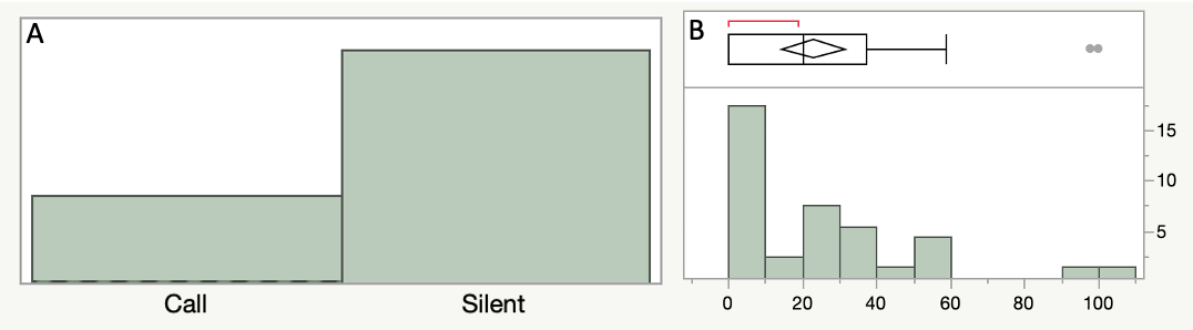

Figure 1.3. Percent of 24-hour period which males signaled. A.) Probability of male signaling; B.) Percent of 24-hour period that each male signaled – some males rarely signaled, but a few signaled every day or almost every day. The horizontal line represents the median, the confidence diamond contains the mean and the upper and lower 95% of the mean, the ends of the box represent the 25th and 75th quantiles respectively, and the red bracket represents the shortest half.

Individual Signaling Variation: Effect of Age and Size

Signaling changed with age (Table 1.2; Figure 1.4). The probability of an individual calling at least once through the night increased with age (Figure 1.5). Age also significantly affected time spent calling and carrier frequency (Table 1.2; Figure 1.5). Specifically, males increased their signaling effort and decreased their carrier frequency as they aged. Body size also explained some of the variation in signaling behaviour. Specifically, smaller males signaled on more nights than larger males (Table 1.2). Smaller males also signaled more often, with more pulses per chirp, longer chirp durations, and longer call durations than larger males (Table 1.2). The interaction between age and size significantly influenced many of the calling components including time spent calling ($p = <0.0001$; Figure 1.6), interpulse duration ($p = 0.0095$; Figure 1.7), interchirp duration ($p = 0.0279$), and carrier frequency ($p = 0.0229$). While smaller male signaling vigour was fairly consistent throughout their lives, they changed their signal quality: they increased their breaks, taking more time between producing pulses and chirps. Conversely, larger males rarely called when they were younger and then ramped up their signaling vigour as they aged: they also start putting more energy into their calls as they aged, signaling with shorter breaks between pulses and chirps. Carrier frequency tended to decline with age across males, regardless of their size.

Our general linear mixed models also showed that individuals varied extensively in several aspects of their calling behaviour (Table 1.3), explaining 27-84% of the variation in the model, trait dependent. Not only did individuals differ in their signaling vigour and signaling quality, they also differed in how their calling changed over time (Figure 1.4 and 1.5). Further, in some cases, this individual variation over time was also dependent on body

size (Table 1.3). These interactions between ID& and age or body size explained up to 85% of the variance among males.

Table 1. 2. Fixed factors influencing signaling behaviour. General linear mixed model results for age, body size and their interaction (*). Bolded terms are significant.

Trait	Fixed Effects	Estimate	SE	DF	t	P
time Spent Calling	Intercept	-2.927	2.817	0.51	-1.04	0.5917
	Age	**0.121**	**0.033**	**60.90**	**3.64**	**0.0006**
	Size	-2.219	0.961	0.68	-2.31	0.3439
	Age*Size	**0.177**	**0.029**	**256.00**	**6.04**	**<0.0001**
pulse Duration	Intercept	**6.884**	**0.464**	**6.21**	**14.83**	**<0.0001**
	Age	0.028	0.014	3.76	1.98	0.1234
	Size	-0.144	0.148	9.10	-0.97	0.3554
	Age*Size	0.010	0.008	2.27	1.28	0.3149
interpulse duration	**Intercept**	**9.162**	**0.943**	**3.67**	**9.72**	**0.0010**
	Age	-0.033	0.029	2.41	-1.13	0.3569
	Size	0.649	0.293	8.65	2.21	0.0555
	Age*Size	**-0.067**	**0.017**	**5.14**	**-4.03**	**0.0095**
pulses per chirp	**Intercept**	**2.092**	**0.217**	**12.70**	**9.63**	**<0.0001**
	Age	0.009	0.007	8.20	1.27	0.2389
	Size	**-0.158**	**0.063**	**21.30**	**-2.52**	**0.0199**
	Age*Size	0.007	0.004	13.90	1.92	0.0750
chirp duration	**Intercept**	**24.100**	**2.594**	**7.34**	**9.29**	**<0.0001**
	Age	0.089	0.084	3.82	1.07	0.3492
	Size	**-1.593**	**0.627**	**11.60**	**-2.54**	**0.0266**
	Age*Size	0.073	0.041	9.44	1.77	0.1097
interchirp duration	Intercept	103.900	26.860	1.46	3.87	0.0980
	Age	-0.568	0.525	3.51	-1.08	0.3480
	Size	16.810	13.200	0.23	1.27	0.6872
	Age*Size	**-1.296**	**0.377**	**3.87**	**-3.43**	**0.0279**
carrier frequency	**Intercept**	**6612.000**	**58.850**	**3.45**	**112.00**	**<0.0001**
	Age	**-3.361**	**0.735**	**36.10**	**-4.57**	**<0.0001**
	Size	55.33	34.300	3.79	1.61	0.1859
	Age*Size	**0.869**	**0.365**	**34.60**	**2.38**	**0.0229**
amplitude	**Intercept**	**27.910**	**3.956**	**5.53**	**7.06**	**0.0006**
	Age	0.153	0.115	3.29	1.33	0.2689
	Size	-1.556	1.180	10.30	-1.32	0.2159
	Age*Size	0.126	0.059	4.10	2.16	0.0954
call duration	Intercept	12.640	6.333	11.10	2	0.0711
	Age	0.221	0.175	5.30	1.26	0.2606
	Size	**-7.167**	**1.250**	**10.40**	**-5.73**	**0.0002**
	Age*Size	-0.022	0.102	3.65	-0.21	0.8428

Table 1. 3. Random factors influencing signaling behaviour. General linear mix model results for random factors and their interaction (*). The models run were: Trait ~ age + size + age*size + |ID + |ID*age +|ID*size + |ID*age*size. Bolded terms are significant.

Trait	Random Effects	Standard Error	Variance Component	Percent of Total	P	R^2 adjusted
time spent calling	**ID**	**0.24**	**-1.9814**	**0**	**< 0.0001**	62%
	ID*Age	**0.00**	**-0.0076**	**0**	**< 0.0001**	
	ID*Size	5.11	4.7994	85	0.3473	
	ID*Age*Size	**0.00**	**0.0178**	**0.3**	**< 0.0001**	
	Residual	0.10	0.8074	14		
pulse duration	ID	0.80	0.6852	75	0.3941	61%
	ID*Age	0.00	0.0001	0	0.8055	
	ID*Size	0.21	-0.0394	0	0.8510	
	ID*Age*Size	0.00	0.0002	0	0.6835	
	Residual	0.03	0.2265	25		
interpulse duration	ID	1.54	1.2263	53	0.4247	80%
	ID*Age	0.01	0.0028	0.1	0.6362	
	ID*Size	**0.06**	**0.5886**	**26**	**< 0.0001**	
	ID*Age*Size	**0.00**	**0.0019**	**0.1**	**< 0.0001**	
	Residual	0.05	0.4745	21		
pulses per chirp	ID	0.12	0.2156	84	0.0613	72%
	ID*Age	0.00	0.0006	0.2	0.3718	
	ID*Size	0.03	-0.0369	0	0.1915	
	ID*Age*Size	0.00	-0.0001	0	0.5806	
	Residual	0.00	0.0397	16		
chirp duration	ID	17.4	32.782	82	0.0601	70%
	ID*Age	0.03	0.0052	0	0.8532	
	ID*Size	3.82	-5.9201	0	0.1208	

	ID*Age*Size	0.01	0.0059	0	0.6124	
	Residual	0.67	6.9621	18		
interchirp duration	**ID**	**28.0**	**-136.95**	**0**	**< 0.0001**	74%
	ID*Age	0.86	-0.1065	0	0.9018	
	ID*Size	502	320.47	85	0.5232	
	ID*Age*Size	1.19	1.1610	0.3	0.3310	
	Residual	11.1	54.229	14		
carrier frequency	**ID**	**1190**	**-13093**	**0**	**< 0.0001**	84%
	ID*Age	**38.8**	**425.01**	**0.9**	**< 0.0001**	
	ID*Size	**7760**	**26833**	**55**	**0.0005**	
	ID*Age*Size	**9.71**	**-106.26**	**0**	**< 0.0001**	
	Residual	1930	21164	44		
amplitude	ID	54.2	55.431	83	0.3061	75%
	ID*Age	0.38	0.2810	0.4	0.4421	
	ID*Size	14.8	-5.9916	0	0.6852	
	ID*Age*Size	0.09	-0.0588	0	0.5083	
	Residual	1.14	10.810	16		
call duration	ID	123	78.110	15	0.5241	27%
	ID*Age	0.17	0.0256	0	0.8770	
	ID*Size	30.2	-18.431	0	0.5419	
	ID*Age*Size	**0.00**	**0.0086**	**0**	**< 0.0001**	
	Residual	41.3	432.09	85		

