Safe and Sound: Studies on the Function and Evolution of Defence Sounds in Bombycoidea Caterpillars

by

Amanda Leia Dookie

A thesis submitted to the Faculty of Graduate and Post-Doctoral Studies for the partial fulfillment of the requirements for the degree of

Doctorate of Philosophy

in

Psychology

Specialization in Neuroscience

School of Psychology

Faculty of Social Sciences

University of Ottawa

Abstract

Defence sounds are widespread and diverse amongst insects. Despite their ubiquity and variability, hypotheses explaining their functions and evolutionary origins have been understudied. My thesis focused on these topics using silk and hawkmoth Bombycoidea caterpillars as a model system. In Chapter Two I investigated why defence sounds have evolved in some caterpillars but not others by testing the hypothesis that large body size is a factor in the evolution of defence sounds. To test this hypothesis, I followed the development of defence sounds in four Bombycoidea species from hatching to pupation. I predicted that early instars would not produce defence sounds, and that within sound producing instars defence sounds would be more likely to occur in larger caterpillars. Results showed that defence sounds were absent in the first and second instar, and that they developed in the third through to the fifth instar in all species. Moreover, the onset of sound production occurred when all species were the same relative size (~1.12 g, ~26.37 mm), despite the fact that the species differed in their final instar size. I concluded that early instar caterpillars do not make defence sounds, and that there is a critical size when defence sounds develop. I further tested the hypothesis that smaller caterpillars do not have enough energy to make defence sounds, by analyzing the relationship between size and several temporal characteristics of the sounds. I predicted that smaller caterpillars would signal less than larger caterpillars, and produce shorter signal units and trains, with lower duty cycles. Results partly supported the hypothesis, showing that in two species there was a positive relationship between size and the number of units produced within two seconds following an attack, the mean number of units per train, and the mean duration of the units in one species. I also tested the hypothesis that sounds of small caterpillars are not in the hearing range of predators. I predicted that there would be a relationship between caterpillar size, and the sound pressure levels and dominant frequencies of the sounds. Results showed no significant relationships with dominant frequencies or sound pressure levels and size. I concluded that the caterpillars made sounds that were within the hearing range of major predators from the onset of sound production. In Chapter Three I followed the other antipredator defences of the four species throughout development. I investigated whether the frequency of defences changed with instar. I found that the caterpillars employed up to seven different secondary defences throughout development. In one species the frequency of dropping and major thrashing increased in the late instars, and in a different species the frequency of regurgitation increased. I concluded that in some cases defence sound production accompanies other secondary defences that increase with the size of caterpillars during development. In Chapter Four I tested the hypothesis that the defensive whistle of the walnut sphinx caterpillar, *Amorpha juglandis* (Sphingidae: Sphinginae), functions to startle birds. I predicted that the birds would startle to the sounds, and habituate upon repeated exposure within a trial. Results showed that play-back recordings of the whistles elicited a startle response in captive red-winged blackbirds (*Agelaius phoeniceus*) and caused them to hesitate and/or flee from prey. I concluded that the whistles function as a startle display. Together, the experiments conducted within my thesis addressed important outstanding questions regarding the evolutionary origins of defence sounds in caterpillars, and their functions in predator-prey interactions.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Jayne Yack. I have had an enriched learning experience with Jayne, whether it was through long conversations over tea or when she would quiz me to help me prepare for my examinations. I came from a background in psychology, and Jayne introduced me to the field of biology and the world of bioacoustics and caterpillars. She has put an incredible amount of work in to helping me make sure my work is of high quality, and I admire her patience in doing so. I will always be grateful to have been mentored by such a remarkable, caring person. Thanks to Jayne I will look back on the years of my PhD as a great educational experience.

I am also very grateful for my co-supervisor, Dr. Nafissa Ismail. Nafissa has provided the support I needed to pursue my program at the School of Psychology, and came to my aide at a very important stage in my academic career. Nafissa's positive approach to my studies has played an integral role in my progress in the program. A big thanks also goes to Dr. Alfonso Abizaid who helped me in my transition from my previous lab to the Yack Lab. Alfie encouraged me to contact Jayne, and I would not have a found a topic I could find both joy and pride in studying without his advice. He has served as a member on my committee, along with Dr. Thomas Sherratt and Dr. Catherine Plowright whom I would like to thank for providing their valuable feedback on my thesis work.

I could not have gotten through the more technical aspects of my experiments without the excellent statistical and engineering helped that I received. I thank Dr. Gilles Lamothe for stepping outside his area of expertise to work on my studies with the birds, and Dr. Dwayne Schindler and Dr. Anthony Rose for their statistical consultations. Thank you to Doug Wong for engineering the sensor used for the bird experiments. I am grateful for everyone who made my field experiments possible, including the welcoming staff at QUBS, notably Frank Phelan, Laura Schoenle-Thomas and Dr. Frances Bonier. I am also grateful towards Dr. Julie Morand-Ferron for letting us watch birds in her backyard, and for being a valuable member of my comprehensive exam committee. Thank you also to Corey Cougle for help with bioacoustic analyses, and Jared Davidson for help with editing.

Thank you to past and present Yack lab members for their help around the lab, feedback and friendship. Thank you to Courtney Young for assisting with my field experiments, braving the elements and living in a cabin with me. Special thanks go to Conrado Rosi-Denadai, Amanda Lindeman, Christian Nathan and Chanchal Yadav who have provided me with useful feedback on my experiments and moral support. I'd would also like to thank past undergraduate students, Chelsea Blackman and Asha Toner, who have helped me with my data collection.

Finally, I would like to thank my family for always believing in my abilities to pursue academia and for their unconditional love and support. And to my friends who made sure I had fun. Special thanks to William Logan who has cared for me every step of the way. Too often animals are thought of as objects for our use rather than beings in their own right, so as a final thought I would like to thank the animals for the wonderful opportunity to glimpse in to their beautiful existence.

Plans for Publications and Co-authorship Statements

Chapter Two: **What makes a caterpillar tick? Acoustic defences are size and instar dependent in Bombycoidea caterpillars by Dookie, A.L.D., Kawahara, A., & Yack, J.E.** The project was conceived by Dookie and Yack. Specimen collection was performed by Kawahara. Data collection and analysis was conducted by Dookie. The manuscript was written by Dookie and edited by Yack.

Chapter Four: **Why do caterpillars whistle at birds? Insect defence sounds startle avian predators by Dookie, A.L., Young, C.A., Lamothe, G., Schoenle-Thomas, L., & Yack, J.E.**

Experiments were conceived by Dookie and Yack. Schnoele-Thomas provided care of the birds and feedback on the experimental set-up within the aviaries. Fieldwork and data collection were completed by Dookie and Young. Data analysis was performed by Dookie and Lamothe. The manuscript was written by Dookie and edited by Yack. All co-authors contributed with feedback.

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

A General Introduction to Insect Defence Sounds and Topics Covered in the Thesis

1.1 Defence sounds in insects

Insects are the main food source for many predators and have thus evolved a wide variety of elaborate and oftentimes stunning defences to protect themselves. These defences target various sensory systems in predators (e.g. visual, olfactory, tactile, gustatory and auditory) and are classified as either primary or secondary modes of defence (Ruxton, Sherratt, & Speed, 2004). Primary defences help insects to avoid detection by predators (Edmunds, 1974). For example, some insects have a camouflaged appearance that helps them blend into the environment or mimic the appearance of an object (Lederhouse, 1990; Ruxton et al., 2004; Skelhorn, Rowland, Speed, & Ruxton, 2010). Secondary defences help insects to avoid attacks by predators after detection (Edmunds, 1974). For example, when attacked some insects will attempt to escape, release a chemical deterrent, feign death or emit a loud sound (Edmunds, 1974; Ruxton et al., 2004). Many of these remarkable defences have fascinated biologists and naturalists for centuries, and have been extensively studied in many different insects (Edmunds, 1974; Ruxton et al., 2004). In 1867, Charles Darwin wrote to fellow naturalist Alfred Wallace asking, “why are caterpillars so beautifully and artistically coloured?” to which Wallace replied explaining that the bright colours helped caterpillars to avoid bird predation by signaling their distastefulness (Darwin, 1887). Since then hundreds of studies have focused on these bright displays in insects, but comparatively little has been reported on the function of defences of the acoustic nature (Conner, 2014).

It is surprising that sounds that aid in deterring predators, hereby referred to as “defence sounds” (Masters, 1980) are understudied because defence sounds are taxonomically diverse amongst insects, occurring in at least 6 major insect orders (Alexander, 1957). They are also acoustically diverse in terms of the mechanisms by which they are produced and transmitted (i.e.

air-borne or solid-borne vibrations) (Alexander, 1957, Claridge, 2006). Mechanisms that produce air-borne vibrations include stridulation, vibration of body parts or tymbals, and air expulsion. Solid-borne vibrations are generally produced by striking body parts against substrates. These different mechanisms produce sounds that are acoustically diverse in terms of temporal, spectral and intensity characteristics, resulting in a variety of perceivably different sound types that have been variously called “chirping,” “buzzing,” “clicking,” “squeaking” and “hissing” (Alexander, 1957; Claridge, 2006; Ewing, 1989; Masters, 1980; Rowe & Halpin, 2013). For example, some species of beetles such as *Ips pini* (Scolytinae), produce defence sounds by creating friction between two body parts (i.e. stridulation) which generates a sound comprised of a series of multiple pulses (i.e. a sound wave consisting of a highly-transient, rapidly decaying sine wave) which is described a “chirp” (Fig. 1.1). On the other hand, some species of caterpillars such as *Antheraea polyphemus* (Bombycoidea) contract and relax their mandibles which generates a sound comprised of only a few pulses, described as a “click” (Barr, 1960; Brown, Boettner, & Yack, 2007; Elsner & Popov, 1978) (Fig. 1.1). While there is a diverse variety of defence sounds produced by a large number of insects, there is still a lack of understanding of the important questions surrounding why defence sounds have evolved in insects and how they function in predator-prey interactions.

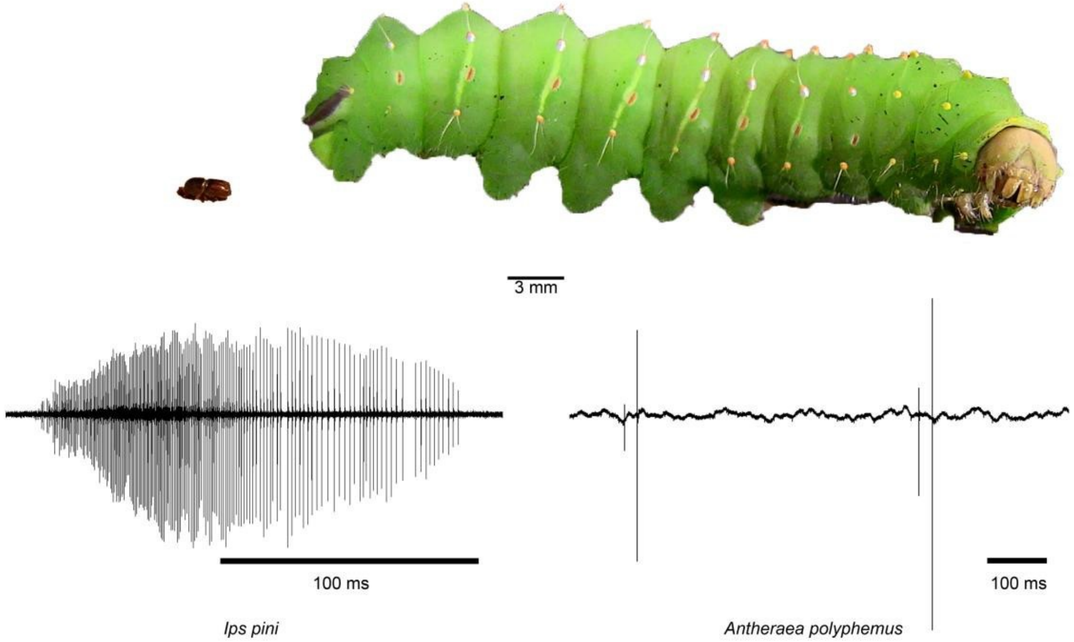


Figure 1.1. Diversity of defence sounds in insects showing on the left, a chirp made by a tiny bark beetle, *Ips pini* (Curculionidae (Scolytinae)), and right, two clicks made by mandibles of a large silkmoth caterpillar, *Antheraea polyphemus* (Saturniidae (Saturniinae)). Reproduced with permission from Young et al., 2015.

1.1.1 Factors involved in the evolution of defence sounds in insects

Despite the wide distribution of defence sounds across the class Insecta, it is not clear why defence sounds have evolved in some insects but not others. Several hypotheses have been proposed. One hypothesis is that some insects have evolved defence sounds because they are hunted by predators with well-developed hearing (e.g. those that hunt at night) such as bats (Hoy, Nolen, & Brodfuehrer, 1989). Another hypothesis is that some insects have evolved defence sounds because they live in habitats that are better suited for transmitting sounds to predators (e.g. insect sounds are better transmitted in dense forests as opposed to open fields) (Romer, 1993). Yet another hypothesis is that some insects do not produce defence sounds because their morphology restricts effective sound production (e.g. small body size can impair an insect's ability to produce sounds that are loud enough to be heard by predators) (Bennet-Clark, 1999; Sanborne & Phillips, 1995). Since there are many factors that may be involved in the evolution of defence sounds in insects, it may be possible that one or more of these hypotheses explains why some insects produce defence sounds while others do not, but few studies have experimentally tested them.

1.1.2 What we know about the function of defence sounds in insects

Despite the large and diverse repertoire of defence sound types and mechanisms distributed across the class Insecta, there is a lack of understanding of how they function to protect prey against predators (Conner, 2014). These insufficiencies in the literature are partially because there has been a greater focus on visual defences, and also because there have been few studies that have tested the effects of defence sounds on live predators (Conner, 2014). Several hypotheses have been proposed which fall in two major categories based on whether they are directed at conspecifics, or predators (Table 1.1). The two main functions that have been proposed for defence sounds that target predators are warning and startle. These two functions are sometimes portrayed as mutually exclusive (Ratcliffe & Fullard, 2005), yet examples of startle and warning have been demonstrated within the same subfamily of some species (i.e. Arctiinae) (Bates & Fenton, 1988; Corcoran, Conner & Barber, 2010; Hristov & Conner, 2005) and there has been some difficulty in distinguishing them (Masters, 1980; Skelhorn, Homes, & Rowe, 2016). It has been suggested that the functions of defence sounds that are directed at predators may vary based on the types of sounds that are produced (Bura, 2010; Bura et al., 2016, Corcoran, Barber, Hristov & Conner, 2011). For example, some sound types such as clicks may be better suited to teaching predators (e.g. acoustic aposematism), whereas other sound types such as whistles may be more suited to deterring predators by scaring them (e.g. deimatic/startle display) (Brown et al., 2007; Bura, Rower, Martin & Yack, 2011; Bura, Kawahara & Yack, 2016). It is important to look at how defence sounds work on the sensory systems of predators to understand how they function to protect prey. However, very few studies have captured these interactions. This thesis is concentrated on the study of insect defence sounds, focusing on why defence sounds have evolved in some species and not others, and how they function to protect prey against predators using caterpillars as a model organism.

Table 1.1. Proposed functions of defence sounds in insects.

Proposed function		**Sender**	**Receiver**	**Context**
Intraspecific	**Warning**	Termite (*Macrotermes natalensis*)	Termite (*Macrotermes natalensis*)	Solid-borne vibrations warning the offspring/conspecifics of predators[1]
	Distress	Treehopper nymph (*Umbonia crassicornis*)	Treehopper nymph (*Umbonia crassicornis*)	Solid-borne vibrations elicit help from conspecifics against predators[2]
Interspecific	**Distress**	Treehopper (*Publilia concava*)	Ant (*Formicidae*) bodyguards	Solid-borne vibrations attract a second predator to interfere with the attacking predator's attack[3]
	Startle	Arctiid moth (*Cycnia tenera*)	Bats (*Eptisicus fuscus*)	Ultra-sonic clicks (air-borne vibrations) startle predators/induce hesitation[4]
	Aposematism	Tiger moth (*Bertholdia trigona*)	Bats (*Eptisicus fuscus*)	Ultra-sonic clicks (air-borne vibrations) warn predators of an impending chemical defence[5]

References: (1) Hager & Kircher, 2013; (2) Cocroft, 1999; (3) Morales et al., 2008; (4) Bates & Fenton, 1988; (5) Corcoran et al., 2011.