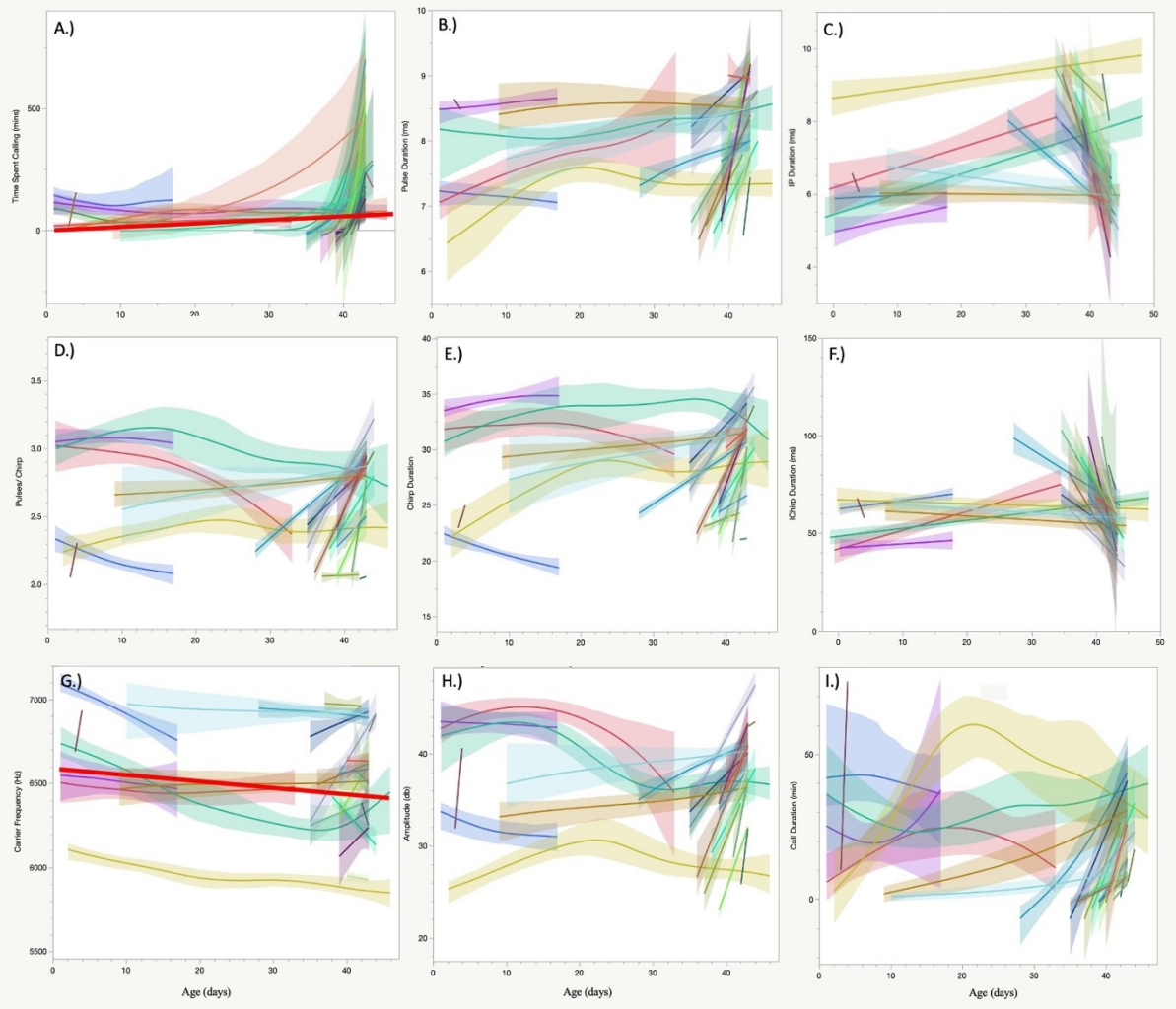

Figure 1.4. Splines from model output showing how call components were influenced by age. A thick red line shows a significant age effect at the population level. Each colour represents how that individual changed his calling trait with age. Solid red lines are the population average for call components with significant effects. Different traits examined include: A) time spent calling, B) pulse duration, C) interpulse duration, D) pulses per chirp, E) chirp duration, F) interchirp duration, G) carrier frequency, H) amplitude, and I) call duration.

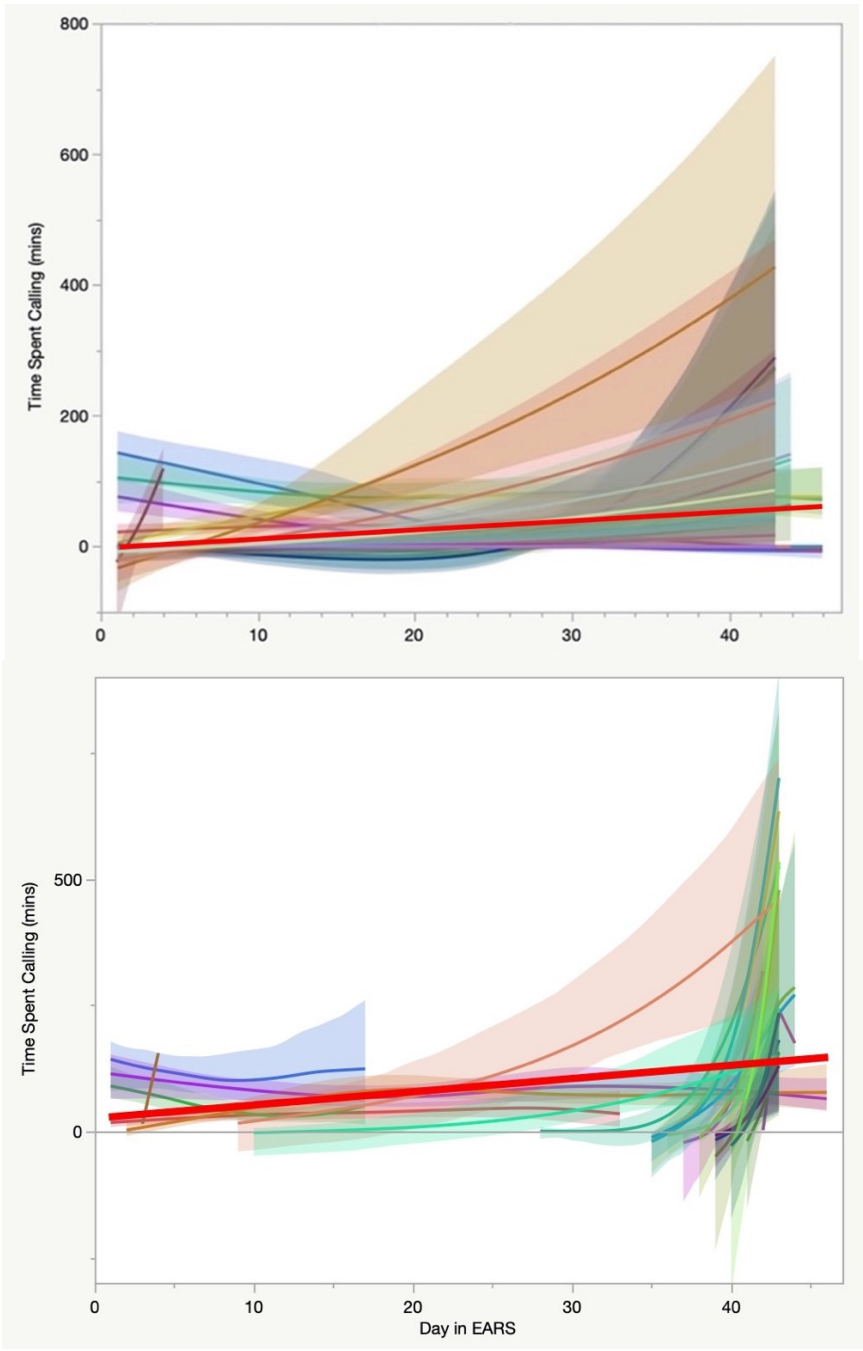

Figure 1.5. Splines from model output showing how time spent calling was influenced by age. A thick red line shows a significant age effect at the population level. (A) shows the spline results from the model output when days not signaling were included in the dataset, while (B) shows the spline results from the model output when only days when males called were included in the dataset. A thick red line shows a significant age effect at the population level. Each colour represents how that individual changed his time spent calling with age.

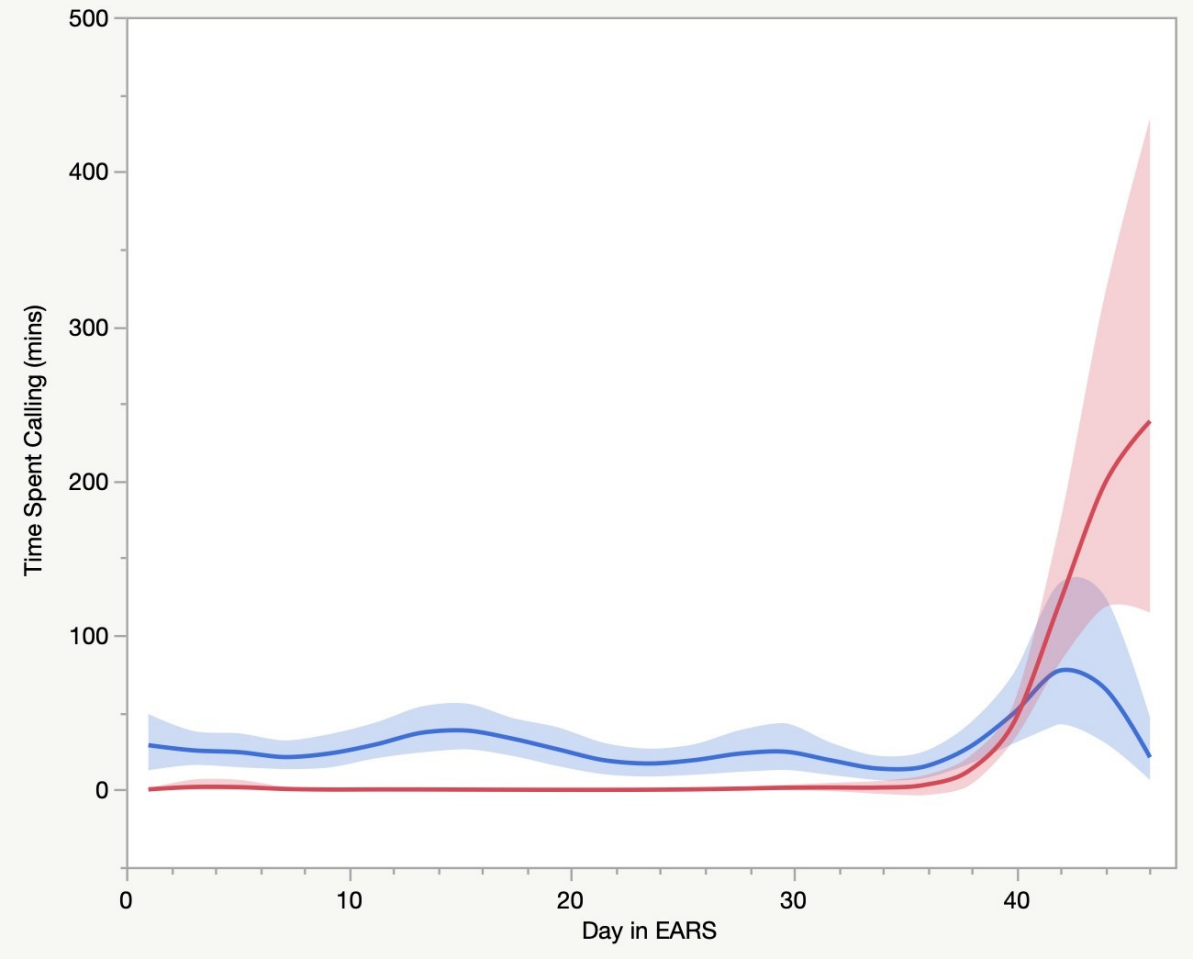

Figure 1.6. A pictural representation of how body size and age interact to influence time spent calling. Blue line represents small males and red line represents large males.

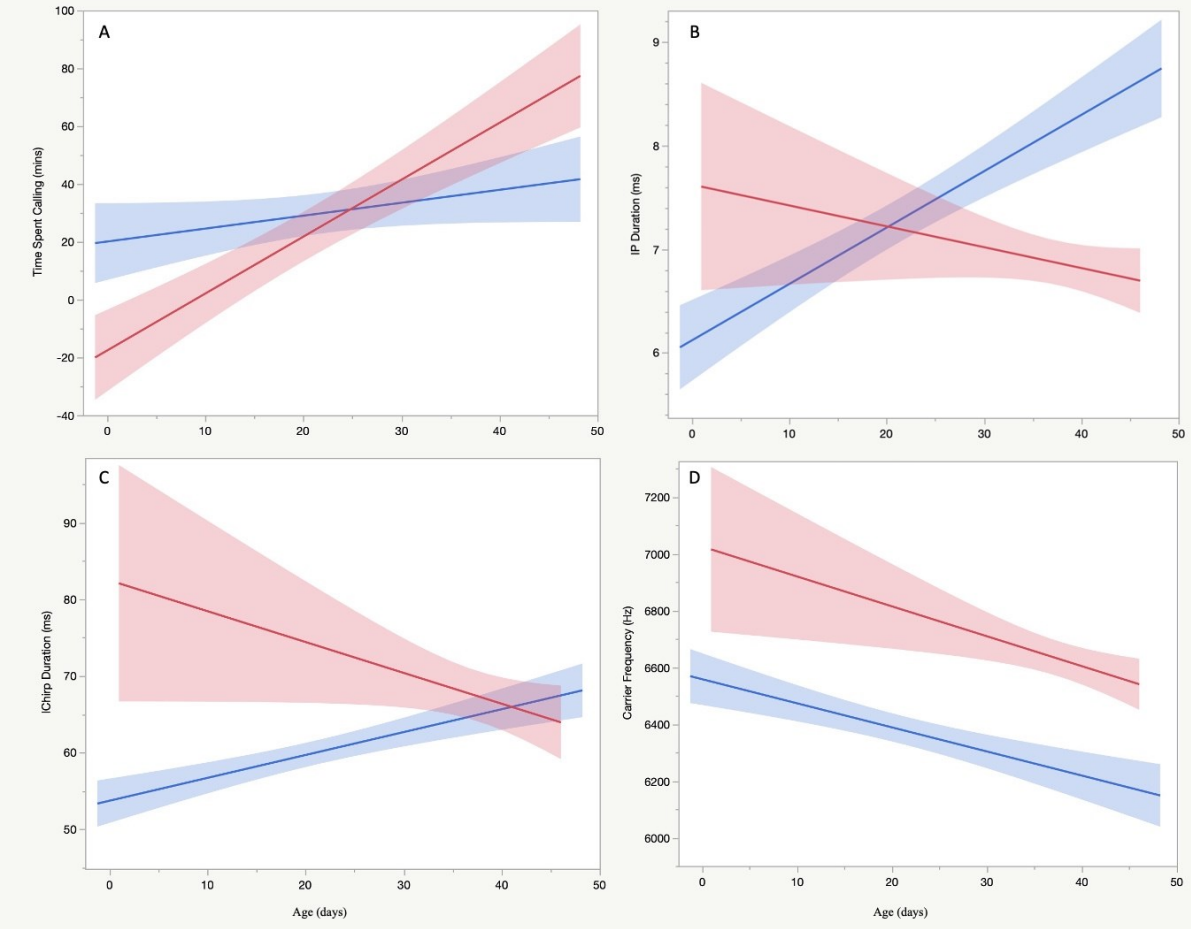

Figure 1.7. The interactive effect of age and body size on call components. Blue lines represent small males and red lines represent large males. A) time spent calling, B) interpulse duration, C) interchirp duration & D) carrier frequency. Only call components with significant interactions between body size and age are shown.

Discussion

Species Level Differences in Signaling Vigour and Fine Scale Components:

Gryllodes sigillatus signal less often compared to other Gryllidae species and their signaling effort is much lower. For example, male *G. sigillatus* males only signaled on 27% of nights, and when they were signaling, they spent only an average of 26 minutes signaling per day. This is far lower than the other Gryllidae species studied to date: *Gryllus veletis* (158

minutes/day; Bertram & Fitzsimmons, 2011), *Gryllus integer* (180-420 minutes/day; Cade, 1991), *Gryllus rubens* (180 minutes/ day; Gray, 2011), *Gryllus pennsylvanicus* (255 minutes/ day; Harrison, et al. 2013), and *Gryllus assimilis* (73 minutes/ day; Bertram, et al. 2021).

Gryllodes sigillatus also differed in what their mate attraction signals look like compared to many other *Gryllus* species. For example, *G. sigillatus* produce calls with an average pulse duration of 8 ms and an interpulse duration of 7 ms, whereas *G. veletis* has an average pulse duration of 16 ms with an interpulse duration of 34 ms (Bertram & Fitzsimmons, 2011). Chirp duration in *G. sigillatus* was much lower than other species, measured at 29 ms for *G. sigillatus*, 69 ms for *G. pennsylvanicus*, and 137 ms for *G. veletis* (Harrison et al. 2013; Bertram & Fitzsimmons, 2011). *Gryllodes sigillatus* had an average of 2.5 pulses/chirp. This value is lower than many of the other species, who range between 3-4 pulses per chirp (*G. pennsylvanicus*; Bertram, et al. 2021), 4 pulses per chirp (*G. veletis*; Bertram & Fitzsimmons, 2011), and 7-9 pulses per chirp (*G. assimilis*; Bertram, et al. 2021). The average amplitude measured in *G. sigillatus* was 36 dB, which is lower than the 62-70 dB measured in other species (*G. pennsylvanicus*; Bertram, et al. 2021 & *G. veletis*; Bertram & Fitzsimmons, 2011; respectively). Average carrier frequency was also much higher in *G. sigillatus* (6460Hz) than in other *Gryllus* species (*G. pennsylvanicus*, 5018Hz; Harrison, et al. 2013; *G. veletis* 5028Hz; Bertram & Fitzsimmons, 2011). These fine scale temporal and spectral species level differences are not surprising, as females typically use the number of pulses per chirp, chirp duration, and carrier frequency as species specific identifiers (Doherty & Storz, 1992).

A previous study that investigated *G. sigillatus* signaling revealed that males signaled with 3-4 pulses per chirp (Champagnon & Cueva del Castillo, 2008). The average pulses per chirp of the males that I monitored is at the bottom range of their findings, as the average

signals I quantified had only 2.5-3 pulses per chirp. There are a few different possibilities for why the values I recorded were lower. The EARS II units are carefully calibrated to pick up male acoustic mate attraction signals. Part of this calibration is inputting threshold numbers for the average signal in a species; this allows the EARS II to determine when to commence recording of a signal. The first pulse that is produced by *G. sigillatus* is a much shorter pulse which is also produced at a much quieter volume than the remainder of the pulses. The EARs often did not pick up the quieter short pulse, suggesting the threshold values inputted were slightly too high, missing the first pulse of signaling data for some of the males.

It is important to note that the population of *G. sigillatus* that I used came from a farm environment, where they rear billions of crickets at a time for human food and livestock feed. These extremely dense rearing conditions, coupled with killing individuals at the five-week mark which is shortly after the crickets reach adulthood, likely causes intense selection. This farm environment may therefore be selecting for more of a scramble competition where everyone mates as quickly as possible, maybe without using signaling to attract potential mates. As a result, there may be selection against calling in this farm reared species. Further, I have no idea how many individuals started the founding population, let alone what proportion of the individuals breed each generation, so it is possible that the *G. sigillatus* crickets I used have limited genetic variation. This farming practice may have eliminated not only variation, but also individuals at the higher end of the range, thus, giving me lower-than-expected results compared to previous studies.

Body Size and Age Effects on Call Components

Signaling changed with male age. As males got older, the probability that they would signal increased. Males only signaled on 27% of nights, reflecting that most males were completely silent in most 24-hour periods. Toward the end of their lifespan, over 50% of males signaled, compared with just 10% in the early days of adulthood. As males aged, they were not only more likely to call, but they also spent more time calling each night. Males exhibited extensive inter-individual variation in how their signaling changed with age. Some males remained at a consistent level of calling effort across their lifespan, but many males only began calling in the last few days of their lives. Time spent calling (Figure 1.5), pulse duration (Figure 1.4), and pulses per chirp all increased with age. Body size significantly influenced the number of pulses per chirp, chirp duration and call duration; smaller males signaled more often and were found to have more pules per chirp, longer chirp duration and longer call duration. Together these results suggest that as individuals grow older, they produce mate attraction signals that are both more energetically costly and are also more attractive compared to when they are younger.

I found a significant interaction effect between age and body size on the following call components: interpulse duration, time spent calling, interchirp duration, and carrier frequency. Smaller males increased their interpulse and interchirp durations as they aged, while larger males decreased their interpulse and interchirp durations as they aged. Smaller males signaled more consistently across their lifespan. Larger males were silent until 38 days at which point, they drastically increased calling to an average of roughly 250 minutes per 24-hour period. Larger male interchirp duration increased. Smaller male signals remained constant with age

while their vigour decreased. Conversely, larger males increased both quantity and quality of their signals with age.