1.2 Defence sounds in caterpillars

Caterpillars are excellent models for the study of antipredator traits in insects. They provide many opportunities to investigate defences because they face selection pressures from numerous predators (e.g. birds, bats, reptiles and rodents) and have thus evolved a wide variety of defences (reviewed in Lederhouse, 1990). Aside from the lack of studies that have focused on defence sounds in caterpillars, they have served as key models for the study for insect defences (Edmunds, 1974; Lederhouse, 1990; Greeney, Dyer & Smilanich, 2012; Ruxton et al., 2004). For instance, some caterpillars employ brightly coloured visual displays that have served as hallmark examples of aposematism (Ruxton et al., 2004). Eyespots on caterpillars have also garnered recent attention as intriguing examples of snake mimicry in insects (Hossie, Skelhorn, Breinholt, Kawahara & Sherratt, 2015). Caterpillars are great models for studying defence sounds because confounding variables such as insect sounds used for mating (e.g. such as is the case for bark beetles or tiger moths) can be ruled out because caterpillars are juveniles. Indeed, when Darwin asked Wallace why caterpillars were so brightly coloured Wallace pointed out that it could not be due to sexual selection since caterpillars are essentially "sexless" (Darwin, 1887, p. 317). As an added benefit, confounding variables such as the use of insect sounds for conspecific communication (e.g. in tiger moths) (Corcoran et al., 2011; Miller & Surlykke, 2001), can most likely be ruled out in Bombycoidea caterpillars (the only superfamily of caterpillars currently known to produce defence sounds) because late instar Bombycoidea caterpillars are usually solitary, and the different types of defence sounds are only elicited when the caterpillars are attacked or disturbed (Brown et al., 2007; Bura et al., 2009; Bura, Hnain, Hick & Yack, 2012, Bura et al., 2016). Due to the aforementioned reasons, caterpillar models have proven to be an excellent opportunity for some much needed investigation on defence sounds in insects.

Compared to defences that target the visual or olfactory systems of predators, defence sounds in caterpillars have been underreported. Early accounts of caterpillar defence sounds date back to 1865, when the naturalist Hy Ullyett carried home several larvae from *Acherontia atropos* (Sphingidae: Sphinginae) on the branch of a lime tree and noted that the caterpillars made a sound "like the tick of a watch" (Ullyett, 1865). Similarly, Federley (1905) reported mandible clicking resembling the "ticking of a watch" in *Antheraea polyphemus* (formerly *Telea polyphemus*) (Saturniidae: Saturniinae) which was audible from several meters away. Federley postulated that the sounds were a means of intimidation against predators. Various other sound types have been reported in caterpillars, including "squeaking" in *Amorpha juglandis* (formerly *Cressonia juglandis*) (Sphingidae: Sphinginae) (Packard, 1904). Despite the fact that defence sounds in caterpillars were first reported over a century ago, they have only very recently been the subjects of experimental testing (i.e. Brown et al., 2007; Bura, Fleming & Yack, 2009; Bura, 2010; Bura et al., 2011; Bura et al., 2012). To date, airborne defence sounds in caterpillars have only been reported within the Bombycoidea superfamily from Lepidoptera. The superfamily consists of silk moths, emperor moths, sphinx moths, hawk moths and relatives. Sound production appears to be both common and widespread throughout the Bombycoidea, and to have evolved multiple times, varying in mechanism and acoustic properties (Fig. 1.2) (Bura et al., 2016). Prior to the start of my studies, a total of 61 species were tested for defence sounds, 20 of which were reported to make sounds (Bura et al., 2016). Since defence sounds in caterpillars are underreported, one of the goals of this thesis was to test as many new species as possible for defence sounds.

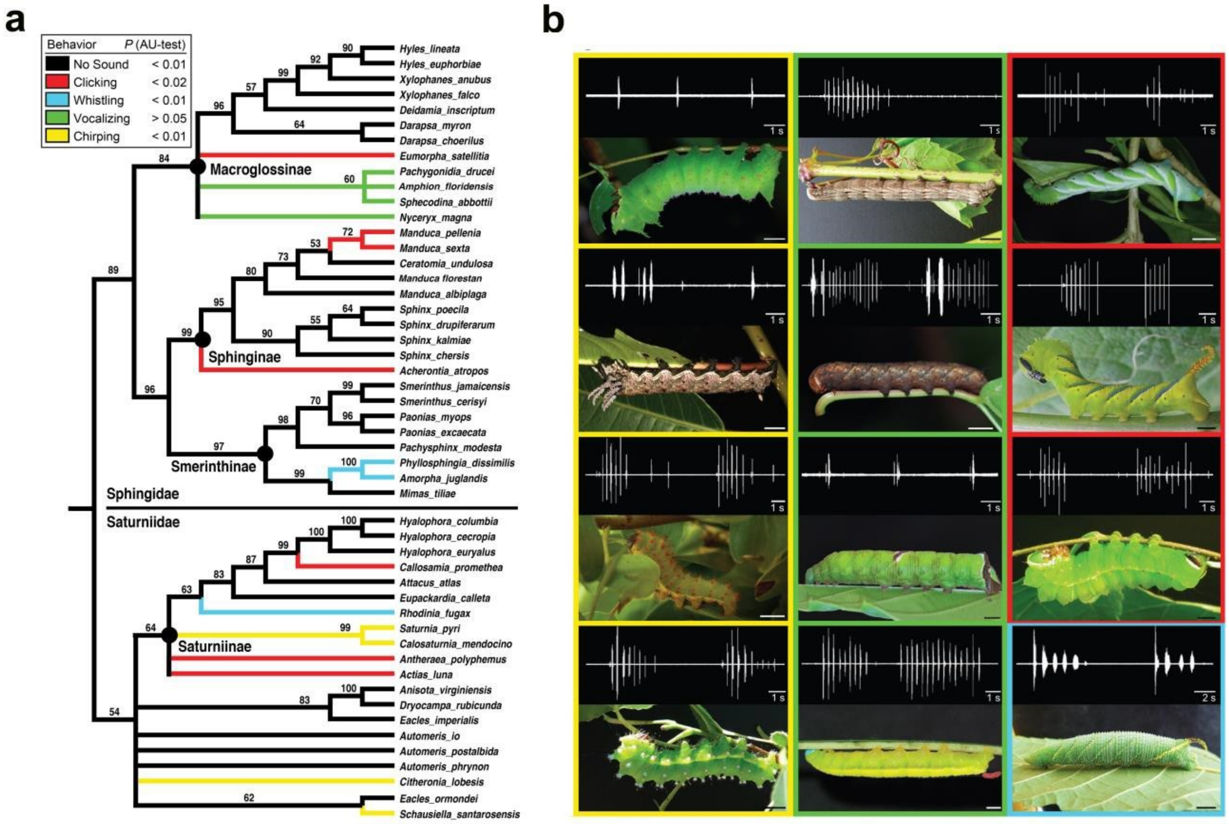


Figure 1.2. Phylogenetic tree showing the evolution and diversity of defensive sounds in Bombycoidea caterpillars, representative species, and corresponding sound trains. The four different sound mechanisms that have been identified in different species are delineated by colour within the phylogeny and in each of the photographs: (i) red, clicking species; (ii) blue, whistling species; (iii) green, vocalizing species; and (iv) yellow, chirping species. Bura et al., 2016. Reprinted with permission.

1.2.1 What we know about the function of defence sounds in caterpillars

An aposematic function has been proposed for defensive clicking in larvae from *Antheraea polyphemus* and *Manduca sexta* (Brown et al., 2007; Bura et al., 2012). In both studies, simulated predator attack trials were performed to correlate sound production with the attacks. In Bura et al.'s (2012) study, it was shown that 77% of larvae tested produced defence sounds when first attacked, which increased to 100% after repeated attacks. The sounds preceded or accompanied regurgitation of a chemical compound 93% of the time on sequential attacks. In Brown et al's (2007) study sound production was also positively correlated with attack, and the regurgitate was found to be unpalatable to ant and mice predators. The significant correlations between both defence sounds and production of a chemical deterrent (regurgitation) provides support for the hypothesis that defence sounds function as a warning signal (i.e. acoustic aposematism).

With regards to the function of defence sounds in caterpillars, support for the startle hypothesis is lacking. Preliminary evidence for a startle function comes from a pilot study by Bura et al. 2011 which showed that the defensive whistles of *Amorpha juglandis* caused Yellow Warblers (*Dendroica petechia*) to dive and fly away. The authors had good reason to suggest the sounds function to startle the birds because of the observable reactions of the birds to the sounds, however there were several limitations to the study. Aside from the small sample size (i.e. 3 birds), experimental testing of the startle response in the birds was not conducted. As well, *A. juglandis* also responded to attacks by sometimes vigorously thrashing the thorax. It was not known whether caterpillars attacked by birds thrashed in this study. Thrashing in caterpillars has also been described as startling, and thus the startle effect of the sound may have been confounded by the thrashing. These limitations are common for researchers studying startle displays and have been the cause of a recent debate over how we should define them (Skelhorn et al., 2016; Umbers & Mappes, 2016). To date, only a handful of studies have tested the effects of an insect defence sound on live predators (Table 1.2), and no studies have tested the startle response of an avian predator to the isolated effect of a caterpillar defence sound. Since experimental testing of startle displays in insects is lacking, one of the goals of my thesis was to experimentally test the startle hypothesis using live avian predators.

1.3 Research Questions and Thesis Objectives

My doctoral research program was designed to address questions about the evolution and function of insect defence sounds, using caterpillars as models. The main research questions addressed in each chapter are summarized below.

1.3.1 Chapter Two: Why do some caterpillars make defence sounds while others do not?

In Chapter Two I addressed questions concerning why some caterpillars produce defence sounds while others do not by testing the hypothesis that body size is an important factor in the evolution of defence sounds. There are several reasons why this hypothesis was promising. First, thus far defence sounds have only been reported in caterpillar species from the Bombycoidea superfamily, and this superfamily is recognized as consisting of some of the largest moth species in the world (Heppner, 2008). It has been noted anecdotally that defence sounds are more common in species that are large, and in late instars (III, IV,V) (Bura, 2010; Federley, 1905). However, the possibility that defence sounds can occur in small caterpillars, or early instars of those species already reported to produce sounds as late instars, cannot be ruled out.

Table 1.2. List of studies that have tested a proposed startle function of acoustic defences in insects using live predators.

Prey	Predator	Findings	Reference
Mantis (*Stagmatoptera biocellata*)	Cowbird (*Molothrus bonariensis*)	Mantis warded off attacks by cowbird.	Maldonado (1970)
Cicada (*Diceroprocta apache*)	Grasshopper mouse (*Onychomys torridus*)	Stridulation reduced predator capture efficiency.	Smith & Langley (1978)
Mutillid wasp (*Dasymutilla lepeletierii*)	Mouse (*Mus musculus*)	Sound producing wasps survived more attacks by mice.	Masters (1979)
Peacock butterfly (*Inachis io*)	Bat (*Plectus auritus, Pipistrellus pipistrellus*)	Predators were startled.	Mohl & Miller (1976)
Tiger moth (*Bertholdia trigona*)	Bat (*Eptesicus fuscus*)	Predators were startled.	Corcoran et al., (2011)
Arctiid moth (*Cycnia tenera*) (recording)	Bat (*Eptesicus fuscus*)	Predators were startled.	Bates & Fenton (1988)
Grasshopper (*Pareuprepocnemis syriaca*)	Gecko (*Ptyodactylus hasselquistii guttatus*)	Higher survival rate in sound producing grasshoppers.	Blondeheim & Frankenberg (1983)
Beetle (*Elaphrus riparius*)	Sandpiper (*Actitis hypoleucos*)	Chirping beetles had a higher survival rate.	Bauer (1976)
Beetle (*Odontotaenius disjunctus*)	Crow (*Corvus brachyrhychos*)	Crows took longer to kill stridulating beetles.	Buchler et al., (1981)
Bush cricket (*Mygalopsis ferruginea*)	Skink (*Egernia napoleonis*)	Stridulation caused skinks to hesitate/recoil.	Sandow & Bailey (1978)

Second, there is reason for larger caterpillars to invest in a secondary defence such as defence sounds because the efficacy of their primary defence (i.e. hiding) may become impaired as large size increases visibility and predation level (Bernays, 1997; Cornell, Stamp & Bowers, 1987; Halpin, Skelhorn & Rowe, 2013). Finally, the hypothesis that body size plays an important role in the evolution of defence sounds was promising because a relationship between size and several other different types of caterpillar defences have been reported (Grant, 2007; Hossie et al., 2015; Sandre, Tammaru, Esperk, Julkunen-Titto, & Mappes, 2007). Since the hypothesis that size plays an important role in the evolution of defence sounds in caterpillars has not been experimentally tested, the main objective of Chapter Two was to follow the development of defence sounds in caterpillars in relation to body size, to determine whether early instars/smaller caterpillars are capable of making defence sounds.

1.3.2 Chapter Three: How do other secondary defences change throughout development in sound producing caterpillars?

In Chapter Three I was interested in knowing whether sound producing caterpillars have other defences throughout development. This question was of interest to me because I wanted to gather further information to explore potential alternative hypotheses for why defence sounds occur in some caterpillars but not others. For example, one hypothesis is that defence sounds are part of a multimodal display; another is that sound production evolved as a communication signals of another secondary defence, like regurgitation. To test these hypotheses it is necessary to document what defences occur and how they change throughout development. I followed the antipredator defences of four Bombycoidea species that make sounds as late instars throughout development. I compared the frequency of secondary defences observed during simulated predator attacks between instars within species. Since the ontogenetic changes in primary and secondary defences in these species has not been followed, the main objective of Chapter Three was to determine whether defences change with instar to lay the ground work for future testing of hypotheses explaining why some caterpillars produce defence sounds while others do not.

1.3.3 Chapter Four: What is the function of defence sounds in insects?

Several hypotheses have been proposed for the function of acoustic defences in insects, however few studies have experimentally tested the startle hypothesis. There are several reasons why this may be. First, there is still a debate over how startle displays should be defined and tested (see Skelhorn et al., 2016; Umbers & Mappes, 2016). Traditionally, startle displays have been identified based on key defining characteristics that make stimuli startling including novelty, rarity, conspicuousness, anomaly and threat (Sargent, 1990). It is argued that this is a problematic way of defining startle displays, because these characteristics are not always exclusive to startle displays (Skelhorn et al., 2016). For example, the defence of the mountain katydid is both aposematic and deimatic (Umbers, Lehtonen, & Mappes, 2015). The consensus is that startle displays should elicit a startle reflex in predators (Skelhorn et al., 2016; Umbers & Mappes, 2016). The startle reflex is ubiquitous across all vertebrates and has been an important model for neuroscientific research (Hoy, 1989). It is presumed to have a protective effect in nature by eliciting an escape response, but experimental support for this hypothesis is lacking because the survival value of the reflex has been hard to demonstrate in the wild (Götz & Janik, 2011; Olofsson, Eriksson, Jakobsson & Wiklund, 2012b). This is because it is often difficult to observe the startle reflex during predator-prey interactions, which are ephemeral in the wild, and proper testing requires baseline behavioural measurements of the prey which are not possible to obtain from wild animals (Sargent, 1990; Skelhorn et al., 2016). The main objective of Chapter Four was to isolate the effect of a caterpillar defence sound, and to experimentally measure the startle response of wild-caught predators to the sound to address these deficiencies in the literature on startle displays.

1.3.4 Summary

The studies conducted within this thesis have led to a more comprehensive understanding of defence sounds in caterpillars, particularly related to an important factor involved in their evolution, body size, as well as their functions in predator-prey interactions. They provide a solid basis for future testing on the evolution and function of defence sounds in insects, particularly in relation to experimental testing of the startle hypothesis, for which experimental support using a caterpillar defence sound has been demonstrated for the first time.

CHAPTER TWO

What makes a Caterpillar Tick? Acoustic Defences are Size and Instar Dependent in Bombycoidea Caterpillars

Abstract

Antipredator defences vary widely between different species and/or developmental stages of insects. Several hypotheses related to life history and habitat have been proposed to explain the evolution of antipredator traits. One hypothesis is that the body size of an insect plays a role in the evolution of insect defences. In this study we seek to explain why some Bombycoidea caterpillars have evolved defence sounds while others have not by following the development of acoustic defences in *Amphion floridensis* (Sphingidae: Macroglossinae)*, Antheraea Polyphemus* (Saturniidae: Saturniinae)*, Antheraea oculea* (Saturniidae: Saturniinae) and *Actias luna* (Saturniidae: Saturniinae). Our results showed that defence sounds did not occur within the first two instars (I-II) for any species, and within instars (III-V) that were capable of producing sounds, individuals were the same mean size at the onset of sound production (1.12 g, 26.37 mm). We then sought to explain why sound production is restricted to larger caterpillars by testing two non-mutually exclusive hypotheses. The first hypothesis is that smaller caterpillars do not have enough energy to make defence sounds. It was predicted that within a sound producing instar, smaller caterpillars would signal less, and produce shorter signals with lower duty cycles, than larger ones. This hypothesis was partially supported; there was a positive relationship between size and (1) the number of units within two seconds following an attack in *A. polyphemus* and *A. luna*; (2) mean number of units per train in *A. polyphemus;* and (3) mean duration of the units in *A. oculea.* The second hypothesis was that smaller caterpillars do not make defence sounds because predators cannot detect their sounds. It was predicted that sounds of small caterpillars do not fall within the frequency hearing range of predators and are not loud enough to be heard by vertebrate predators at an average attacking distance of two to eight cm. No significant relationships were found between sound frequencies or sound pressure levels and

caterpillar size. All of the caterpillars that did make sounds made sounds that would be detectable by a predator at an average attacking distance based on our knowledge of predator hearing. We conclude that size plays an important role in the evolution of defence sounds in Bombycoidea caterpillars.

2.1 Introduction

Insects employ a variety of primary and secondary defences against predators (reviewed in Edmunds, 1974; Lederhouse, 1990; Ruxton et al., 2004). Primary defences (e.g. camouflage, masquerade, hiding, adaptive silence) help insects to avoid detection (Conner, 2014; Edmunds, 1974; Skelhorn et al., 2010; Stevens & Merliata, 2011). Secondary defences (e.g. aposematism, deimatic displays, escape, retaliation) help insects to survive attacks from predators following detection (Edmunds, 1974; Ruxton et al., 2004). Several factors have been proposed to play a role in the evolution of insect defences, including selection pressures from predators, environmental conditions, or foraging habits of prey (Edmunds, 1974; Prudic, Oliver & Sperling, 2007; Reinhold, 2010; Sherratt & Wilkinson, 2002; Skelhorn & Ruxton, 2008). Another factor which has been proposed to impact the evolution of insect defences is body size.

Many studies have demonstrated a relationship between body size and defence (e.g. crypsis, aposematism, mimicry, eyspots, escape and thanatosis) in insects (Grant, 2007; Hozumi & Miyatake, 2005; Marden & Chai, 1991; Sandre et al., 2007; Skelhorn et al., 2010). Examples of size/defence trait relationships, along with proposed explanations for why these relationships exist are reported here. A relationship between size and changes between crypsis and aposematism have been reported in *Saucrobotys futilalis* (Crambidae: Pyraustinae) (Grant, 2007). It is suggested that large size increases predation level because as larvae become larger they spend more time feeding in areas that are exposed to predators, are more visible to predators against the substrate, and are more desirable to predators because they are more profitable (Berger, Walters & Gotthard, 2000; Bernays, 1997, Blanckenhorn, 2000, Greeney et al., 2012; Halpin et al., 2013; Mänd, Tammaru & Mappes, 2007; Remmel & Tammaru, 2009). A relationship between size and flying/escape behaviour has been demonstrated in several Neotropical butterfly species (Marden & Chai, 1991). It is suggested that large size increases muscle strength that is required to evade predators, and that large butterflies have a better ability to fly away from predators because they have stronger muscles (Marden & Chai, 1991; Srygley & Chai, 1990). A relationship between size and the efficacy of eyespots in caterpillars has been proposed by Hossie et al. (2015). It is suggested that in order for the eyespots to effectively mimic a dangerous predator the caterpillars need to be large enough to appear threatening to predators. These examples illustrate how various ecological and biological factors play a role in the relationship between body size and insect defences. In this study, we will assess whether there is a relationship between acoustic defences and size in caterpillars, and if so, explore possible explanations for why these relationships exist.

Defence sounds, defined as sound or vibrational signals generated by prey when attacked or threatened by predators, are diverse and occur throughout the class Insecta (Alexander, 1957; Chapman, 2013; Claridge, 2006; Ewing, 1989; Masters, 1979). Despite the diversity and ubiquity of such sounds, little research has focused on hypotheses explaining why they occur in some species and not in others, or why they differ in signal characteristics (Conner, 2014). Like for the relationships between body size and behavioural, morphological and visual defences, a few factors have been proposed to play a role in the evolution of acoustic defences in insects, such as: (1) the environment, which may affect how well insect sounds are transmitted. For example, insect sounds that are high in frequency attenuate faster in dense forests than they do in open fields (Bennet-Clark, 1999); (2) Predator sensory ecology, because some insects face higher selection pressures from predators that rely on sound rather than vision when hunting (Conner, 2014). For example, as a result of predation pressure from bat predators, moths have evolved the ability to produce defence sounds that deter bats (Hristov & Conner, 2005); (3) Morphological features such as the size of body parts and musculature, because it improves the efficacy of the sounds (Bennet-Clark, 1999; Cocroft & De Luca, 2006). For example, larger cicadas (Cicadidae: Cicadinae) produce louder defence sounds (Sanborn & Philips, 1995); (4) Preadaptations, which equip insects with the organs required for sound production that stem from other behaviours. For example, Bura (2010) suggests that defensive whistles in caterpillars may have stemmed from movements to increase ventilation. In this study we specifically examined the relationship between defence sounds and morphology (i.e. body size) in caterpillars.

Relationships between insect body size and defence sounds, or defence sound characteristics have received little experimental attention. A few examples are reported here, along with possible explanations for why these relationships may exist. Preliminary observations from a comparative study of Bombycoidea caterpillars suggest larger species tend to produce sounds moreso than smaller species (Bura, 2010), and that within species of bush crickets (*Pantecphylus cerambycinus* (Tettigonidea: Pseudophyllidae) larger individuals tend to produce stridulatory defence sounds moreso than smaller individuals (Heller, 1996). In both examples it is suggested that defence sounds may be more common in larger species/individuals because they lack the ability to escape quickly from predators (Bura, 2010; Heller, 1996). Relationships between insect body size and defence sound characteristics are also reported. For example, in *Mantis religiosa* (Mantodae: Mantidae), males are smaller than females, and consequently produce higher frequency defence sounds (Hill, 2007). One explanation for why smaller insects produce higher frequency sounds is that frequency range is restricted by the size of the sound producing structure in insects, such that smaller structures are incapable of producing low frequency sounds (Bennet-Clark, 1999). In another example, a relationship between body mass and the sound pressure levels of defence sounds has been demonstrated in *Tibicen pronotalis* (Cicadidae: Cicadinae) (Sanborn & Philips, 1995). It is suggested that smaller insects have lower muscle strength which restricts sound pressure levels (Bennet-Clark, 1999). In a final example, a relationship between body size and temporal characteristics of defence sounds (i.e. duration, sub- pulse rates) was reported in beetles of the genus *Typocopris* (Coleoptera: Geotrupidae). It was suggested that the duration and sub-pulse rates are correlated to the length of the file used by the beetles to produce the defence sounds (Carisio, Claudia & Rolando, 2004). Here, we test the hypothesis that body size plays a role in determining whether defence sounds occur in caterpillars, by following the development of the sounds in four species of Bombycoidea caterpillars. Like for the evolution of visual displays and body size, we need to first determine whether a relationship exists between defence sounds and size, and if so, why this relationship exists.

Bombycoidea caterpillars are excellent models for investigating the relationship between size and acoustic defences. The Bombycoidea superfamily contains worldwide distribution of approximately 4,723 known species (Holloway, 1987; van Nieukerken et al., 2011). A total of 20 species have recently been reported to make defence sounds, representing 33% of all Bombycoidea species tested for defence sounds to date (Bura et al., 2016). Four distinct types of sounds are produced by different species (clicking, whistling, vocalizing, and chirping) (see

Fig. 1.2). Thus, it appears that defence sounds are variable amongst Bombycoidea caterpillars, and not all species produce defence sounds. Aside from the great variability in the occurrence and types of defence sounds in Bombycoidea caterpillars, this group serves as an excellent model for investigating the evolution of defence sounds because they are juveniles, and therefore confounding variables such as the use of sounds for mating can be ruled out. In this study we approach the question of size and defence sound relationships by focusing on the ontogenetic changes of defence sounds, in four Bombycoidea species known to produce defence signals upon attack in their late instar phases of development.

There were three main goals for this study. The first was to explain why some caterpillar species produce defence sounds while some do not. We tested the hypothesis that body size plays a role in the development of defence sounds in Bombycoidea caterpillars. There were two predictions: 1A: Early instars will not produce defence sounds; and 1B: within a sound producing instar, defence sounds are more likely to occur in large caterpillars (Table 2.1, H1). To test our predictions for this first goal the development of defence sounds was followed from hatching to pupation, noting both instar and body size every 3-4 days. The second goal was to explore why smaller caterpillars do not make defence sounds. We tested the hypothesis that smaller caterpillars do not have enough energy to make defence sounds. We had five predictions: 2A: Smaller caterpillars will produce shorter trains per attack; 2B: Smaller caterpillars will produce fewer units per train; 2C: Smaller caterpillars will produce fewer units of sound per two seconds following an attack; 2D: Smaller caterpillars will produce signals with shorter unit durations; 2E: Smaller caterpillars will produce signals with lower duty cycles (Table 2.1, H2). To test our predictions, the relationship between a combined measure of mass and length for each individual, the Scaled Mass Index (SMI), and each of the aforementioned acoustic characteristics were analyzed (i.e. number of units per attack, unit duration, train duration, duty cycle). Our third goal was to explore why larger caterpillars produce defence sounds. We tested the hypothesis that defence sounds are limited to larger caterpillars because predators cannot detect the sounds of small caterpillars. We had two predictions: 3A: Sounds of small caterpillars are not loud enough to be heard by predators within an attacking distance of 2-8 cm; and 3B: Sounds of smaller caterpillars do not match the frequency hearing range of major predators (e.g. birds, bats, rodents) (Table 2.1, H3). To test our predictions, the relationship between the Scaled Mass Index (SMI) and both sound pressure level and dominant frequency was analyzed. Together, the experiments conducted in this study seek to explore the potential relationship between the evolution of defence sounds and body size in insects, as well as possible explanations for why these relationships may exist.

2.2 Methods

2.2.1 Animals and rearing conditions

A total of 11 species of Bombycoidea caterpillars were reared for this study. Larvae were selected based on availability and previous knowledge of (or lack thereof) sound producing capabilities as late instars. Larvae tested represented two families and three subfamilies (Table 2.2). Four of the 11 species were found to produce defence sounds (see results). The details of these sound-producing species are reported in this chapter and include 20 *Amphion floridensis* (Sphingidae: Macroglossinae), 20 *Antheraea polyphemus* (Saturniidae: Saturniinae), 12 *Antheraea oculea* (Saturniidae: Saturniinae) and 25 *Actias luna* (Saturniidae: Saturniinae). See Appendix A for defences in non-sound producing species.

Table 2.1. Outline of hypotheses and predictions tested in Chapter Two.

Hypothesis	**Prediction**
H1: Size plays a role in the evolution of defence sounds in Bombycoidea caterpillars.	1A: Early instars will not produce defence sounds.
	1B: Within a sound producing instar, regardless of instar, the onset of defence sound production is more likely to occur in large caterpillars.
H2: Smaller caterpillars do not have enough energy to make defence sounds.	2A: Smaller caterpillars will produce shorter trains per attack.
	2B: Smaller caterpillars will produce fewer units per train.
	2C: Smaller caterpillars signal less by producing fewer units of sound per two seconds following an attack.
	2D: Smaller caterpillars will produce signals with shorter unit durations.
	2E: Smaller caterpillars will produce trains with lower duty cycles
H3: Defence sounds are limited to larger caterpillars because predators cannot detect the sounds of small caterpillars.	3A: Sounds of small caterpillars are not loud enough for major predators (i.e. birds, bats) to detect within attacking distance of 2 - 8 cm.
	3B: Sounds of smaller caterpillars do not match the frequency hearing range of major predators.

Eggs were obtained during the months of May to October 2013 and July to August 2014 (Table 2.2). Sample sizes were not the same in each instar in each species because in some cases larvae died during the course of development. In other cases, the sample sizes increased in later instars because results from additional individuals that were followed by undergraduate students were added to the analysis to increase the size. Eggs and larvae were housed at an insect rearing facility at Carleton University at an average temperature of 20-23°C. Eggs were kept in 2-ounce plastic containers with holes in the lid for ventilation. Upon hatching, larvae were transferred, using a fine-toothed paintbrush, to sprigs of host plants held in plastic water filled vials inside individual marked 3.8 L glass mason jars. Host plants were replaced every 2-4 days, at which time the mason jars were also cleaned to remove frass, old plant material, and head capsules.

2.2.2 Morphological measurements throughout development

Development of larvae was documented by measuring mass and length, as well as instar phase for individuals. This was done to determine if sound production was associated with size and/or instar transitions. Changes in head capsule sizes were used to determine instar (Dyar, 1890). To do this, individuals were kept individually in mason jars and when they molted head capsules were collected. Head capsule width measurements were also taken from the live larvae using an Olympus dissection microscope (SZX12; Olympus, Japan) equipped with a Zeiss PixeLINK Megapixel FireWire camera (PL-A642) and Zeiss Axiovision Microscopy Software (Jena, Germany), measured (+/- 0.01 mm) to the level of the most anterior ocelli (Etile & Despland, 2008).

Table 2.2. List of species tested showing host plant, source, number of individuals tested and sound production.

Family	Subfamily	Species	Source	Host plant	N	Date Tested	Previous knowledge of sound? (Y/N)	Sound (Y/N)
Sphingidae	Macroglossinae	*Xylophanes falco* (Walker, 1856)	Akito Kawahara (University of Florida)	*Bouvardia glaberrima* (Rubiaceae)	2	04/14/2013	N	N
	Macroglossinae	*Amphion floridensis* (Clark, 1920)	Akito Kawahara (University of Florida)	*Vitis* (Vitaceae)	20	05/16/2013 – 06/06/2013 07/29/2014 -08/26/2014	Y (Rosi-Denada & Yack, 2015, Bura et al., 2016)	Y
	Macroglossinae	*Darapsa myron* (Clark, 1780)	Akito Kawahara (University of Florida)	*Vitis* (Vitaceae)	8	09/10/2013 -09/19/ 2013	N	N
Saturniidae	Ceratocampinae	*Citheronia splendens* (Druce, 1886)	Akito Kawahara (University of Florida)	*Rhus typhina* (Anacardiaceae)	10	09/06/2013 -09/24/2013	N	N
	Ceratocampinae	*Eacles oslari* (Rothschild, 1907)	Akito Kawahara (University of Florida)	*Quercus* (Fagaceae)	2	09/25/2013	N	N
	Saturniinae	*Antheraea polyphemus* (Cramer, 1775)	Bill Oehlke (Prince Edward Island, Canada)	*Quercus* (Fagaceae)	20	07/19/2013 -08/05/2013 07/15/2013-09/13 2014	Y (Brown et al., 2007)	Y
	Saturniinae	*Antheraea oculea (Neumoegen, 1883)*	Akito Kawahara (University of Florida, FL, USA)	*Quercus* (Fagaceae)	12	09/07/2013 -10/10/ 2013	N	Y
	Saturniinae	*Actias luna* (Linnaeus, 1758)	Bill Oehlke (Prince Edward Island, Canada)	*Betula* (Betulaceae)	25	07/15/2014 – 08/05/2014	Y (Brown et al., 2007)	Y
	Saturniinae	*Hyalophora columbia* (Neumoegen, 1891)	Akito Kawahara (University of Florida)	*Betula* (Betulaceae)	8	06/04/2013 – 07/12/2013	N	N
	Saturniinae	*Hyalophora cecropia* (Linnaeus, 1758)	Bill Oehlke (Prince Edward Island, Canada)	*Betula* (Betulacea)	10	07/22/2013	N	N
	Saturniinae	*Eupackardia calleta* (Westwood, 1853)	Akito Kawahara (University of Florida)	*Salix* (Salicaceae)	5	06/05/2013	N	N

The physical appearance of the caterpillars on the host plant was also recorded by photographing the caterpillars undisturbed in greenhouse light with a Canon PowerShot G7 X camera (Canon Canada Inc., Mississauga, ON) every 3-4 days. Larval mass was measured using a Sartorius CP2245 Analytical Balance (Sartorius Stedim Biotech, Gottingen, Germany). Length (mm) was measured by taking a photograph of the caterpillar with a ruler placed beside the caterpillar. Photographs were subsequently analyzed using ImageJ Image Processing and Analysis in Java Software (Open Source, Public Domain). Length was calculated by generating the mm to pixel ratio within ImageJ, using the ruler within the image as a scale.