Males appeared to have different signaling strategies in relation to body size. This coupled with the change in calling with age in larger males lends support to the terminal investment hypothesis, as signals became more vigorous and more frequent as individuals got older. As females have been shown to prefer males with more vigorous signals (e.g., Wagner & Hoback, 1999; Champagnon & Cueva del Castillo, 2008; Bertram et al., 2021), males may be altering their calls to appear more attractive to potential mates (Smith, 1991). As signaling is energetically costly (Wagner & Hoback, 1999), individuals can only pay the associated costs if they are in good condition (Smith, 1991). As all individuals used were virgin, they should have been increasingly desperate to secure a mate as they aged and came closer to death. My findings in smaller males support the theory of age-related reproductive senescence, where males would be expected to deteriorate as they age due to factors such as energy constraints or loss of bodily functioning (Archer & Hunt, 2015). As I found a reduction in signal quality in smaller males, it is possible that this is due to age related degradation of signaling organs. Smaller males had to take more breaks (high interpulse and interchirp durations) indicating that they may not have the necessary energy requirements to pay the cost of signaling and therefore had to decrease their signaling effort. As smaller males may be at an automatic mating disadvantage due to their size, they may be utilizing strategies to appear more attractive to potential mates early in life (i.e., vigorous and consistent signaling). By investing a consistent amount of energy into daily signaling across their lifespans, and by having a more vigorous signal, it may increase their odds of finding a potential mate. However, they are unlikely to be able to maintain this higher level of calling, which is why they do not

ramp up their calling as much later in life as the larger males do. Further, it may also explain why smaller males take longer breaks between their pulses and chirps as they get older, while larger males take shorter breaks between their pulses and chirps. Reproductive senescence might have been observed in larger males, had I observed them for longer than 48 days post-imaginal eclosion (which would have been more than twice the length that they typically live-in nature).

An interesting finding was that smaller males had lower carrier frequency when compared with larger males. This is quite strange as larger males in other species typically have lower carrier frequencies due to their larger signaling organs (Scheuber et al., 2003). My finding indicates that perhaps females in this species do not prefer lower carrier frequency or that these farmed individuals may not have had to rely on signaling as much and instead rely on visual or chemical cues (Mullen, et al. 2007). Since the individuals used are from such a dense environment, it is possible that selection has favoured more intimate mate attraction methods, which larger males would utilize.

As not a lot of work has been done on quantifying this species' signaling behaviour, future studies should aim to examine different aspects of male signaling, age and body size more in depth. Individuals should be analyzed until their natural death to get the full spectrum of male calling across their entire lifespan. This would help shed further light on which hypothesis (i.e., terminal investment hypothesis, senescence or a combination) is most closely supported in this species and help to further explain carrier frequency. It would also be helpful for a study to compare the signaling vigour and fine scale spectral and temporal properties of *G. sigillatus* in their natural environment, to ascertain whether the reduction in signaling vigour that we see relative to other *Gryllus* species is a *Gryllodes sigillatus* trait, or whether it

is an artifact of using farm reared insects. It would also be helpful for future work to be done in teasing apart the effects of body size on mate strategy. Chemical, visual and courtship displays could also be analyzed in this species.

Chapter 2 – How Acoustic Mate Attraction Signaling Is Influenced by Foreign Body Ingestion

Mate attraction signaling in many animals is condition dependent (Holzer et al., 2003; Martin & Lopez, 2010; Takeshita et al., 2018). As a result, immunity, age and nutrition likely all factor into an individual's reproductive strategy and may help to explain variation within and across courters. Reproductive behaviour may trade-off with immunity when courters experience illness or parasitism, and this trade-off may be age and condition dependent. Specifically, courters in high condition may be better able to afford to continue to pour resources into reproduction even in the face of immunity challenges, while lower condition courters may be unable to handle the same immunity challenges, and as a result may reduce their overall signaling effort or quality.

Challenging courter condition can and does negatively impact courter acoustic mate attraction signaling behaviour. This negative relationship between parasite infestation and courter sexual displays has been observed in many anuran species. For example, Moretti et al (2014) quantified the relationship between calling vigour and helminth parasite intensity in the Brazilian treefrog, *Hypsiboas prasinus*. They determined that male calling characteristics were negatively correlated with parasite intensity and, importantly, that the relationship between calling vigour and parasite load was more pronounced in calling properties pertaining to vigour than in other aspects of male calls. The relationship is also observable in field crickets. Orozco and Bertram (2004) showed that male Texas field crickets (*Gryllus texensis*) parasitized with *Ormia ochracea* (Diptera, Tachinidae, Ormiini) exhibited significantly reduced total signaling times, and produced calls with shorter duration at slower trilling bout rates than unparasitized males.

In *Gryllodes sigillatus,* males reproduce by attracting females from a distance using acoustic mate attraction signals and then, when females are nearby, they switch to a short-range courtship call to encourage females to mount them (see Overview for details). Little has been published about how immune challenges impact acoustic mate attraction signaling in *G. sigillatus*. The only published research I am aware of investigated whether there was a relationship between lytic activity and courtship call rate (Ketola et al. 2009). Ketola et al. (2009) found no relationship between courtship calling and lytic activity. To my knowledge, mate attraction signaling, and immune challenges have never been investigated.

The effects of parasitism and other immune challenges on spermatophylax production has, however, been extensively investigated using *G. sigillatus* as a model organism. Courting male *G. sigillatus* can increase their reproductive effort by altering the amino acid composition of their spermatophylaxes. Changes in the amino acid composition presumably increases the gustatory appeal to choosy females (Duffield et al., 2015). Immune challenges impact spermatophylaxes. Kerr et al. (2010) experimentally injected male *G. sigillatus* to simulate an immune response; they also experimentally injected some male courters to increase spermatophore yield. Courters with a high immune response produced smaller spermatophores, while courters with large spermatophores had lower immunity (Kerr et al. 2010). Kerr et al's (2010) findings suggest that *G. sigillatus* courting males exhibit a fundamental trade-off between immunity and reproduction. As a result, the spermatophylax may act as a condition-dependent indicator (Kerr et al., 2010). *Gryllodes sigillatus* populations can also become infected with the sexually transmitted parasite *Mehdinema alii* (Nematoda: Diplogasterida). While this parasite uses choosy females as hosts, they can infect courting males during host mating (Luong, 2004). Choosers do not appear to exhibit mate choice for

courters based on male infection status (Luong & Kaya, 2005). Male *Gryllodes sigillatus* with an *M. alii* infection produce smaller spermatophores (Luong, 2004). Courters in higher condition may be better able to fight off and better compensate when dealing with parasites than courters in poorer condition. To complement these strong findings on how immune challenges impact reproduction in *G. sigillatus*, here I investigate how interacting with a pest species impacts acoustic courters mate attraction signaling vigour and quality using the edible cricket species, *Gryllodes sigillatus*.

Rearing crickets for food and feed has recently become profitable in western countries, in part because the nutritional value in rearing and consuming crickets is higher than that of traditional meats and results in substantially less environmental damage (Magara et al., 2021). There are several established farms in North America that are raising *Gryllodes sigillatus* both for human consumption and for consumption by pets and one of these farms (Entomo Farms) is located here in Ontario. As a spin-off to the Bertram and MacMillan Labs research with Entomo Farms, I wanted to investigate how adult male signaling behaviour might be influenced by the ingestion of a foreign body (hastisetae from *Dermestes ater*). The beetle *Dermestes ater* is often found in stored food products and is a scavenger of dead insects and moult (Ruzzier et al., 2020). The dense farm environment results in an accumulation of moults and dead insects, providing an abundant and rich sustenance for dermestids. Dermestids are problematic to crickets because, during their larval stage, dermestids are covered in a dense layer of setae called hastisetae (Figure 2.1). The setae are small, barbed, and are thought to be used as a mechanical defense against predation by arthropods (Ruzzier et al., 2020). These hastisetae are easily removed with any kind of mechanical force (Ruzzier et al., 2020) and are

therefore likely to be found in any area inhabited by dermestid beetles, especially in the crickets' feed.

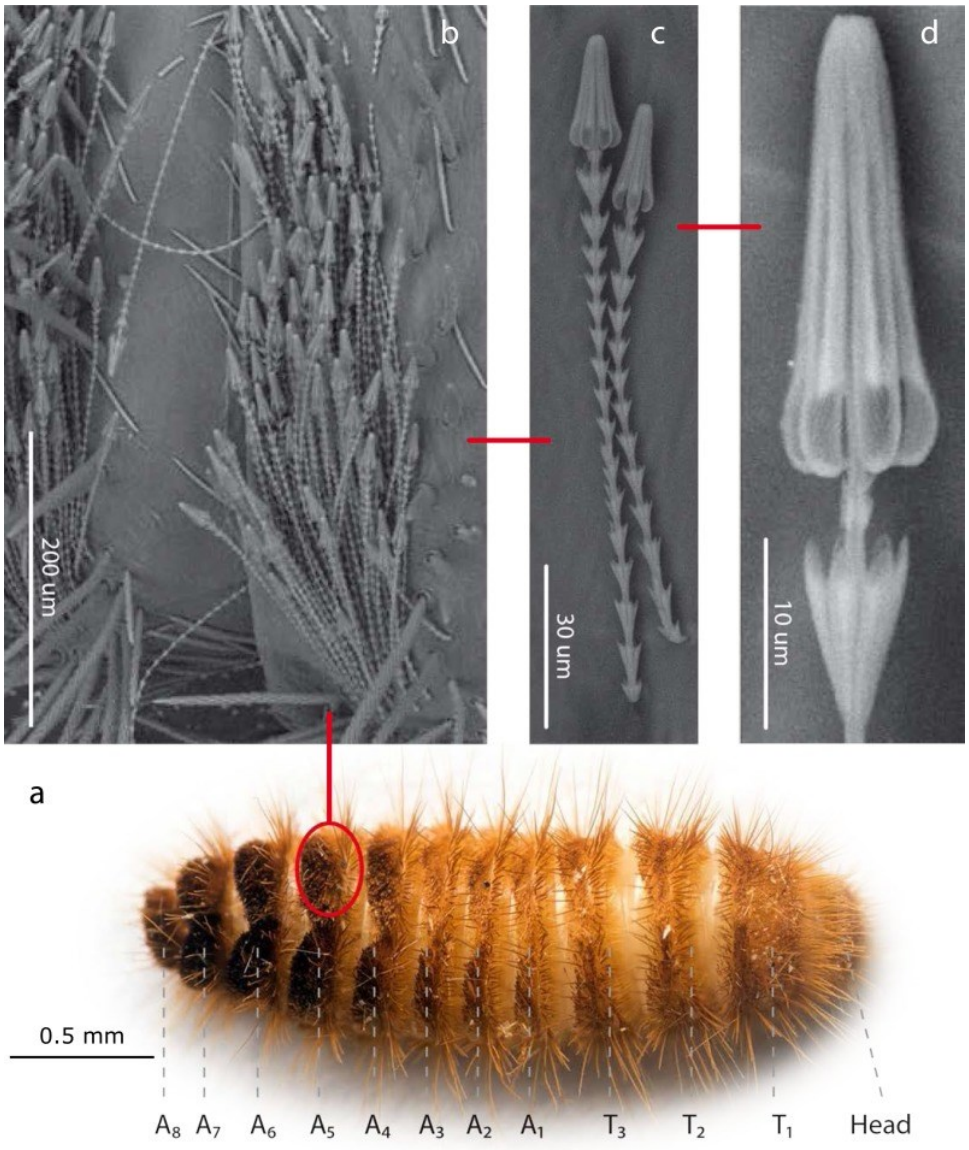

Figure 2.1. "(A). *Megatominae* larva (dorsal view), with abdominal and thoracic segments labelled. (B). Hastisetae as they are seen on abdominal segments. (C). Lateral view of hastisetae. (D). Depicts head of hastisetae." Photograph taken from Ruzzier et al., 2020.

Little is known about how hastisetae ingestion influences crickets or other insects. Nutting & Spangler (1969) fed dermestid larvae to earwigs that had not been fed for several

hours. The earwigs ate 5-6 larvae over several days, and then refused to eat. Upon dissection, their foregut was observed to be infested with hastisetae. While this study suffers from a small sample size (N = 2), it suggests that dermestid hastisetae may cause problems for insect guts. I suspect that farm-reared *G. sigillatus* are regularly ingesting hastisetae as they consume their food at the farm. A preliminary analysis performed by Marshall Ritchie in the MacMillan lab at Carleton University revealed structures in the guts of farm reared *Gryllodes sigillatus* similar to the hastisetae of dermestid (Figure 2.2). Further, the hastisetae appear to have ruptured the cricket guts, warranting further investigation as a leaky gut could lead to a decreased ability for males to adequately absorb nutrients, thus leading to lower condition. Additionally, parasitism may impact male condition due to trade-offs between reproduction and immunity – with gut health also affecting immune activation in crickets. As male signaling is condition-dependent, it is important to understand the potential impact that the presence of hastisetae may have on male fitness.

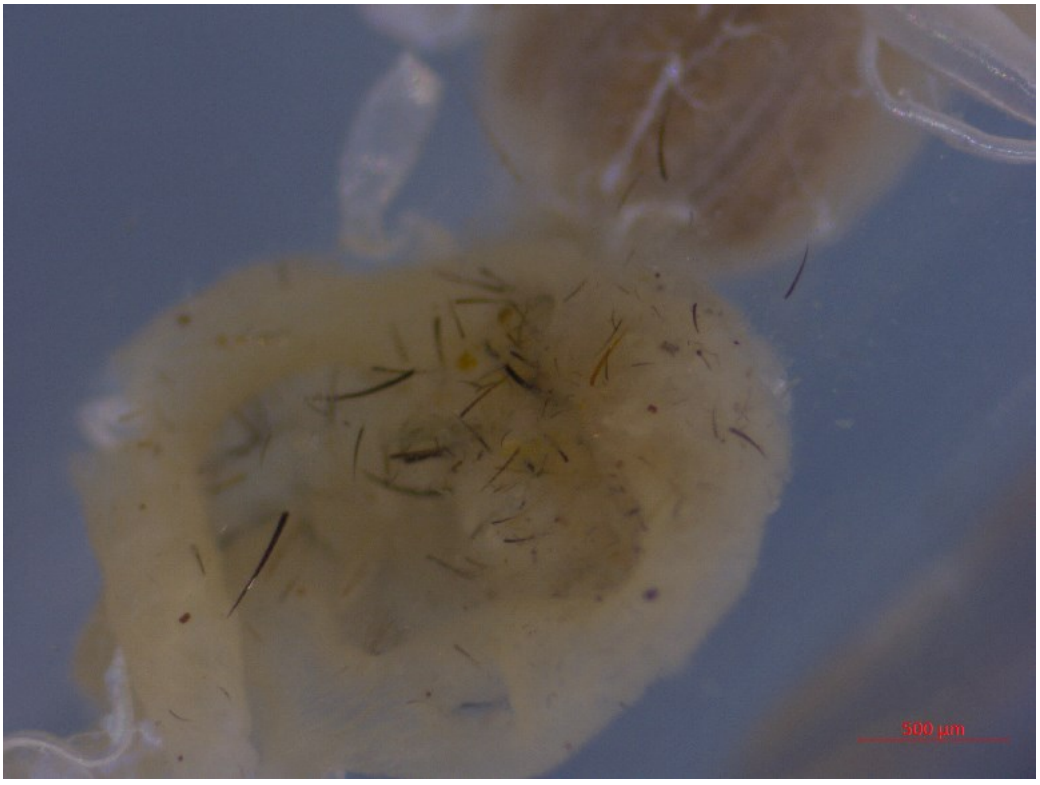

Figure 2.2. *Gryllodes sigillatus* crop showing hastisetae infestation.

While puncture and perforation of an insect's gut is common and has been explored in the context of healing and the associated mechanisms, little literature exists on the long-term effects of repeated gut punctures on insect fitness. Invertebrates have the capacity to heal wounds in the gut (Huang et al., 2016), however, the effects of repeated chronic punctures have rarely been investigated. One exception to this general observation is the work on damselflies. Siva-Jothy et al (2001) used phenol oxidase to measure the immune system of damselflies that had a chronic mid-gut infection by eugregarine trophozooites parasites. The researchers induced an acute haemolymph effect by inserting a small nylon filament. They found that when two physiological compartments were challenged, the phenol oxidase levels in the midgut were significantly reduced indicating that maintaining phenol oxidase levels across two or more compartments was costly (Siva-Jothy et al., 2001). As the gut microbiome of insects plays an important role in immune system functioning (Smith et al., 2017), the

chronic onslaught of punctures by dermestid hastisetae may impact an individual's immune function, and as a result their overall condition. Given signaling is often influenced by condition, I wanted to investigate whether repeated exposure to dermestid hastisetae impacts male signaling vigour.

Here I investigated how ingestion of the pest species *Dermestes ater*'s hastisetae impacts within and among male variation in signaling vigour. Hastisetae may cause "leaky" or damaged guts, thereby lowering the males' ability to effectively absorb nutrients from their food, and as a result likely impacting their signaling vigour. I hypothesized that males exposed to hastisetae will have decreased fitness (H_1). I hypothesized that males reared on food containing hastisetae will signal less vigorously and that their signals will be less attractive than males reared on food containing no hastisetae. I predicted that their signals will overall be less attractive with higher carrier frequencies, quieter chirps and that they will signal less often.

Methods

I conducted this research with several other members of the Bertram and MacMillan labs, including Matthew Muzzatti and Marshall Ritchie. I therefore use plural (we) when discussing the methods. Matt and Marshall reared the crickets and will conduct analyses on how diets containing hastisetae impact development time, survival to adulthood, and overall adult body size. I then took the males and quantified how diets containing hastisetae impact male acoustic mate attraction signaling behaviour. Here I present just my findings on how hastisetae contaminants in the diet impact adult male acoustic mate attraction signaling.

Juvenile males of unknown age were delivered to Carleton University from Entomo Farms during Spring 2022. We housed the males in individual containers (540 mL) with *ad libitum* food and water, under a 16:8-hour light cycle, at 30 ± 2 °C. When males reach their penultimate moult, they were assigned to one of three conditions (control n = 9, high n = 13, very high n = 6) with *ad libitum* food and water, under a 16:8-hour light cycle, at 30 ± 2°C. The control diet had no hastisetae present, the high diet had 1 dermestid/gram of food, and the very high diet had 2 dermestids/gram of food. These diets were prepared with dermestid larvae supplied by Entomo Farms (Norwood, Ontario, Canada). These dermestids were frozen and then dried at the farm by the resident entomologist, Dr. Lee Bess. Once they arrived at Carleton, we put them in a centrifuge tube and vortexed them to encourage the hastisetae to dislodge. The hastisetae were then added to the food (Earth's Harvest Organic Cricket Grower).

Once males reached their final moult they were weighed (OHAUS Pioneer Analytical Balance Model: Adventurer SL AS64; SE = 0.0001g) and then I placed them in the Electronic Acoustic Recording system (EARs) to quantify their long-distance mate attraction signals. Courters were placed in the EARS for up to 3 weeks to have their signaling behaviour quantified, or until death, whichever came first. During this time, the EARS continually recorded signaling, as described in Chapter 1. I analyzed the signaling data contained in the EARS files using the Spike2 (designed by Cambridge Electronic Design, Cambridge, UK) computer program; this program parsed each data file into useable data.

Following acoustic recording, any courters still alive were cold euthanized by placing them in the freezer. We photographed the courting males dorsal side up (Carl Zeiss, Stereo Microscope Stemi 305 KMAT) using ImageJ, and then quantified head width, pronotum

width, and pronotum length (Figure 1.1). I then used these morphological values as covariates in the model to determine the effect that concentration of hastisetae has on male signaling ability and on male survival.

Statistical Analysis

I used JMP 16.0.0 to determine if hastisetae in the diet impacted courter male acoustic mate attraction signaling behaviour. I ran general linear mixed models using the following as fixed effect predictors: body size (a combined measure of pronotum size and body mass), presence of hastisetae (Diet), and age. I included an interaction between age and hastisetae presence as well as an interaction between size and hastisetae presence. I did not include the three-way interaction between size, age and hastisetae presence in the diet because my sample size was too limited. Individual (ID) was included as a random effect to account for repeated measures. I included the body size and age components, as Chapter 1 results indicated that both explain some of the variation in signaling behaviour. I log-transformed time spent calling data to meet Gaussian model fit assumptions. To determine whether the probability of calling or not throughout a 24-hour period was influenced by diet, I ran a logistic regression using the same fixed and random effects as described above.