2.2.3 Simulated predator attack trials and sound measurements throughout development

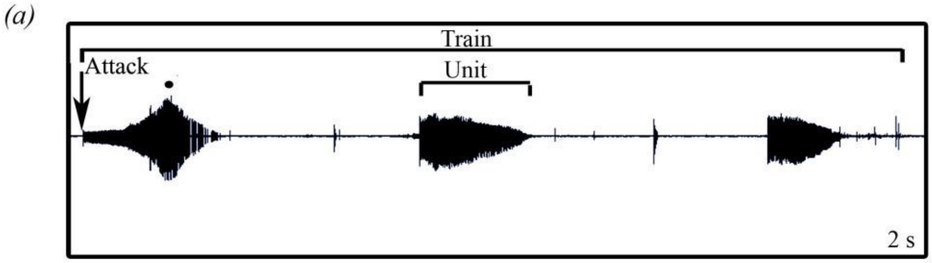
Simulated predator attack trials were conducted to note the presence/absence of defence sounds during development, relate the temporal characteristics of the defence sounds to simulated attacks, and to record sounds for acoustic analysis to determine if there was an association between the acoustic characteristics and body size. Trials were accompanied by sound and video recordings and were performed every 3-4 days in *AF* (N = 20), *AP* (N = 20), *AO* (N = 12) and *AL* (N = 25). First, the larva was removed from the jar on the plant cutting, which was placed on a stand affixed with a clamp to secure the plant. The larva was recorded undisturbed for 30 seconds prior to the trial using a Sony DCR-TRV19 camera (Tokyo, Japan) equipped with a Sony ECM- MS908C microphone located 2 cm from the animal. Another broad frequency microphone (4 Hz -40 kHz) (Earthworks QTC40, Milford, NH, USA) was also used for subsequent acoustic analysis. The microphone was oriented perpendicular to the head of the caterpillar at a distance of 2 cm. Simulated predator attacks were performed by lightly pinching the posterior of the abdomen four times at five second intervals with blunt forceps, a technique commonly used to simulate an attack by a bird (Grant, 2007; Bura et al., 2009). The presence or absence of defence sound production was noted for each attack. Sounds were recorded to a Fostex FR-2 data recorder at a sampling rate of 192 kHz. All sound and video recordings were conducted in a sound-attenuated acoustic chamber (Eckel Industries, Cambridge, MA, USA).

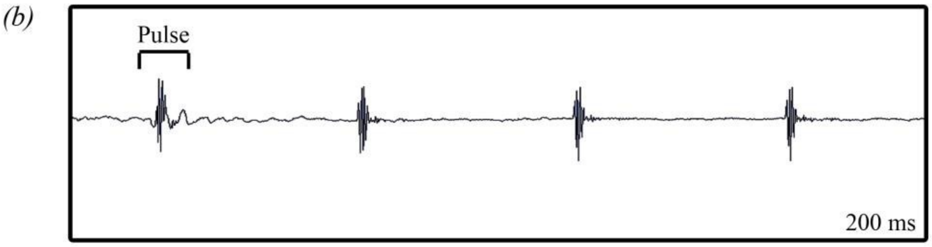
A second set of sound recordings was performed to measure the amplitude and spectral characteristics of the defence sounds in *AF* N= 10; *AP* N = 10; *AO* N= 12; and *AL* N = 5 using a Brüel & Kjær (Naerum, Denmark) 1/4" microphone type 4939 (grid on) amplified using a Brüel & Kjær Nexus conditioning amplifier type 2690 connected to a Tektronix THS720A oscilloscope. Defence sounds were recorded at 2 cm and 8 cm from the microphone. To elicit the sounds, the same four-pinch procedure was followed as outlined above. Following each pinch, peak-to-peak measurements of voltages were recorded on the oscilloscope for subsequent calculation of dB SPL values (see *2.2.4 Acoustic measurements and nomenclature*). The sounds were recorded onto a Fostex FR-2 Field Memory Recorder (Gardena, CA, USA) at a sampling rate of 192 kHz and spectral characteristics were analyzed using AviSoft SASlab Pro (Avisoft Bioacoustics, Berlin, Germany) (see *2.2.4 Acoustic measurements and nomenclature*).

2.2.4 Acoustic measurements and nomenclature

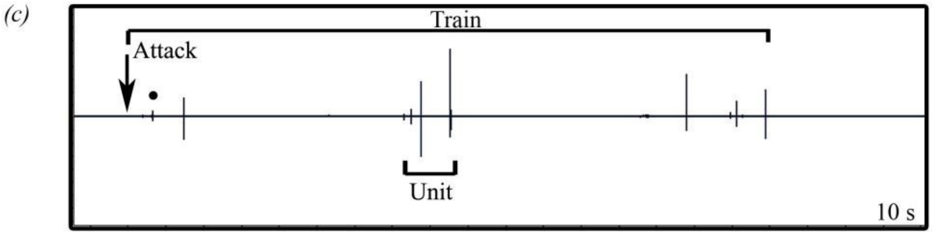
Several acoustic characteristics were measured including temporal parameters (unit duration, train duration, duty cycle, number of units per train, number of units within the first 2 seconds following an attack), amplitude parameters (sound pressure levels), and spectral (dominant frequency). A **train** was defined as the total time (s) from the onset of sound production to the end of sound production preceding an attack (Fig. 2.1a). A **unit** was defined as the smallest unit of sound distinguishable by the human ear (Fig. 2.1b) (Broughton, 1963).

Vocalizing mechanism





Clicking mechanism



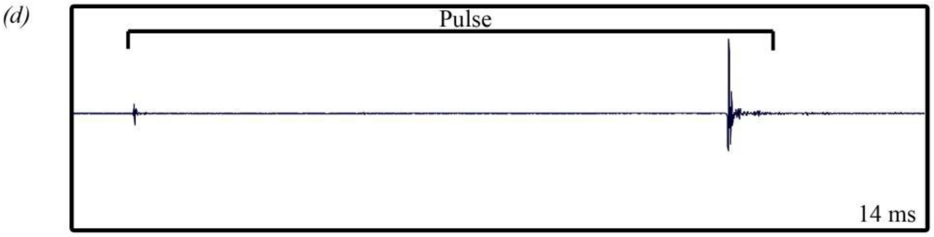


Figure 2.1. Examples of sound trains with constituent sound units produced by the two different mechanisms: (a, c) oscillograms of a sound train following attack in a vocalizing species *(A. floridensis*); and a clicking species (*A. polyphemus*). (b, d) expanded time scales of the area represented by the black circle of the train, showing: (b) Four pulses produced by vocalizing; and (d) Two pulses in a single click.

Duty cycle was measured as the total amount of time occupied by the units following the first 2 seconds after an attack (Morris, DeLuca, Norton & Mason, 2001). To calculate the **sound pressure level**, voltages were converted to Pascal's (Pa) based on the sensitivity output of the Nexus amplifier (1 V/Pa) and subsequently transformed to dB SPL values using the following equation: Sound pressure level (dB SPL) = 20log (P_i/P_o) where P_i is the pressure in Pascal's and P_o is $2x10^{-5}$ Pa (threshold of human hearing at 1kHz). To measure **dominant frequency,** logarithmic power spectra were produced using a 1024-point Fast Fourier Transform (FFT) (Hanning window) for the first 5 units at 2 and 8 cm for each individual measured at -10 dB below peak frequency (Ewing, 1989). All analyses were conducted using Avisoft-SASLab Pro Sound Analysis and Synthesis Software (Avisoft Bioacoustics, Glenicke, Germany).

2.2.5 Data collection and analysis

Sound production measurements and analyses

The purpose of these measurements and analyses was to test Hypothesis 1, that body size plays a role in the evolution of defence sounds (see Table 2.3 for list of predictions). To test prediction 1A, the number of individuals in each species that produced defence sounds at least once per instar was recorded to determine which instars produced defence sounds. We also measured how many trials resulted in defence sound production in each sound producing instar. To do this we calculated the proportion of trials that resulted in sound production for each individual that made defence sounds at least once per instar. Since each individual was tested a different number of times, a weighted average was first generated for each individual based on the number of times they were tested in each instar. Then, the proportion of trials that resulted in sound production was calculated by dividing the weighted averages by the total number of trials conducted in each instar. To test prediction 1B, the mass and length of individuals at the onset of sound production, and the mean mass and length of individuals in the final instar, were compared between species. To test whether there was a relationship between mass and length and the onset of defence sound production regardless of species, the mass and length of individuals at the onset of defence sound production was compared between species using a one-way between subject's analysis of variance in GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA). Similarly, to test whether species differed in their final instar sizes individual mass and length measurements were sampled every 3-4 days and were used to calculate mean mass and length values for each species in the final instar. These values were then compared between species using a one-way between subject's analysis of variance in GraphPad Prism 7 (Table 2.3).

Analysis of body size (SMI) to test Hypothesis 2 and 3

To address Hypothesis 2, that small caterpillars do not have enough energy to make defence sounds, a Scaled Mass Index (SMI) was created, which has been previously used an indicator of the amount of energy reserves that an organism has, and has been found to be a better predictor of body composition than the more commonly used OLS regression (Peig & Green, 2009). To create the index, mass and length measurements were combined for each individual using the following formula:

$$\text{scaled mass index: } \hat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

Where Mi = raw mass of individual, L0 = mean raw length, Li = raw length of individual, and bsma = bols (ln mass, ln length)/R2 (ln mass, ln length), where bols = ordinary least squares slope.

Table 2.3. Summary of predictions, data sampling and analyses used to test each prediction.

Prediction	Data sampling	Analysis
1A: Early instars will not produce defence sounds.	Percentage (%) of individuals that produced sounds at least once per instar.	See Fig. 2.10
1B: Within a sound producing instar, regardless of instar, the onset of defence sound production is more likely to occur in large caterpillars.	Mass (g) and length (mm) of individuals at the onset of defence sound production, and in the final instar.	One way between subjects ANOVAs
2A: Smaller caterpillars will produce shorter trains per attack.	Variable 1: Mean train duration sampled from the first 3 trains following the first three attacks from each individual. Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test
2B: Smaller caterpillars will produce fewer units per train.	Variable 1: Mean number of units per train sampled from the first three trains from each individual Variable 2: SMI* of each individual on each day of testing	Pearson's R correlation test
2C: Smaller caterpillars signal less than larger caterpillars by producing fewer units of sound per two seconds following an attack per train.	Variable 1: Mean number of units sampled from the total number of units following the first two seconds after the first attack from each individual. Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test
2D: Smaller caterpillars will produce signals with shorter unit durations.	Variable 1: Mean unit duration sampled from the first 10 units following the first three attacks from each individual. Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test
2E: Smaller caterpillars will produce trains with lower duty cycles	Variable 1: Duty cycle (%) sampled from the ratio of total amount of time occupied by the units/the first two seconds after an attack from each individual. Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test
3A: Sounds of small caterpillars are not loud enough for major predators (i.e. birds, bats) to detect within attacking distance of 2-8 cm.	Variable 1: Peak-to-peak voltages of sound units were sampled from sounds recorded at 2 and 8 cm from the individual. Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test
3B: Defence sounds are limited to larger caterpillars because predators cannot detect the sounds of small caterpillars.	Variable 1: Dominant frequency of the units were sampled from recordings at 2 and 8 cm from each individual Variable 2: SMI* of each individual on each day of testing.	Pearson's R correlation test

* Scaled Mass Index (see *Analysis of body size (SMI) to test Hypothesis 2 and 3)*

Temporal measurements and analysis

To test predictions 2A-2E of Hypothesis 2 the relationship between the size (SMI) of individuals and several temporal characteristics (i.e. the number of units following the first two seconds after an attack, unit duration, the number of units per train, train duration, duty cycle) of their defence sounds was analyzed using Pearson's R correlation tests (see Table 2.3 for list of predictions and sampling). Analyses on the number of units following the first 2 seconds after an attack, the number of units per train, and duty cycle included individuals that did not make defence sounds, were recorded as zero. All tests were performed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA).

Sound levels and spectral analysis

To test predictions 3A-3B of Hypothesis 3, that defence sounds are limited to larger caterpillars, the relationship between the size (SMI) of individuals and spectral characteristics (i.e. sound levels, dominant frequency) of the defence sounds produced by individuals was compared using Pearson's R correlation tests (see Table 2.3 for list of predictions and sampling). All tests were performed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA).

2.3 Results

2.3.1 Ontogenetic development

Growth and development of sound producing species

Of the 11 species, four produced defence sounds (Fig 2.2) and results on these species are reported below (see Appendix A for information on non-sound producing species).

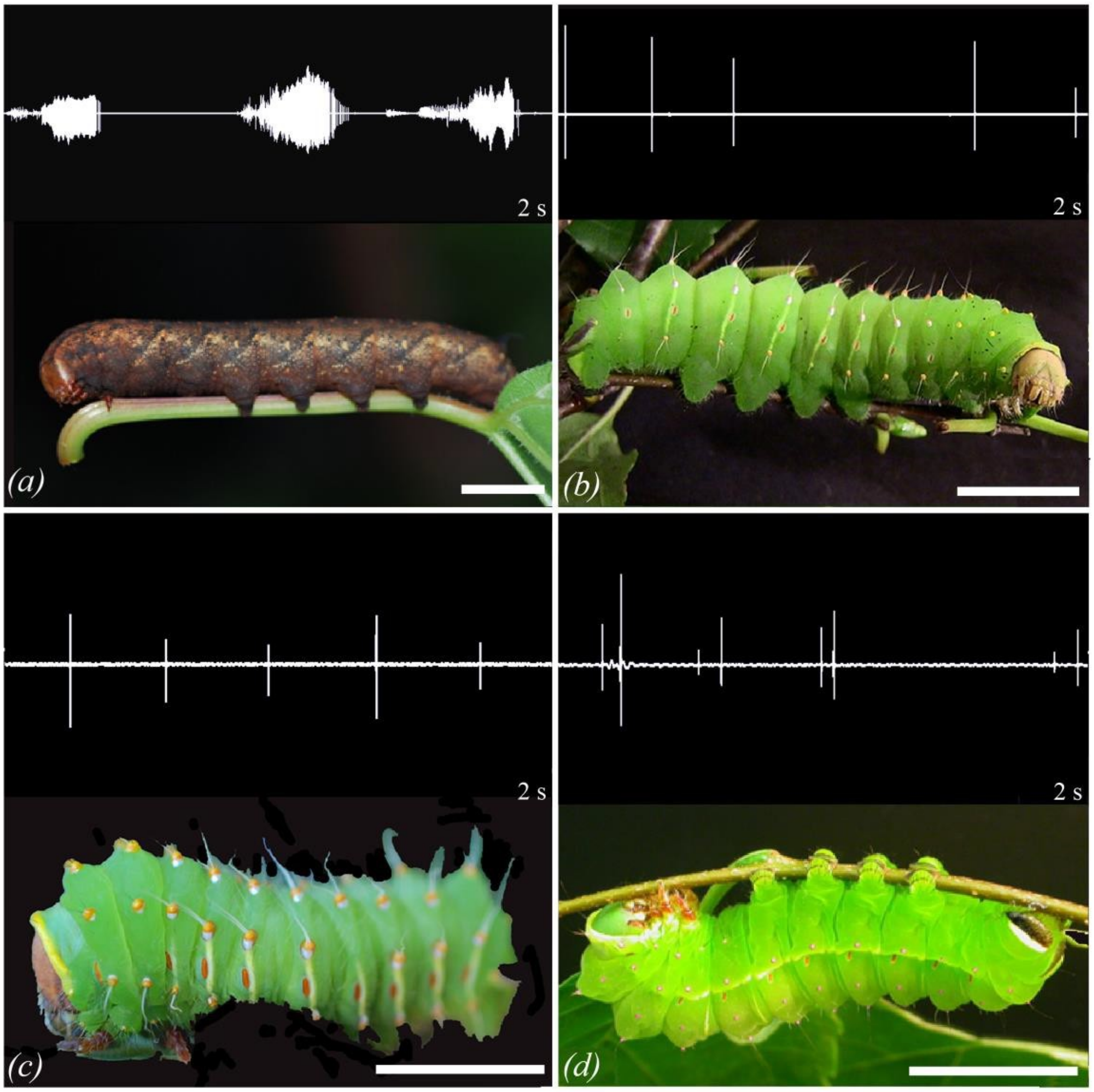


Figure 2.2. Final instar larvae and corresponding oscillograms of multiple defence sound units generated over 2 seconds. Sound production mechanism varies in each species: vocalization in (a) *Amphion floridensis*; mandible clicking in (b) *Antheraea polyphemus*; (c) *Antheraea oculea*; and (d) *Actias luna*. Scale bar 10 mm.

Instar identification and appearance

The number of instars confirmed for each species was as follows: four for *A. floridensis* and five for *A. polyphemus*, *A. oculea* and *A. luna* (Fig. 2.3). Mean head capsule widths did not overlap between instars (Table 2.4, Fig. 2.4). Each species changed in their general appearance throughout development. In instar I through III, the majority of *A. floridensis* appeared green and rested on the underside of the leaf. In instar IV, the larvae underwent a colour change to brown and rested on the branch of the host plant (Fig. 2.5a). The remaining species, *A. polyphemus, A. oculea* and *A. luna,* appeared green throughout all instars, resting on the underside of the leaf (Fig. 2.5b-c).

Mass throughout development

The mass of individuals was followed throughout development (Fig. 2.6, Table 2.5). Mean masses were also calculated for each instar (Fig. 2.7). In general, mass in *A. floridensis, A. polyphemus* and *A. luna* increased from first to last instar by a factor of: 1.5, 2.3 and 0.92 respectively (Fig. 2.7). In *A. oculea*, mass was only measured in instars II → V and increased by a factor of 3.1.

Length throughout development

The length of individuals was followed throughout development (Fig. 2.8, Table 2.6). Mean lengths were also calculated for each instar (Fig. 2.9). In general, *A. floridensis, A. polyphemus, and A. luna* length increased from first to last instar by a factor of: 24.7, 19.5 and 16.9 respectively (Fig. 2.9). In *A. oculea*, length was only measured in instars II → V and increased by a factor of 29.6.

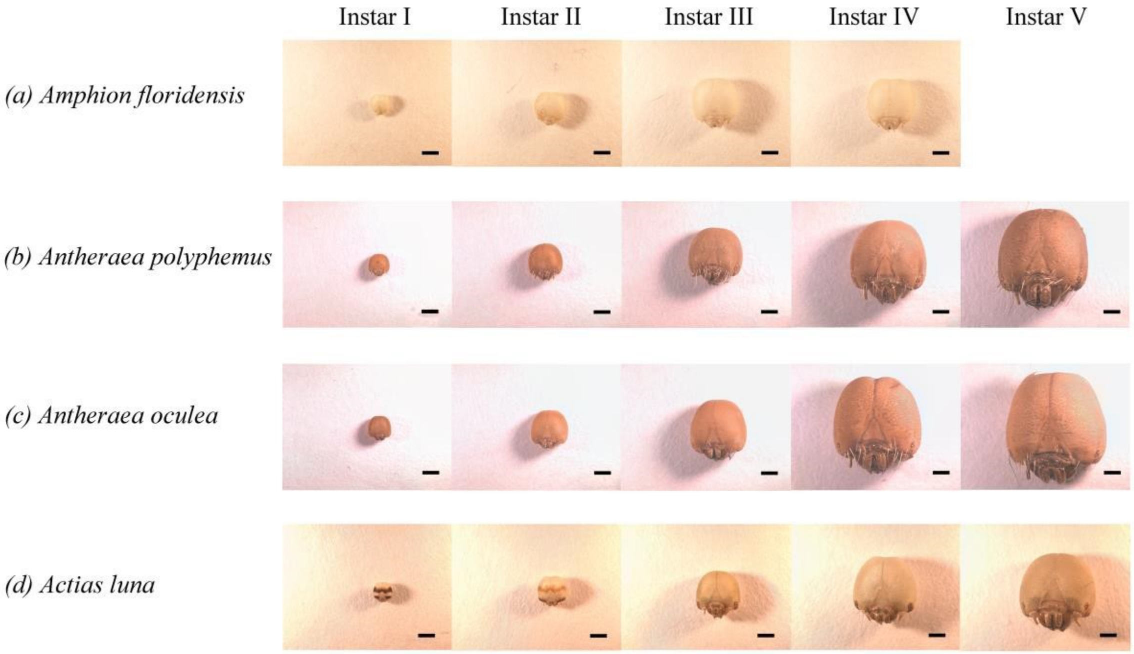


Figure 2.3. Head capsules representing each instar for each species. Scale bar 1 mm.

Table 2.4. Mean head capsule width (mm) of individuals in each instar, standard error of the means.

Species	**Instar I**			**Instar II**			**Instar III**			**Instar IV**			**Instar V**		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
Amphion floridensis	1.14	0.04	15	1.97	0.03	19	2.68	0.13	19	4.21	0.08	13	NA	NA	NA
Antheraea polyphemus	0.98	0.14	6	1.9	0.06	20	3.45	0.14	20	5.35	0.22	12	5.97 ±	0.14	20
Antheraea oculea	0.14	0.01	4	0.48	0.28	4	2.39	0.21	11	3.24	0.39	10	6.07	0.78	4
Actias luna	1.53	0.02	25	2.24	0.15	11	3.08	0.23	8	4.09	0.08	8	5.78	0.17	7

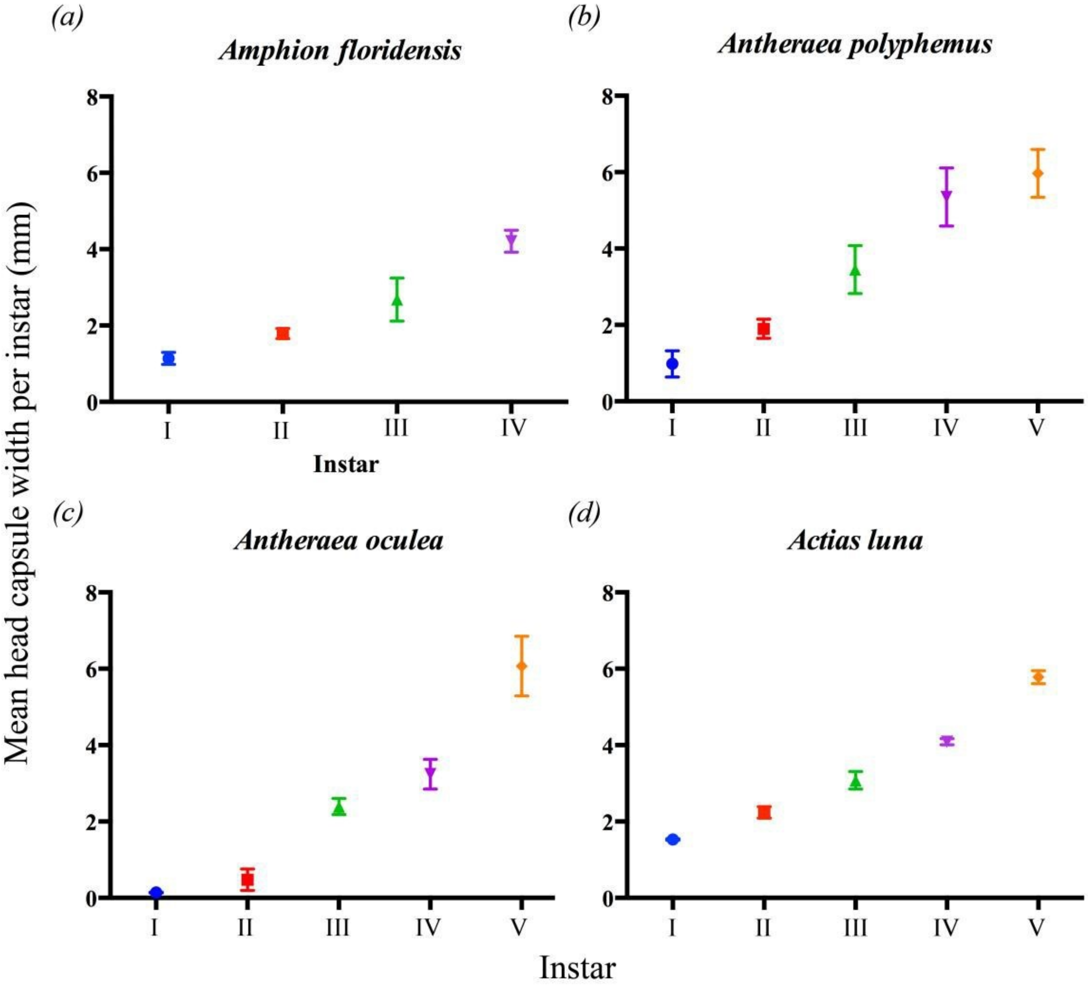


Figure 2.4. Mean head capsule widths per instar for each species. See Table 2.4 for sample sizes.

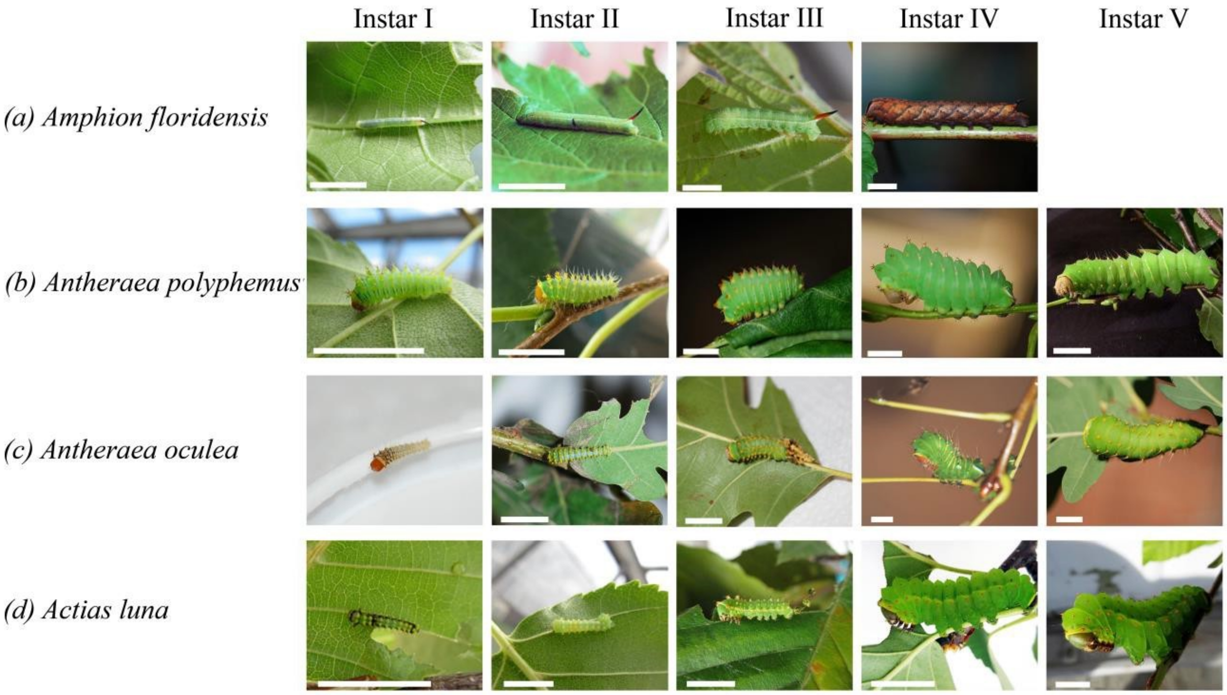


Figure 2.5. Appearance of larvae representing instar stages in each species. Each scale bar represents 10 mm. Scale bar *A. oculea* is not available.

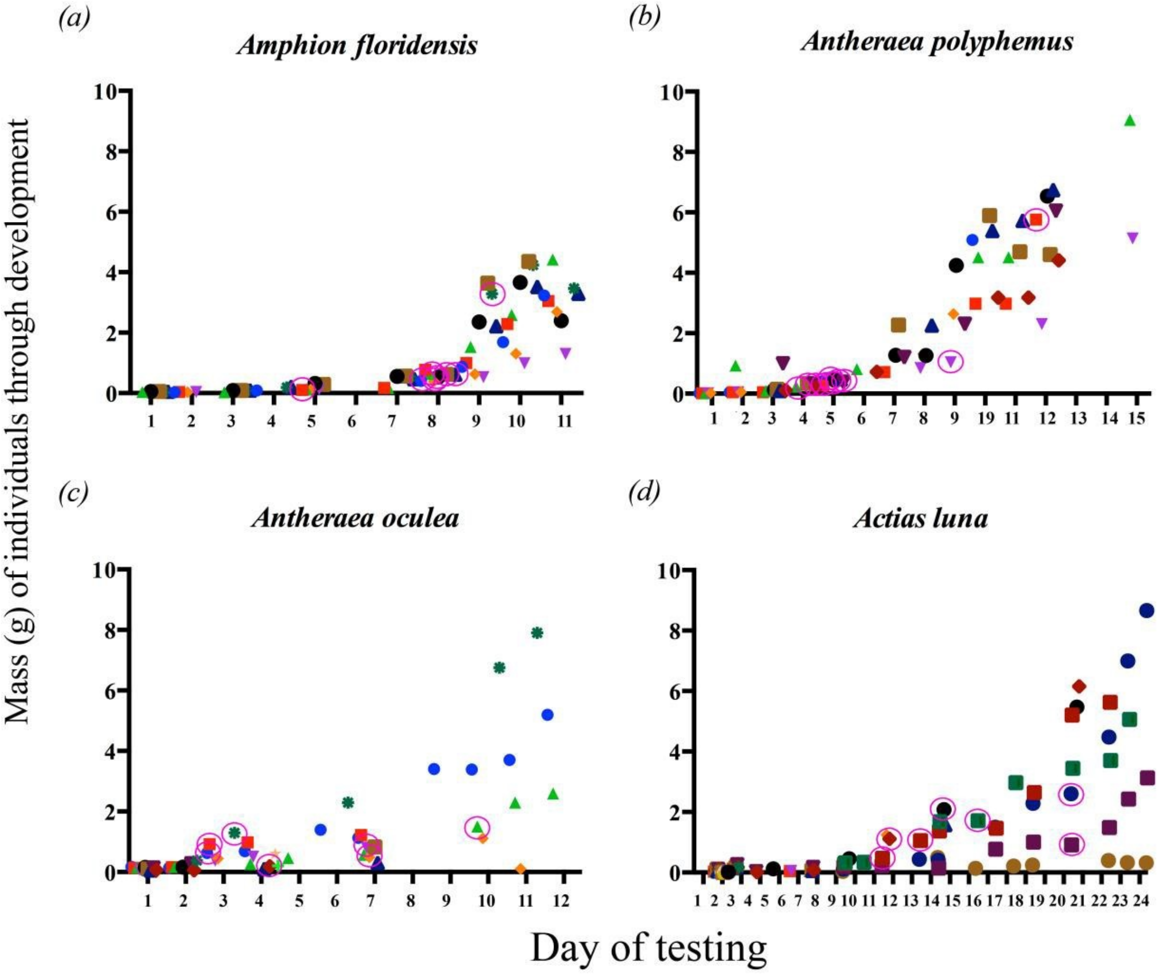


Figure 2.6. Mass of individuals increased during development in each species. Each symbol represents a different individual and the X axis represents the number of times each individual was tested in chronological order from the first to the last day of testing. There were 3-4 days between test days. Open circles represent the mass of each individual at the onset of defence sound production.