Results

Diet infestation with hastisetae on its own did not significantly influence any of the mate attraction signal components, although there were significant interactions with other terms and diet, described below (Table 2.1). Age, on its own, influenced interchirp duration ($p = 0.0191$; Figure 2.3) and call duration ($p = 0.0026$; Table 2.1). Specifically, call duration

increased with age, while interchirp duration decreased with age. Size, on its own, did not significantly influence any of the mate attraction signal components (Table 2.1). There were significant interactions between diet infestation with hastisetae, age and body size, that influenced male signaling traits. Specifically, an interaction between diet and body size influenced time spent calling ($p = 0.0473$; Figure 2.4), interpulse duration ($p = 0.0487$) and carrier frequency ($p = 0.0189$). These interactions can best be explained in the following way: larger males signaled with higher time spent calling than smaller males when fed diets containing no or few hastisetae (Figure 2.4A). However, the opposite occurred for males fed diets containing very high levels of hastisetae. Under this diet treatment, smaller males signalled most often, with medium sized males not signaling often at all and very large males not surviving to adulthood. The same patterns occurred with interpulse duration and carrier frequency. Larger males fed diets containing no or lower levels of hastisetae produced more attractive signals (lower interpulse durations and lower carrier frequencies) compared to smaller males. However, smaller males that were fed the very high levels of hastisetae produced the more attractive calls, signaling with lower interpulse durations and carrier frequencies. These findings suggest that smaller males fed food containing very high levels of hastisetae greatly increased their signaling vigour while making their calls more attractive compared to medium sized males.

I also found an interaction between age and body size influenced amplitude ($p = 0.0047$; Figure 2.5). Here, smaller males decreased their amplitude as they aged, while larger males increased their amplitude as they aged.

I included ID as a random effect to control for repeated measures. It is important to note that ID explained an extensive amount of variation in each of the linear mixed models, accounting for between 51-70% of the variance in the models (Table 2.2).

The likelihood of signaling was analyzed using a logistic regression to determine the effect that diet infestation with hastisetae, age and body size have (Table 2.3). Significant effects were found for age ($p < 0.0001$) and body size ($p < 0.0001$). I also found significant interactions between age and body size ($p < 0.0001$), and diet infestation with hastisetae and body size ($p < 0.0001$). Overall, as males aged, they were more likely to signal. Smaller males signalled consistently, and larger males increased the likelihood of signaling with age.

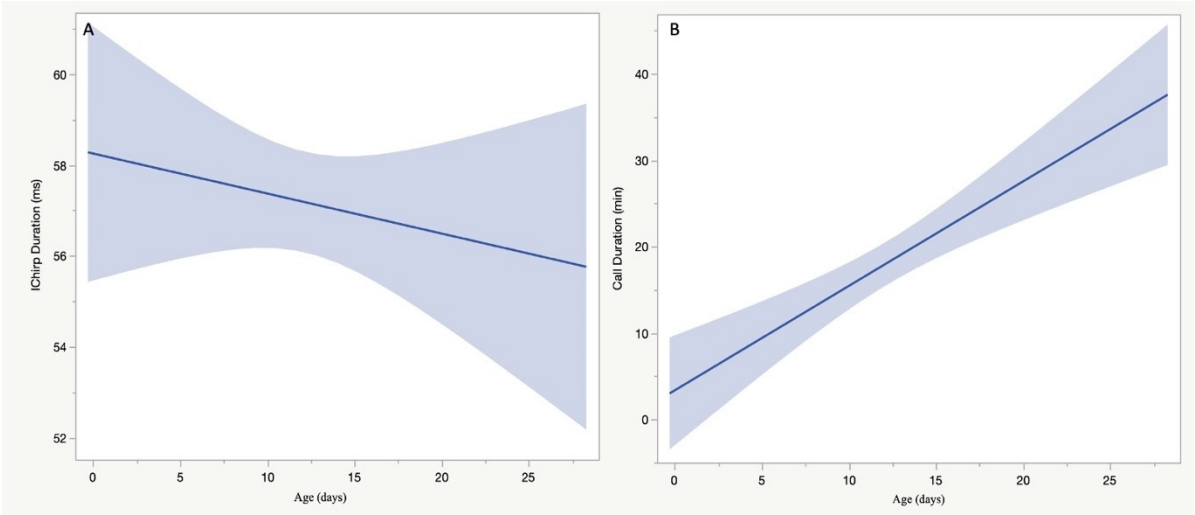

Figure 2.3. Significant effect of age across life span on call components: A.) interchirp duration, & B.) call duration.

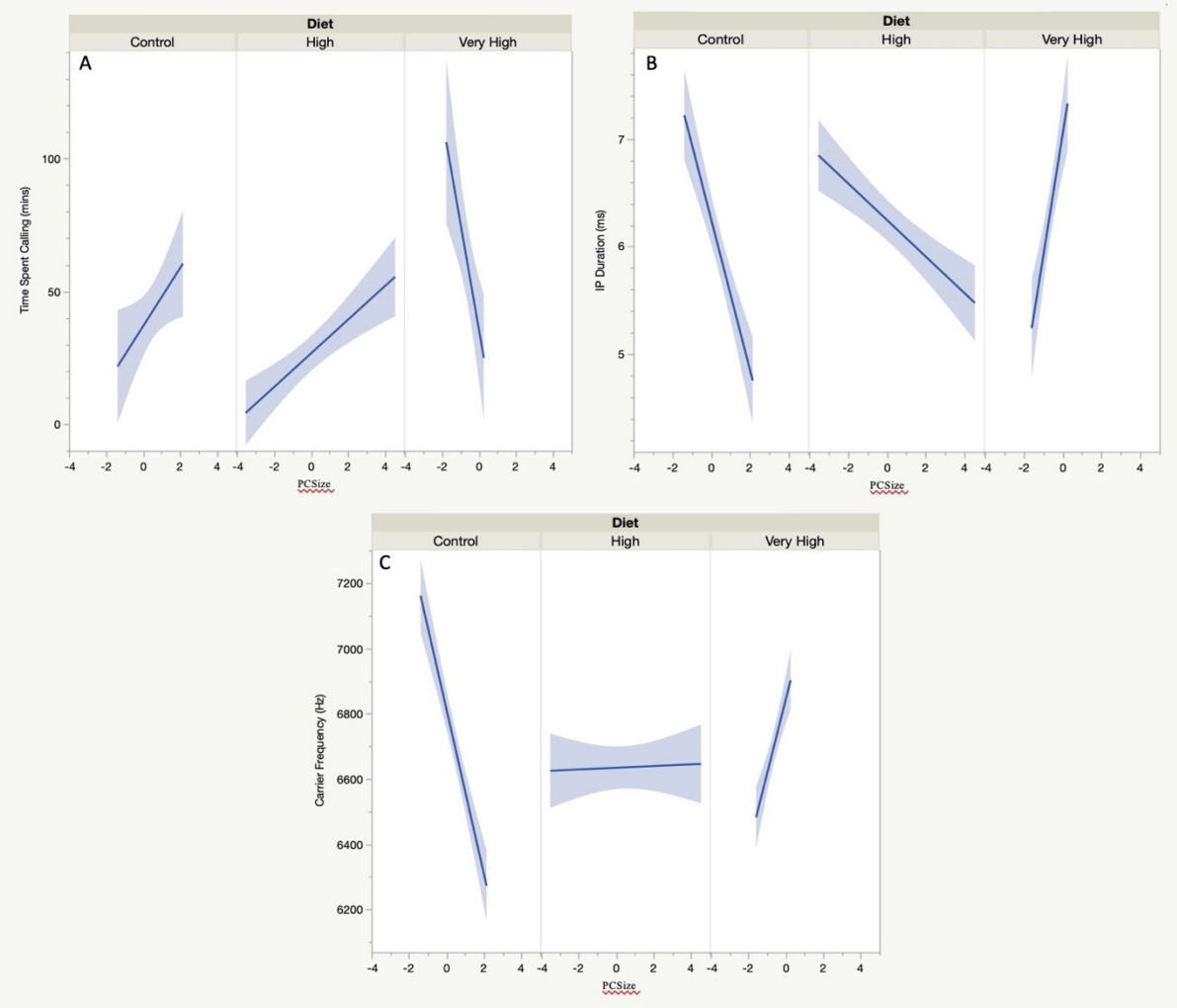

Figure 2.4. Significant interaction effect of diet and body size on call components: A.) time spent calling, B.) interpulse duration, & C.) carrier frequency.

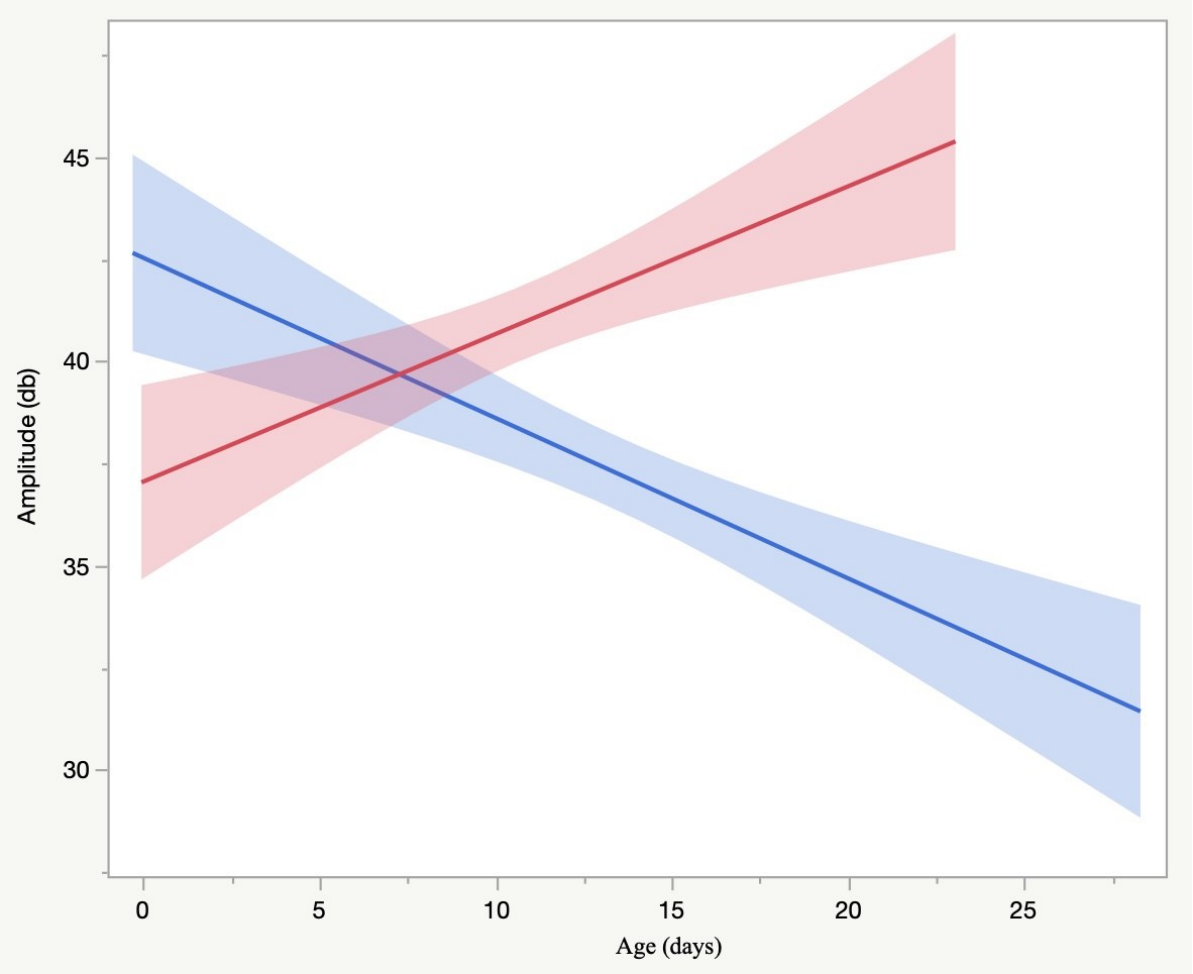

Figure 2.5. Significant interaction effect of age and body size on amplitude. Blue lines represent small males and red lines represent large males.

Table 2.1. Fixed factors influencing signaling behaviour. General linear mixed model results for age, body size, diet and their interaction (*). Bolded terms are significant.

Trait	Fixed Effects	Estimate	SE	DF	t	P
time spent calling	**Intercept**	**2.565**	**0.530**	**18.4**	**4.84**	**0.0001**
	Diet [Control]	0.283	0.327	12.3	0.87	0.4033
	Diet [High]	0.200	0.319	12.8	0.63	0.5414
	Age	0.051	0.040	12.1	1.28	0.2243
	Diet [Control]*Age	-0.019	0.049	12.2	-0.39	0.7036
	Diet [High]*Age	0.047	0.048	12.2	0.98	0.3447
	Size	-0.405	0.247	12.0	-1.64	0.1267
	Diet [Control]*Size	0.595	0.307	11.7	1.94	0.0773
	Diet [High]*Size	**0.597**	**0.257**	**12.1**	**2.32**	**0.0384**
	Age*Size	0.029	0.037	11.6	0.77	0.4541
pulse duration	**Intercept**	**8.164**	**0.217**	**29.9**	**37.7**	**< 0.0001**
	Diet [Control]	0.418	0.224	15.0	1.87	0.0815

	Diet [High]	0.008	0.217	15.4	0.04	0.9709
	Age	-0.007	0.012	17.9	-0.58	0.5660
	Diet [Control]*Age	0.005	0.015	19.8	0.33	0.7449
	Diet [High]*Age	0.011	0.014	14.6	0.75	0.4676
	Size	-0.090	0.169	15.0	-0.53	0.6029
	Diet [Control]*Size	0.266	0.212	14.6	1.26	0.2282
	Diet [High]*Size	0.294	0.177	15.0	1.66	0.1170
	Age*Size	0.001	0.011	18.1	0.13	0.8968
interpulse duration	**Intercept**	**6.889**	**0.302**	**27.6**	**22.8**	**< 0.0001**
	Diet [Control]	-0.356	0.297	14.8	-1.20	0.2496
	Diet [High]	-0.390	0.288	15.2	-1.35	0.1964
	Age	-0.029	0.018	13.7	-1.65	0.1212
	Diet [Control]*Age	-0.007	0.022	14.4	-0.32	0.7548
	Diet [High]*Age	-0.026	0.021	12.8	-1.22	0.2449
	Size	0.104	0.225	14.7	0.46	0.6509
	Diet [Control]*Size	**-0.740**	**0.281**	**14.4**	**-2.63**	**0.0193**
	Diet [High]*Size	-0.336	0.234	14.8	-1.43	0.1729
	Age*Size	0.013	0.016	13.1	0.82	0.4250
pulses per chirp	Intercept	2.602	2.602	2.60	2.60	2.6020
	Diet [Control]	0.049	0.071	13.6	0.69	0.5010
	Diet [High]	-0.038	0.069	14.1	-0.55	0.5909
	Age	0.010	0.005	15.5	1.94	0.0705
	Diet [Control]*Age	-0.004	0.006	16.3	-0.64	0.5318
	Diet [High]*Age	0.001	0.006	14.6	0.15	0.8827
	Size	0.015	0.054	13.5	0.28	0.7845
	Diet [Control]*Size	0.049	0.067	13.2	0.74	0.4749
	Diet [High]*Size	0.030	0.056	13.5	0.53	0.6019
	Age*Size	0.004	0.004	14.8	0.89	0.3893
chirp duration	**Intercept**	**29.70**	**1.107**	**33.2**	**26.8**	**< 0.0001**
	Diet [Control]	1.149	1.108	14.3	1.04	0.3172
	Diet [High]	-1.205	1.074	14.6	-1.12	0.2802
	Age	0.046	0.064	18.4	0.71	0.4841
	Diet [Control]*Age	-0.076	0.079	19.4	-0.97	0.3451
	Diet [High]*Age	0.029	0.076	17.4	0.38	0.7119
	Size	0.252	0.838	14.2	0.30	0.7679
	Diet [Control]*Size	0.235	1.049	13.9	0.22	0.8259
	Diet [High]*Size	0.433	0.874	14.2	0.50	0.6278
	Age*Size	0.088	0.058	17.6	1.51	0.1480
interchirp duration	**Intercept**	**62.79**	**2.657**	**30.8**	**23.6**	**< 0.0001**
	Diet [Control]	-0.017	2.420	13.2	-0.01	0.9945
	Diet [High]	-0.872	2.349	13.6	-0.37	0.7163
	Age	**-0.441**	**0.168**	**14.9**	**-2.63**	**0.0191**
	Diet [Control]*Age	0.139	0.207	15.5	0.67	0.5098
	Diet [High]*Age	-0.407	0.201	14.4	-2.03	0.0617
	Size	-1.736	1.829	13.1	-0.95	0.3597
	Diet [Control]*Size	-1.094	2.287	12.8	-0.48	0.6403

	Diet [High]*Size	-0.392	1.907	13.1	-0.21	0.8404
	Age*Size	0.091	0.154	14.1	0.59	0.5650
carrier frequency	**Intercept**	**6736**	**93.02**	**23.5**	**72.4**	**< 0.0001**
	Diet [Control]	7.893	90.51	14.4	0.09	0.9317
	Diet [High]	-71.90	87.67	14.7	-0.82	0.4253
	Age	1.782	5.528	9.88	0.32	0.7540
	Diet [Control]*Age	-9.540	6.795	10.2	-1.40	0.1900
	Diet [High]*Age	12.14	6.624	9.71	1.83	0.0976
	Size	-25.29	68.44	14.3	-0.37	0.7172
	Diet [Control]*Size	**-249.8**	**85.77**	**14.0**	**-2.91**	**0.0114**
	Diet [High]*Size	47.49	71.35	14.3	0.67	0.5163
	Age*Size	1.518	5.061	9.36	0.30	0.7709
amplitude	**Intercept**	**36.20**	**1.588**	**26.4**	**22.8**	**< 0.0001**
	Diet [Control]	1.255	1.637	14.3	0.77	0.4558
	Diet [High]	0.053	1.586	14.6	0.03	0.9737
	Age	0.182	0.088	14.7	2.06	0.0578
	Diet [Control]*Age	**-0.239**	**0.109**	**15.5**	**-2.19**	**0.0438**
	Diet [High]*Age	0.063	0.106	13.8	0.59	0.5636
	Size	0.679	1.238	14.3	0.55	0.5922
	Diet [Control]*Size	1.268	1.551	14.0	0.82	0.4273
	Diet [High]*Size	0.705	1.291	14.3	0.55	0.5935
	Age*Size	**0.270**	**0.080**	**14.0**	**3.36**	**0.0047**
call duration	Intercept	-1.947	5.990	21.0	-0.32	0.7484
	Diet [Control]	-2.464	5.156	14.5	-0.48	0.6399
	Diet [High]	-0.186	5.025	15.1	-0.04	0.9709
	Age	**1.506**	**0.394**	**11.6**	**3.82**	**0.0026**
	Diet [Control]*Age	-0.445	0.483	12.0	-0.92	0.3756
	Diet [High]*Age	-0.062	0.473	11.1	-0.13	0.8980
	Size	-2.407	3.885	14.4	-0.62	0.5453
	Diet [Control]*Size	4.154	4.851	14.0	0.86	0.4062
	Diet [High]*Size	4.582	4.051	14.4	1.13	0.2766
	Age*Size	0.082	0.356	10.8	0.23	0.8214

Table 2. 2. Random factors influencing signaling behaviour. General linear mix model results for random factors and their interaction (*). The models run were: Trait ~ age + |ID + |ID*age. Bolded terms are significant.

Trait	Random Effects	Standard Error	Variance Component	Percent of Total	P	R^2 adjusted
time spent calling	**ID**	**0.33**	**0.6934**	**34**	**0.0373**	51%
	ID*Age	0.01	0.0104	0.6	0.0912	
	Residual	0.10	1.0368	60		
pulse duration	**ID**	**0.14**	**0.3498**	**52**	**0.0142**	60%
	ID*Age	0.00	-0.0001	0	0.9098	
	Residual	0.03	0.3263	48		

interpulse duration	**ID**	**0.25**	**0.6242**	**55**	**0.0139**	65%
	ID*Age	0.00	0.0007	0.1	0.4936	
	Residual	0.05	0.5052	45		
pulses per chirp	**ID**	**0.01**	**0.0343**	**48**	**0.0219**	51%
	ID*Age	0.00	0.0001	0	0.4231	
	Residual	0.00	0.0374	52		
chirp duration	**ID**	**3.60**	**8.7519**	**57**	**0.0151**	61%
	ID*Age	0.01	0.0100	0	0.4105	
	Residual	0.62	6.5114	43		
interchirp duration	**ID**	**17.8**	**40.703**	**52**	**0.0220**	56%
	ID*Age	0.09	0.0979	0.1	0.3006	
	Residual	3.63	38.034	48		
carrier frequency	**ID**	**24000**	**59332**	**62**	**0.0133**	70%
	ID*Age	128	128.62	0.1	0.3139	
	Residual	3450	35657	37		
amplitude	**ID**	**7.85**	**19.353**	**61**	**0.0136**	66%
	ID*Age	0.03	0.0193	0.1	0.4619	
	Residual	1.18	12.314	39		
call duration	**ID**	**76.2**	**176.87**	**44**	**0.0202**	52%
	ID*Age	0.59	0.4430	0.1	0.4526	
	Residual	22.1	223.06	56		

Table 2. 3. Effects of age, body size, diet and their interaction (*) on likelihood of male signaling in a 24-hour period. Bolded terms are significant effects.