Table 2.5. Mean mass of individuals in each instar, standard deviations

Species				Instar II			Instar III			Instar IV			Instar V		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
Amphion floridensis	0.04	0.013	9	0.16	0.03	9	0.85	0.31	8	2.75	0.29	9	NA	NA	NA
Antheraea polyphemus	0.12	0.09	5	0.17	0.07	8	0.85	0.14	10	3.16	0.68	5	5.24	0.52	10
Antheraea oculea	NA	NA	NA	0.12	0.01	10	0.26	0.05	9	1.77	0.62	9	3.9 ±	1.3	2
Actias luna	0.04	0.01	25	0.14	0.03	10	0.39	0.18	9	1.05	0.19	8	4.35	0.56	7

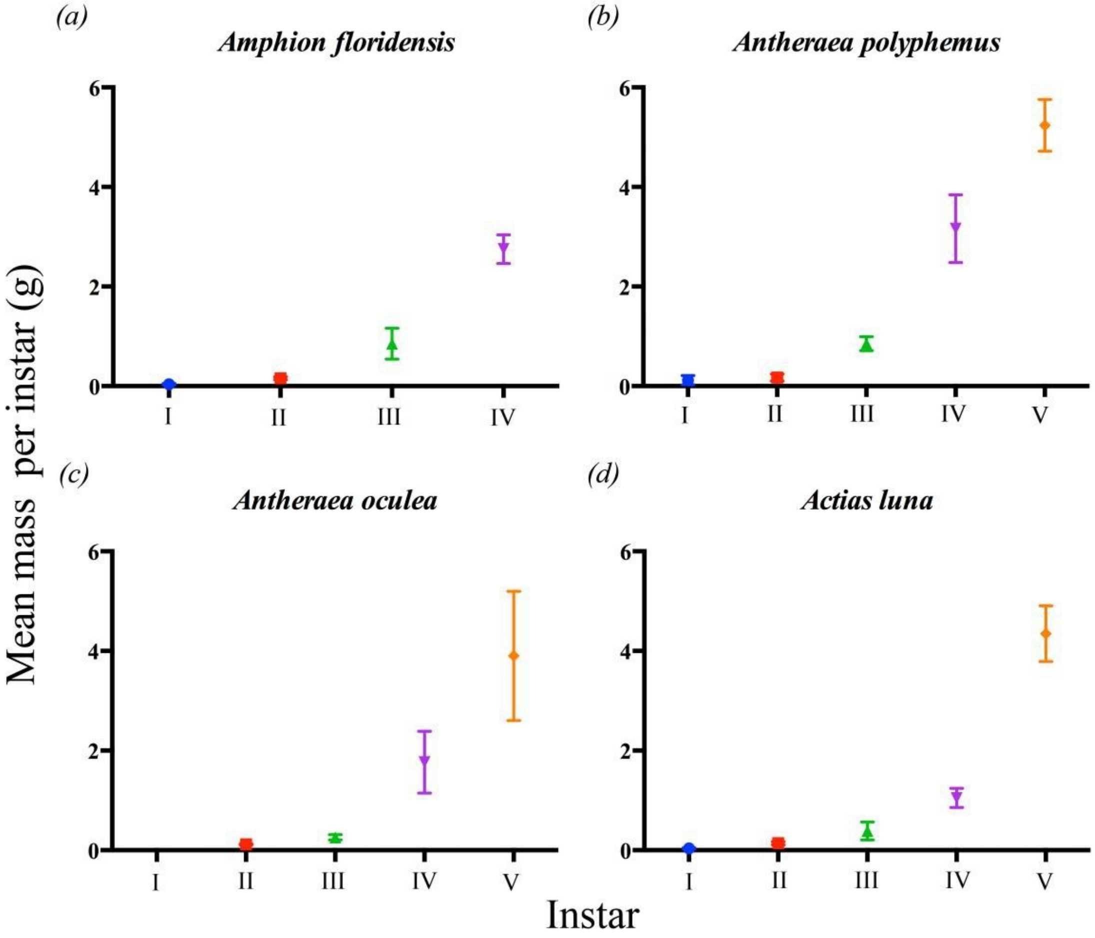


Figure 2.7. Mean mass of individuals for each instar. See Table 2.5 for sample sizes.

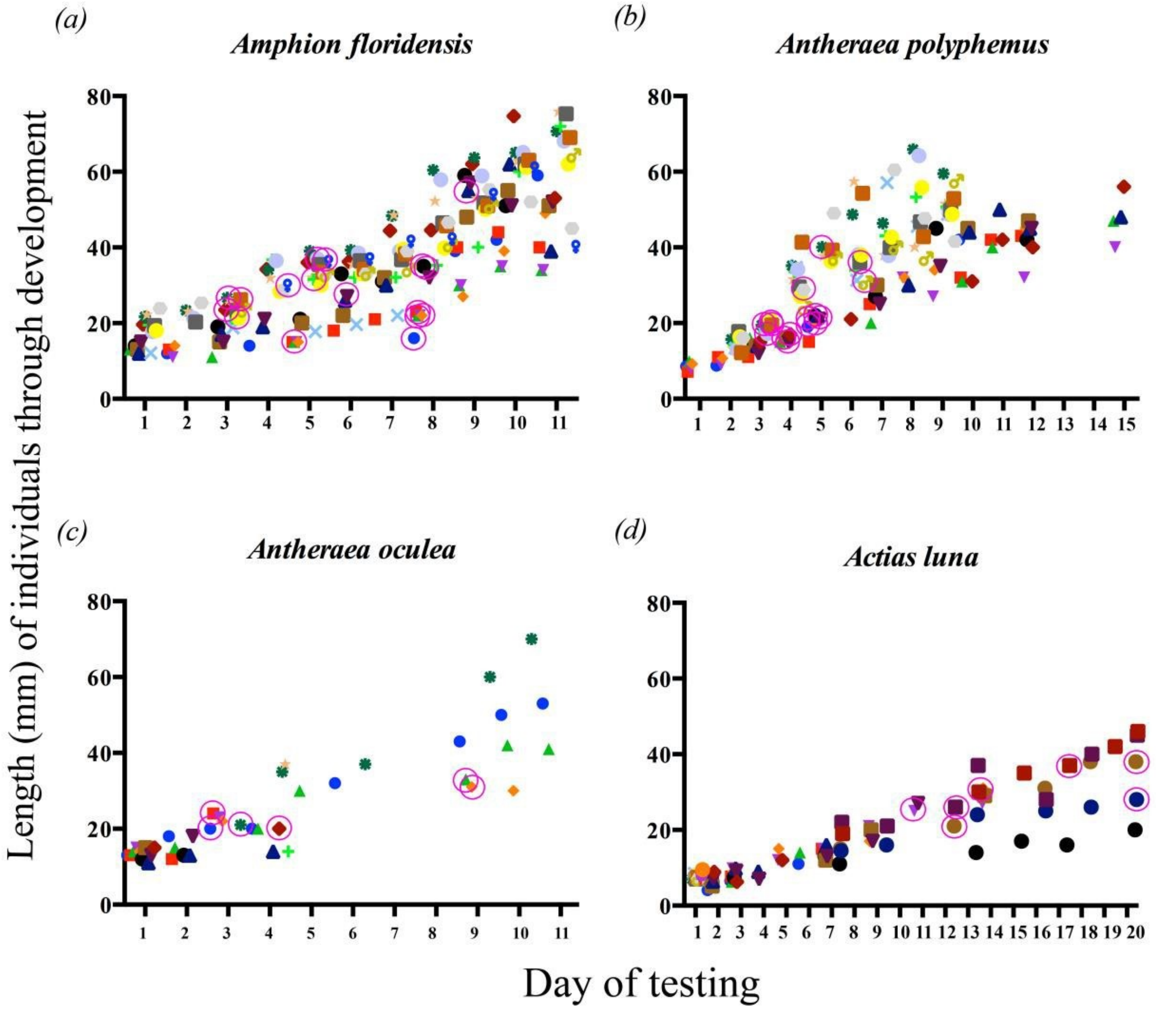


Figure 2.8. Lengths (mm) of individuals increased during development in each species. Each symbol represents a different individual and the X axis represents the number of times each individual was tested in chronological order from the first to the last day of testing. There were 3-4 days between test days. Open circles represent the length of each individual at the onset of sound production.

Table 2.6. Mean length of individuals in each instar, standard deviations.

Species	**Instar I**			**Instar II**			**Instar III**			**Instar IV**			**Instar V**		
	Mean (mm)	**SD**	**N**	**Mean (mm)**	**SD**	**N**	**Mean (mm)**	**SD**	**N**	**Mean (mm)**	**SD**	**N**	**Mean (mm)**	**SD**	**N**
Amphion floridensis	13	0.41	4	18.53	0.2	17	32.46	0.52	17	52.44	0.37	20	NA	NA	NA
Antheraea polyphemus	9.16	0.29	5	14.34	0.43	16	24.62	0.92	12	29.7	0.83	12	46.95	1.37	20
Antheraea oculea	NA	NA	NA	13.45	0.46	10	18.64	0.65	11	31.96	1.81	6	45.5	4.5	2
Actias luna	7.29	0.25	25	13.43	1.22	11	16.77	2.06	8	26.5	2.12	8	43.2	2.51	7

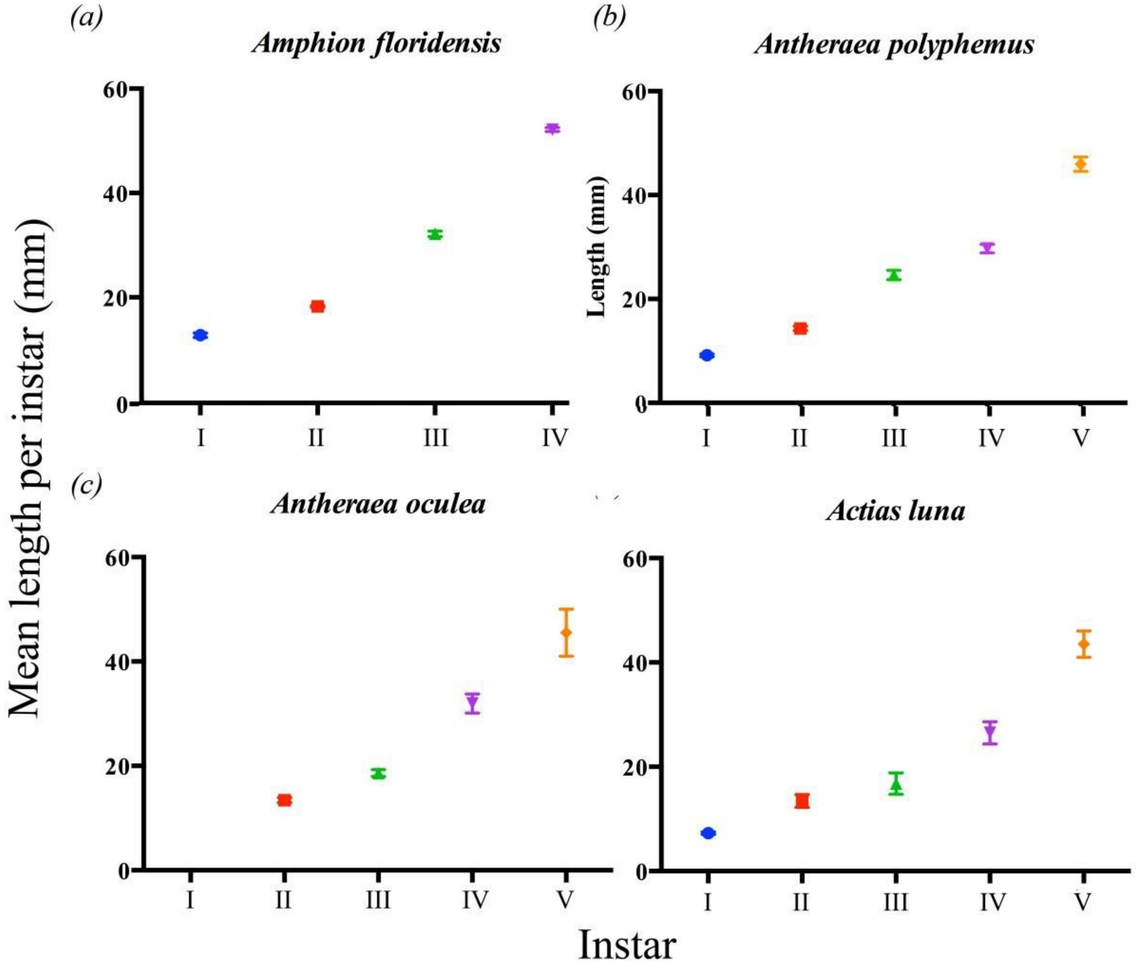


Figure 2.9. Mean length of individuals in each instar. Mean length did not overlap between instars. See Table 2.6 for sample sizes.

2.3.2 Which instars produce sounds?

The percentage of individuals that produced defence sounds at least once per instar, the proportion of trials that resulted in sound production per instar, and how often individuals made sounds throughout development was measured in each species. In all species, the onset of defence sound production occurred in instar III, and sounds were only produced by late instars (III→V) (Table 2.7). Not all individuals produced defence sounds in each instar. For example, in instar IV, 100% of *A. floridensis* and only 12.5% of *A. luna* individuals produced defence sounds at least once (Fig 2.10). Within an instar that generates sounds, the proportion of trials that resulted in defence sounds was also different in each instar (Table 2.8). For example, in IV instar, 55% of *A. floridensis* trials resulted in defence sounds, but only 7% in *A. luna* (Fig. 2.11). Defence sound production was not consistently produced by individuals on each day of testing (Fig. 2.12). Overall, only late instar caterpillars produced defence sounds (instar III → V) and the incidence of defence sound production (i.e. proportion of trials that resulted in sound production) increased throughout development in individuals.

2.3.3 What is the relationship between defence sounds and size (mass, length), regardless of instar?

To test prediction 1B, the masses of individuals at the onset of defence sound production, regardless of instar, were compared between species using a one-way analysis of variance (Table 2.9). The mean mass of individuals at the onset of defence sound production did not vary between species $F(3, 29) = 1.15$, $P = 0.35$. However, the mean mass of individuals in the final instar was different between species $F(3, 28) = 7.35$, $P < 0.05$ (Fig. 2.13a).

Table 2.7. Percentage of individuals that produced defence sounds at least once per instar (%).

Species	**Instar I**		**Instar II**		**Instar III**		**Instar IV**		**Instar V**	
	# of individuals (%)	**N**	**# of individuals (%)**	**N**	**# of individuals (%)**	**N**	**# of individuals (%)**	**N**	**# of individuals (%)**	**N**
Amphion floridensis	0	4	0	17	95	17	100	20	NA	NA
Antheraea polyphemus	0	5	0	16	70	19	75	12	85	20
Antheraea oculea	NA	NA	0	10	18	11	30	6	100	2
Actias luna	0	25	0	11	25	8	12.5	8	42	7

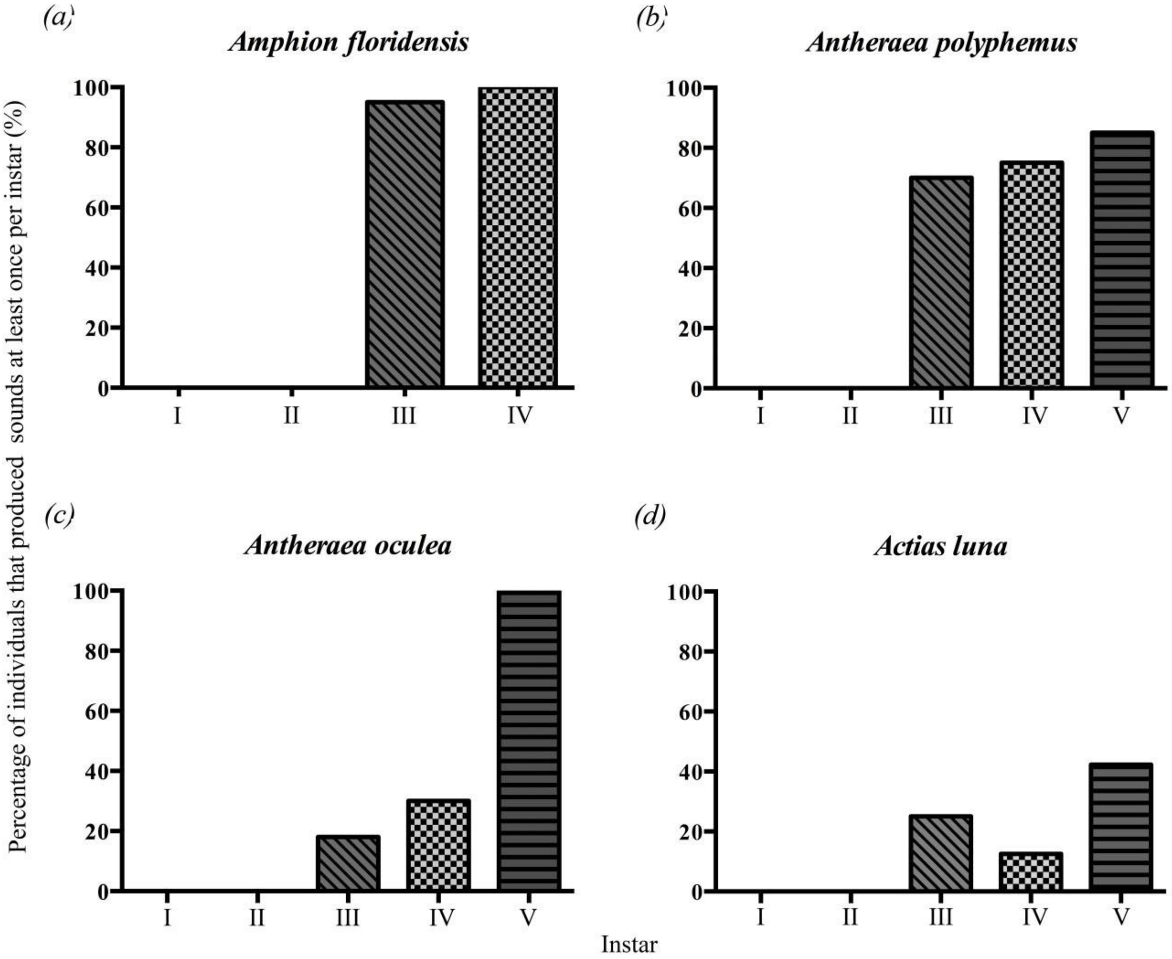


Figure 2.10. Percentage of individuals that produced sounds at least once per instar. See Table 2.7 for sample sizes.