Fixed Effects	ChiSquare	P
Age	**72.600**	**<0.0001**
PCSize	**16.388**	**<0.0001**
Age*PCSize	**20.515**	**<0.0001**
Diet	4.470	0.1070
Diet*Age	0.055	0.9729
Diet*PCSize	**33.343**	**<0.0001**

Discussion

While diet did not significantly impact signaling traits on its own, diet interacted with age and body size to influence several of the signaling traits. Males in the control and high

treatments did not differ from each other in how they signalled, while males in the very high behaved differently from the other two treatments.

In support of my Chapter 1 findings, in Chapter 2 I also found that smaller control and high treatment males signaled consistently across their lives, while larger control and high treatment males increased signaling with age. Interestingly, males in the very high treatment behaved opposite to this, suggesting they may be responding strongly to the presence of the hastisetae. Specifically, smaller individuals produced much more frequent and more attractive signals while larger individuals rarely signalled and, when they did, they produced less attractive signals. Why might small individuals that experience very high hastisetae infestation greatly ramp up their signaling vigour as they age? It is possible that because of leaky guts they can somehow detect that they are at risk and that they ramp up their signaling effort early in life so that they have an opportunity to mate prior to dying. Essentially, these males appear to be using a live fast, die young strategy. The live fast, die young hypothesis postulates that signallers allocate all resources into calling earlier in life and as a result, die much sooner than their lower effort signaling conspecifics (Okada, et al. 2011).

Interestingly, none of the larger males in either of the three treatments lived past 19 days. While it was a much smaller sample size than I had hoped for (n = 14: control = 6, high = 6, very high = 2), there were males in each of the three treatments that became large adults. Large males in the very high treatment did not increase their signal quality and quantity with age and, in the case of amplitude, instead reduced signal quality with age. This finding suggests a possible trade-off between immune challenges (i.e., parasitism) and reproduction, but that the trade-off may be size dependent. Similarly, *Gryllus texensis* (Texas field cricket) has also been shown to significantly reduce signaling effort when parasitized (Orozco &

Bertram, 2004). Replicating this study using a comparative population of wild caught individuals would give an excellent baseline for comparison to see if crickets from the farm population have experienced artificial selection as a result of the farm pressures, and whether these results change with size effects.

Smaller males in the very high treatment were high effort signallers. This finding contrasted with my prediction that individuals ingesting more hastisetae would be lower effort callers. I discovered after completion of trials that our method of hastisetae removal altered the structure of the hastisetae, specifically the barbed head portion (Figure 2.1). This is the portion of hastisetae that I suspect may be responsible for imbedding itself in the cricket gut. With this portion of the hastisetae compromised, it is possible that the intended effect (i.e., cricket crops becoming infested with hastisetae) was structurally less able to occur. Hastisetae are mainly comprised of chitin and protein, though their exact composition is unknown (Ruzzier, et al. 2020; Ruzzier, et al. 2021).

It is also possible that the protein contained in the composition of hastisetae may have fortified the cricket's diet, thereby giving them more nutrients to fuel their energetically costly signaling displays; studies have previously found that males signal more on protein rich diets (Hunt, et al. 2004). It is also possible that the treatments were simply not a high enough concentration of dermestid hastisetae to elicit the expected results.

Another possible explanation for the measured differences between smaller and larger males is the theory of reproductive compensation. In this theory, individuals may be forced to settle for mates that are outside of their preferences due to environmental or social forces (such as parasitism) and may therefore compensate for this (Gowaty, 2008). Reproductive compensation has been investigated before in different host-parasite systems within *Gryllidae*

species. One study (Kelly et al. 2015) found that when attractive *G. texensis* callers were immune challenged, they drastically increased signaling effort compared with unattractive calls. Kelly et al. (2015) posited that higher condition males were able to pay the associated costs of investing more into reproduction and as a result, terminally invested to offset future decreased reproduction. This finding is similar to the effect that I found in large males within the high diet treatment that signaled identically to their control (non-immune challenged) counterparts. As these individuals were in otherwise good condition, they could afford to allocate their resources into reproduction but at the cost of dying younger. Alternatively, other studies have found that male crickets did not exhibit compensation in host-parasitism systems and in the case of one study (Kolluru et al., 2002), male *T. oceanicus* were found to decrease their reproductive effort when parasitized. As seen in large males in the very high treatment, they may have been unable to pay the costs associated with increasing signaling.

Future studies should aim to examine the effects of hastisetae ingestion on signaling behaviour across all individuals natural lifespans in both wild-caught and farm-reared populations. As well, improving hastisetae collection and increasing the amount of hastisetae ingested may shed further light on the fitness costs associated.

Conclusions

Overall, I found that as courter individuals age, they spend more time calling but the quality and vigour of these calls is dependent on individual body size and hastisetae infestation. Smaller courters tended to maintain their signaling effort with age, with the exception that when they are heavily infested with hastisetae they increased their signaling effort. Conversely, larger courters rarely signalled early in adulthood and then increased their signaling vigour and signal quality. Ingestion of dermestid foreign bodies did not significantly

impact courter male signaling in the high treatment, but had an effect in the very high treatment, but the effects of hastisetae can only be seen when body size is taken into consideration. Smaller courters that were heavily infested with hastisetae signalled much more vigorously with age than smaller individuals that were not infested. Conversely, larger courters that were heavily infested with hastisetae did not signal as often, while larger courters who were not exposed (or exposed lightly) to hastisetae increased their signaling vigour and quality with age. Overall, there is strong support of the effects of age and size on signaling behaviour across the two chapters of research.

I found an unexpected result with carrier frequency. Larger males in Chapter 1 had a higher carrier frequency when compared with smaller males – this is opposite to what occurs in other species. Conversely, in Chapter 2, males in the control and very high treatments behaved as expected with larger males having lower carrier frequencies than smaller males. However, males in the high diet behaved as males in Chapter 1, with larger males having higher carrier frequencies. A possible explanation for these rather strange findings is that individuals used in Chapter 1 came directly from the farm as pinheads and could have ingested dermestid hastisetae during their nymph development. This is something that needs to be explored further in this species to better understand the effect of body size on carrier frequency. Conversely, because male *G. sigillatus* signal so little compared to other cricket species, it could be that the EARs are not able to properly capture signaling carrier frequency.

Chapter 2 (effect of foreign body ingestion) should be replicated with a much larger sample size to allow for a survival analysis. I had originally planned on conducting a survival analysis, however with all of the equipment issues (see Supplemental Data) I had, I was unable to have a sample size that was large enough to run one. I anecdotally found that larger males

did not live as long as smaller males. Future work should also aim to further determine if there is a relationship between body size, ingestion of dermestid foreign bodies and survival, to ascertain if courters opt for different strategies dependent on either their body size, condition, or their exposure to pests. A larger sample size and comparison with a wild caught population would greatly help to further our understanding of the extensive interspecific and intraspecific variation in signaling behaviour in courter male *Gryllodes sigillatus*.

Supplemental Data: The Universe Strives for Maximum Chaos

Table S.1. Everything that can go wrong – will.

Date	Attempt	What Went Wrong	What I Learned
Fall 2020	My Masters started during the pandemic. I originally planned on expanding upon my Undergraduate work by studying female mate preference.	Due to lockdowns and the uncertainty, we opted against studying females as it required more in-lab time.	I learned to be flexible and willing to change parts of my study as issues arose which proved to be an invaluable lesson.
Fall 2020	Got my first few shipments from Entomo of juveniles and adults to use males in EARS trials.	Every time that males were separated from females, all males died within a few days.	I suspected a sex specific issue; maybe that males were infected with a sex specific virus.
Winter 2021	Started hatching eggs to rear to adulthood using food straight from Entomo; this food was often infested with dermestids.	Survival was better than juveniles or adults straight from farm – but we still had a drastic drop in mortality. Thinking it was the greenhouse temperature fluctuations, we switched to rearing in an environmental chamber.	My lab mate was working at Entomo at this time and developed an allergy to the dermestids infesting the barns. Began to suspect that maybe dermestids were impacting mortality.
Spring 2021	Switched food to be straight from supplier – no dermestids.	Survival was great, had many eggs hatching that reached	Decided to investigate the effects of dermestid infestation more

		adulthood and went into the EARS.	closely – could it be because they are ingesting hastisetae? Marshall Ritchie and Matthew Muzzatti discovered cricket crops infested with hastisetae.
Spring & Summer 2021	Males successfully surviving to adulthood and being placed in EARS to record signaling.	EARS malfunctioned and were shutting off (completely unusable data). Tried switching computers, adding cooling fans, etc.	Discovered the EARS units were overheating because they were not being internally cooled; internal fans needed to be replaced as a result of sitting idle for too long during the pandemic.
Fall 2021	Began EARS data analysis.	All signaling parameters came back as 0 after analysis (i.e., no signaling data).	This was a quick coding fix to switch threshold values for *Gryllodes* species, as the Bertram lab had never quantified signaling behaviour in *Gryllodes* before.
Winter 2022	Got final shipment (or so I thought) of eggs from Entomo for trials.	Had massive die off soon after hatching; few males survived to be added to the EARS.	Discovered the environmental chamber was broken and was not holding temperature consistently. Switched to a smaller, more consistent incubator.
Spring 2022	Got final, final (really this time) shipment of eggs from Entomo for trials.	Re-ran all my studies and pulled together data for both Chapter 1 and Chapter 2 at the last minute.	RESILIENCE, and an MSc thesis can be conducted in 2 semesters when you are really desperate and everything that can go wrong has already gone wrong.

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