Table 2.8. Proportion of trials resulting in defence sounds per instar.

Species	**Instar I**		**Instar II**		**Instar III**		**Instar IV**		**Instar V**	
	# trials (%)	**N**	**# trials (%)**	**N**	**# trials (%)**	**N**	**# trials (%)**	**N**	**# trials (%)**	**N**
Amphion floridensis	0	4	0	17	35	17	55	20	NA	NA
Antheraea polyphemus	0	5	0	16	47	19	76	12	70	20
Antheraea oculea	NA	NA	0	10	15	11	53	6	50	2
Actias luna	0	25	0	11	20	8	7	8	36	7

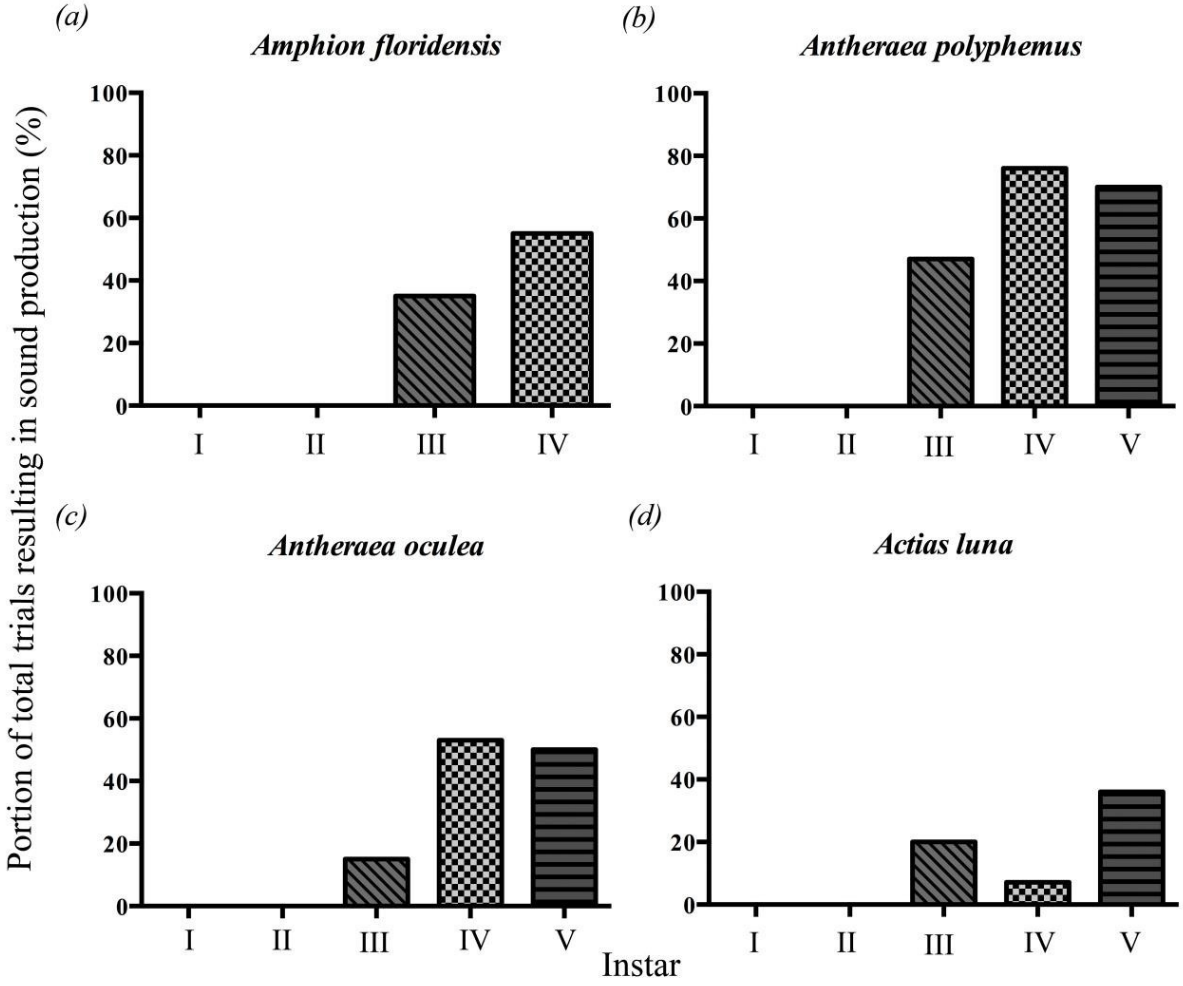


Figure 2.11. Proportion of trials that resulted in defence sound production per instar. See Table 2.8 for sample sizes.

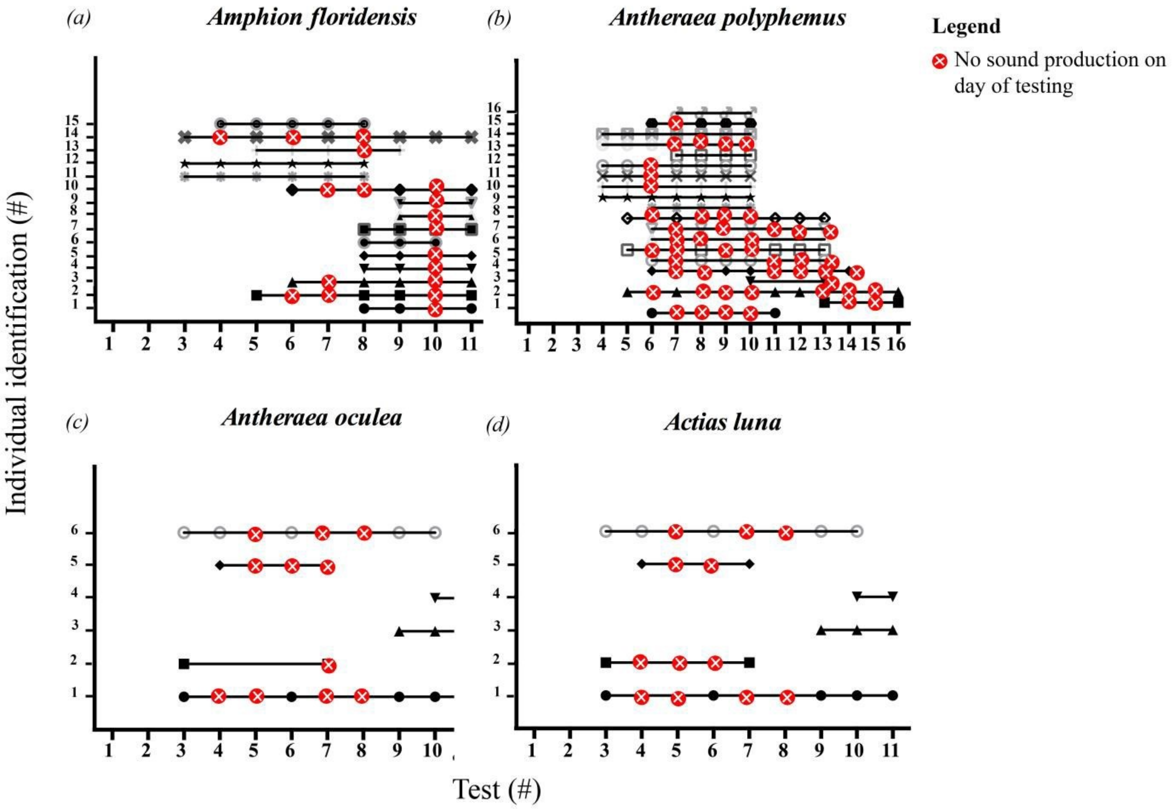
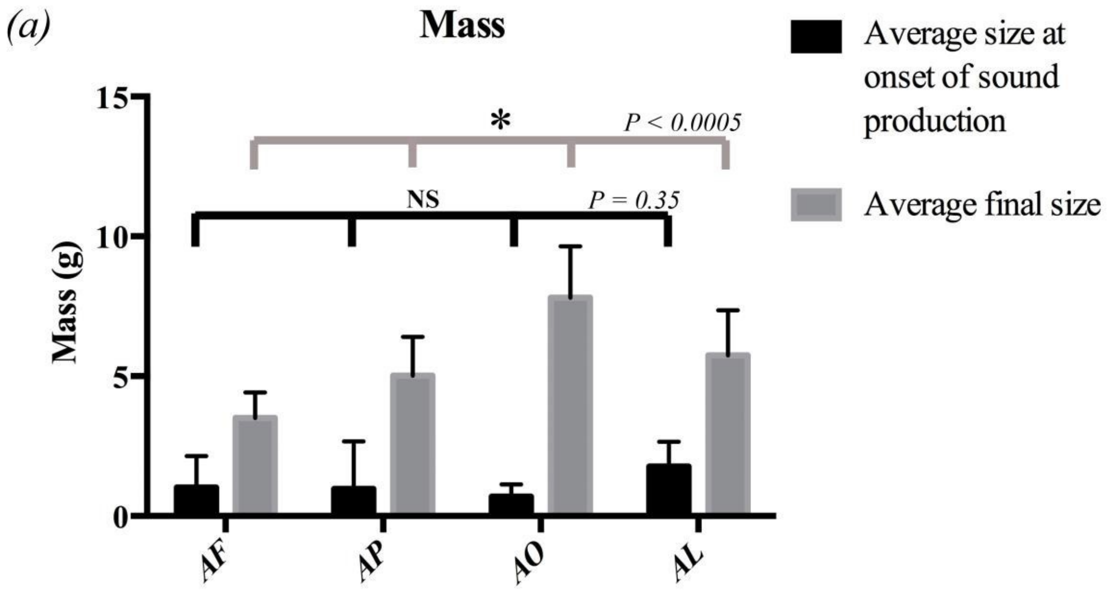


Figure 2.12. The presence or absence of defence sound production in each individual on each day tested. Not all individuals produced defence sounds consistently throughout development. Large circles with an "X" marking represent the absence of sound production on that day of testing.

Table 2.9. Mean mass and length, of individuals in each species at the onset of defence sound production.

Species	**Mass (g)**			**Length**		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**
Amphion floridensis	1.02	1.12	8	26.81	8.58	19
Antheraea polyphemus	0.97	1.7	10	23.35	9.36	17
Antheraea oculea	0.7	0.43	7	24.83	5.78	6
Actias luna	1.77	0.89	8	32.25	7.46	8
Mean of all individuals combined regardless of species	1.12	1.2	33	26.27	8.73	50



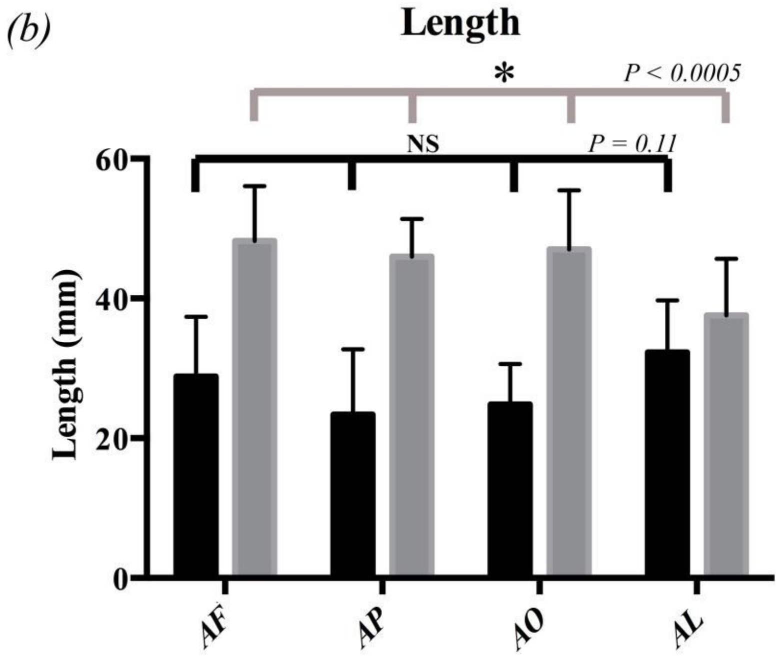


Figure 2.13. Mean (a) mass and (b) length at the onset of sound production in each species. See Table 2.9 for sample sizes. There were no significant differences in mass ($P = 0.35$) or length ($P = 0.11$) at the onset of sound production between species, even though the mean mass and length of individuals in the final instar significantly differed between species ($P < 0.0005$).

Follow-up pairwise comparisons were performed to determine which species differed in their mean final instar mass. The analysis revealed that the mean mass of the final instar differed between *AF* (Mean ± SD: 2.75 ± 0.29 g) and *AO* (Mean ± SD: 3.9 ± 1.3 g) ($P < 0.05$), and between *AF* (Mean ± SD: 2.75 ± 0.29 g) and *AL* (Mean ± SD: 4.35 ± 0.58 g) ($P < 0.05$).

To further test prediction 1B, the length of individuals at the onset of defence sound production regardless of instar was compared between species using a one-way analysis of variance (Table 2.9). The mean length of individuals at the onset of defence sound production did not vary between species $F\,(3, 46) = 2.1$, $P = 0.11$. However, the mean length of individuals in the final instar was different between species $F\,(3, 49) = 4.04$, $P < 0.05$) (Fig. 2.13b). Follow-up pairwise comparisons were performed to determine which species differed in their final instar lengths. Results showed that the mean length of final instars differed between *AF* (Mean ± SD: 52.44 ± 0.37) and *AL* (Mean ± SD: 43.2 ± 2.51) ($P < 0.05$), and *AP* (Mean ± SD: 46.95 ± 1.37) and *AL* (Mean ± SD: 43.2 ± 2.51) ($P < 0.05$).

2.3.4 What is the relationship between size and temporal characteristics of the sounds?

Do smaller caterpillars produce defence sounds with shorter trains?

To test prediction 2A, the relationship between mean train durations measured over the course of the first three attacks, and size (SMI), was analyzed across individuals from each species. Overall, mean train durations were longest in *AO* (i.e.10.56 s) and lowest in *AL* (i.e. 1.44 s) (Table 2.10). There were no significant correlations between mean train duration and size (SMI) in *AF* ($R^2 = 0.04561$, $P = 0.295$, N = 36), *AP* ($R^{2 =}$ 0.0043, $P = 0.803$, N = 17), *AO* ($R^2 = 0.0214$, $P = 0.575$, N = 17), or *AL* ($R^2 = 0.0023$, $P = 0.86$, N = 16) (Fig. 2.14).

Table 2.10. Mean train durations, standard deviations of the means, minimum and maximum train durations and sample sizes (N) in each species.

Species	Mean (s)	SD	Min (s)	Max (s)	Number of pairs*
Amphion floridensis	6.57	4.23	0.91	16.87	29
Antheraea polyphemus	4.44	2.69	0.17	7.33	26
Antheraea oculea	10.56	9.4	0.43	40.03	17
Actias luna	1.44	1.4	0.0002	6.38	21

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

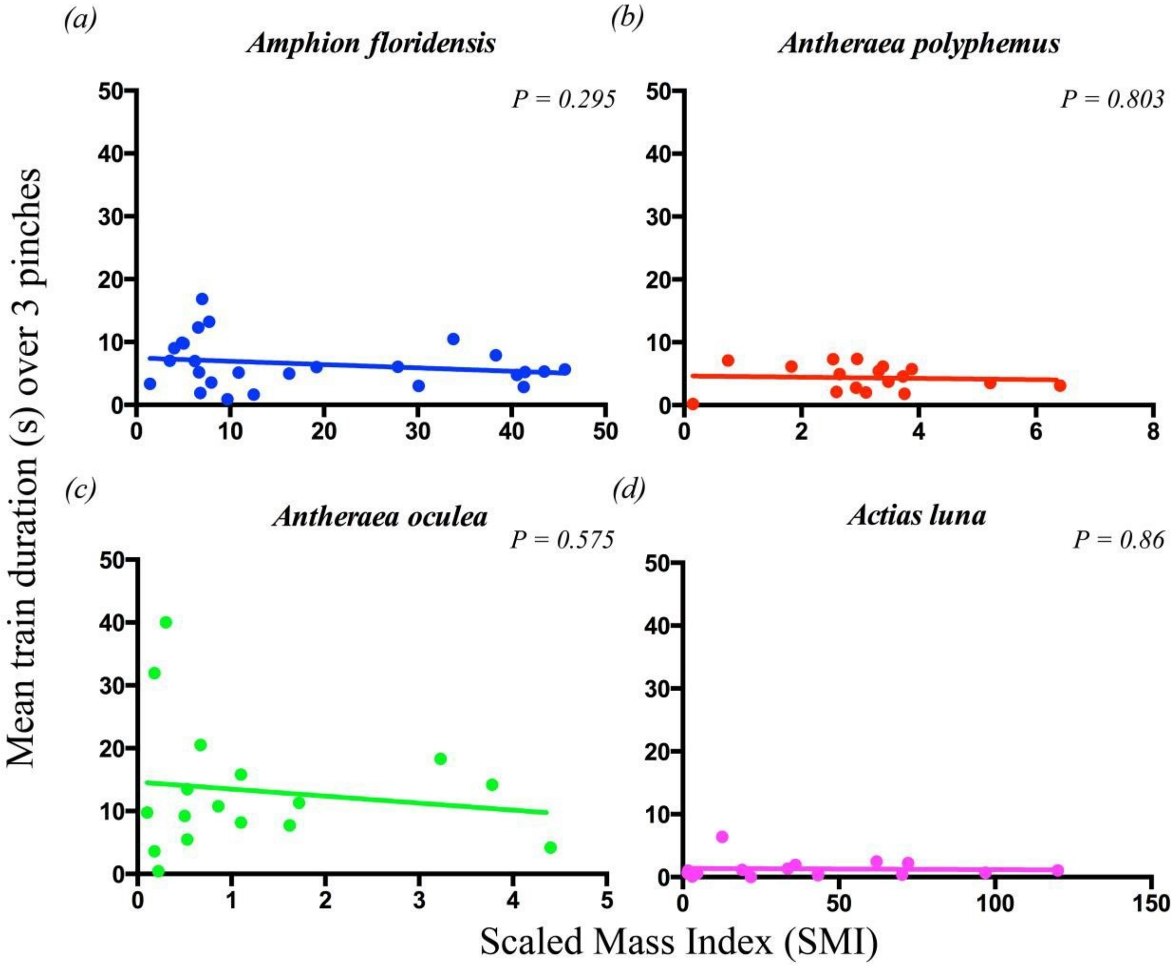


Figure 2.14. Relationship between the mean train durations over the first three attacks within a trial and size (SMI) in each species. There were no significant relationships between the mean train duration and size. See Table 2.10 for sample sizes.

Do smaller caterpillars produce defence sounds with a lower number of units of per train?

To test prediction 2B, the relationship between the number of units per train and size (SMI) was analyzed in individuals from each species. Overall, the mean number of units per train was highest in *AF* and *AO* (i.e. 5 units per train) and lowest in *AP* (i.e. 2 units per train) (Table 2.11). There were no significant correlations between the mean number of units per train and size (SMI) in *AF* ($R^2 = 0.001$, $P = 0.883$, N = 30), *AO* ($R^2 = 0.0016$, $P = 0.862$, N = 21) or *AL* ($R^2 = 0.0011$, $P = 0.872$, N = 25). There was a significant positive correlation between the mean number of units per train and SMI in *AP* ($R^2 = 0.3530$, $P < 0.0001$, N = 36) (Fig. 2.15).

Do smaller caterpillars produce fewer defence sound units following the first two seconds after an attack?

To test prediction 2C, the relationship between the mean number of sound units that occurred within the first two seconds after the first attack, and size (SMI), was analyzed across individuals from each species. Overall, the mean number of units was highest in *AF* (i.e. 9 units), and lowest in *AL* (i.e. 4 units) (Table 2.12). There were no significant correlations between the mean number of units and size (SMI) in *AF* ($R^2 = 0.056$, P = 0.414, N = 14) and *AO* $R^2 = 0.021$, P = 0.639, N = 13). There was a significant positive correlation between the mean number of units and size (SMI) in *AP* ($R^2 = 0.2832$, $P = 0.009$, N = 23) and *AL* ($R^2 = 0.3986$, $P = 0.004$, N = 19) (Fig. 2.16).

Table 2.11. Mean number of units per train standard deviations of the means, minimum and maximum number of units per train, and sample sizes (N) in each species.

Species	Mean (#)	SD	Min (#)	Max (#)	Number of pairs*
Amphion floridensis	5	4.5	0	17	29
Antheraea polyphemus	2	2.5	0	8	26
Antheraea oculea	5	4.66	0	18	17
Actias luna	4	1.79	0	9	21

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

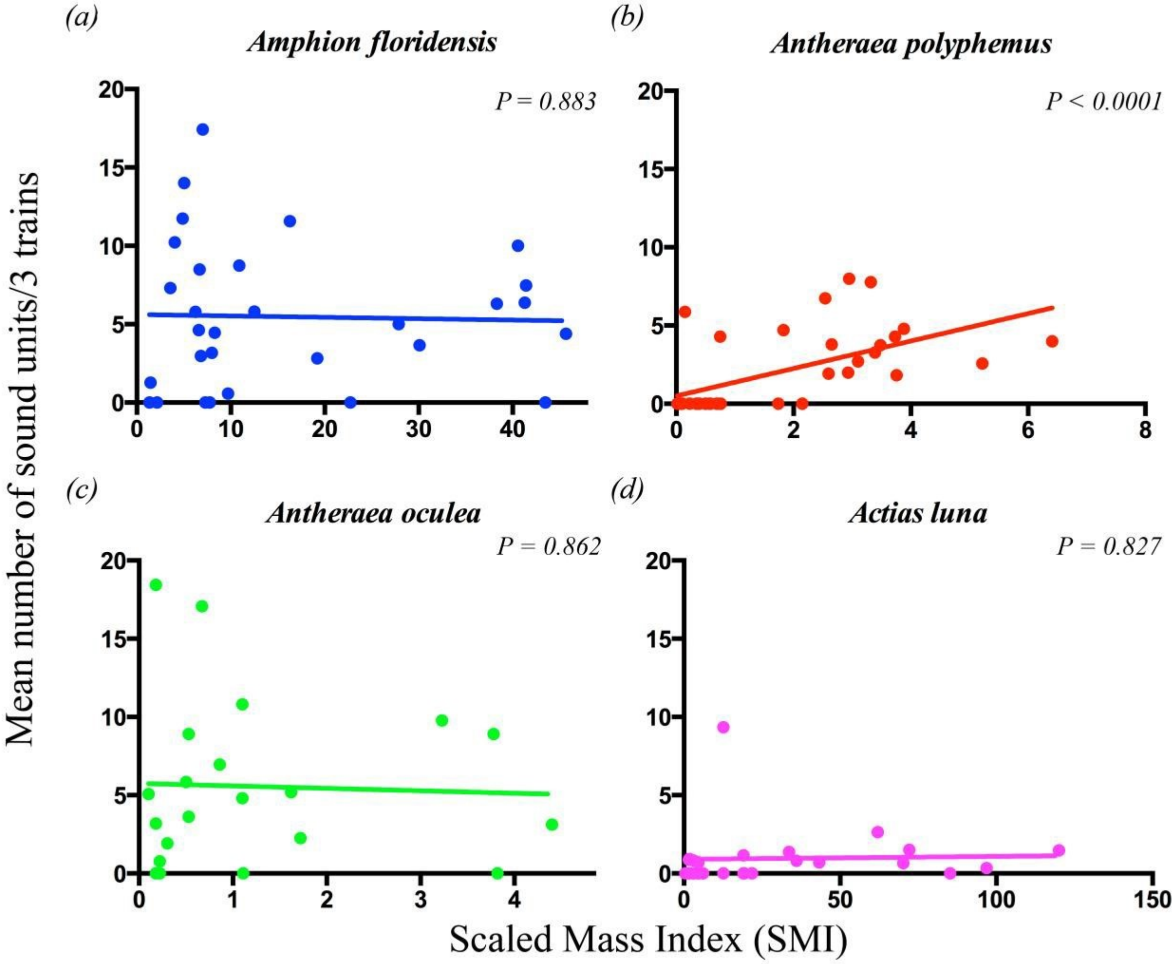


Figure 2.15. Relationship between the mean number of units per train and size (SMI) in each species. There was a significant positive relationship between the mean number of units per train and size (SMI) in *Antheraea polyphemus* (*P* < 0.0001). See Table 2.11 for sample sizes.

Table 2.12. Mean number of units within the first two seconds following the first attack, standard deviations of the means, minimum and maximum number of units following the first two seconds after the first attack and sample sizes (N) in each species.

Species	**Mean (#)**	**SD**	**Min (#)**	**Max (#)**	**Number of pairs***
Amphion floridensis	4.36	2.79	0	9	14
Antheraea polyphemus	1.74	1.98	0	6	13
Antheraea oculea	1.79	1.63	0	6	23
Actias luna	1.26	1.19	0	4	19

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

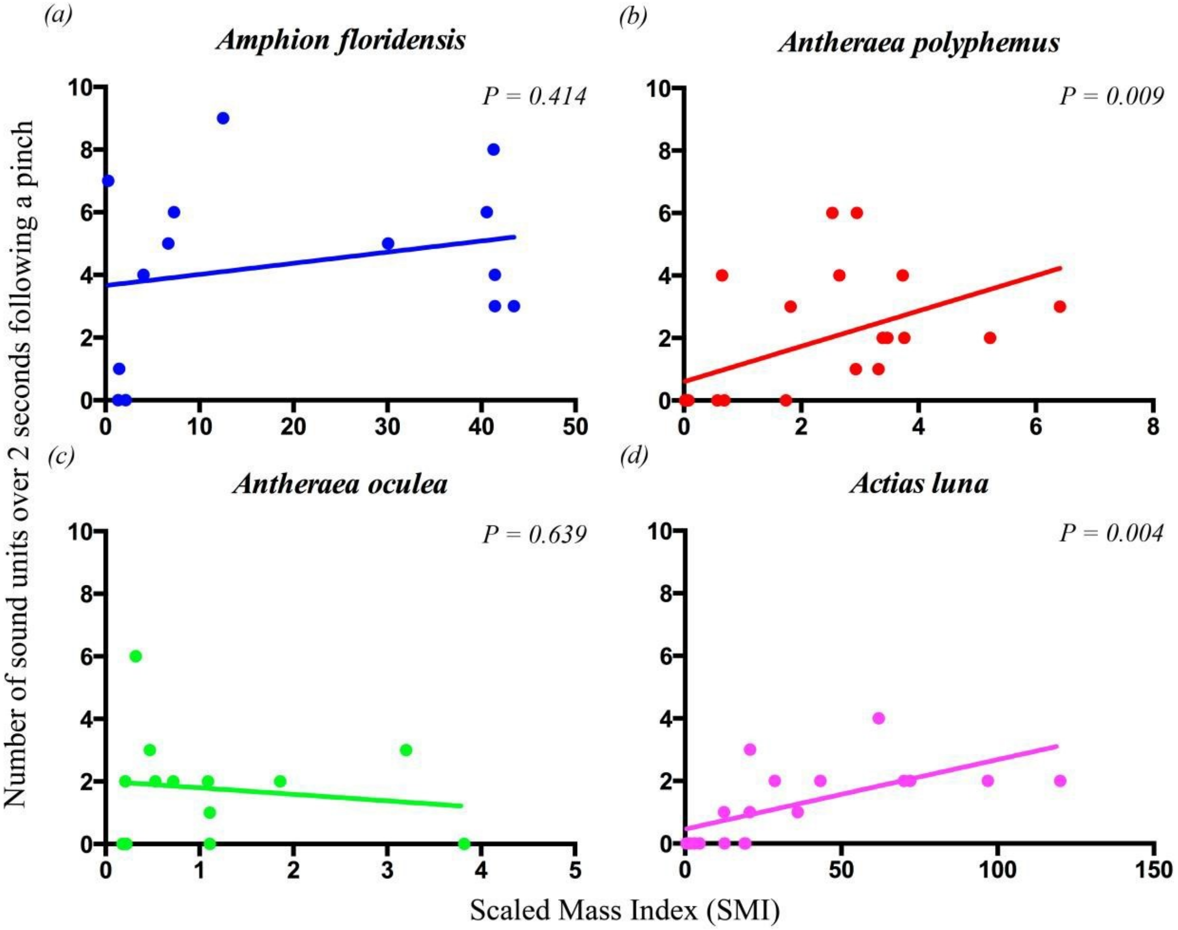


Figure 2.16. Relationship between the mean number of units following the first two seconds after an attack, and size (SMI) in each species. There was a significant positive relationship with size (SMI) in *A. polyphemus* (*P* = 0.639 and *A. luna (P* = 0.004). See Table 2.12 for sample sizes.

Do smaller caterpillars produce defence sounds with shorter unit durations?

To test prediction 2D, the relationship between the mean unit durations, measured from up to the first 10 units following the first 3 attacks, and size (SMI) was analyzed in individuals from each species. Overall, mean unit durations were longest in *AF* (i.e. 0.13 s) and shortest in *AL* (i.e. 0.005 s) (Table 2.13). There were no significant correlations between mean unit duration and size (SMI) in *AF* ($R^2 = 0.0242$, $P = 0.429$, N = 28), *AP* ($R^2 = 0.0563$, $P = 0.243$, N = 26) and *AL* ($R^2 = 0.0182$, $P = 0.559$, N = 21). There was a significant positive correlation between mean unit duration and SMI in *AO* ($R^2 = 0.4952$, $P = 0.016$, N = 17) (Fig. 2.17).

Do smaller caterpillars produce defence sounds with lower duty cycles?

To test prediction 2E, the relationship between duty cycle (% of signal over two seconds) and size (SMI) was analyzed in individuals from each species. Overall, duty cycles were highest in *AF* (i.e. 46%) and lowest in *AO* and *AL* (i.e. 1%) (Table 2.14). There were no significant correlations between duty cycle and size (SMI) in *AF* ($R^2 = 0.1066$, $P = 0.255$, N = 14), *AP* ($R^2 = 0.0501$, $P = 0.304$, N = 23), *AO* ($R^2 = 0.0002$, $P = 0.963$, N = 13) or *AL* ($R^2 = 0.0797$, $P = 0.242$, N = 19) (Fig. 2.18).

2.3.5 What is the relationship between size and intensity/spectral characteristics of the sounds?

Do smaller caterpillars produce defence sounds that are loud enough to be heard by predators?

To test prediction 3A, the relationships between size (SMI) and sound pressure levels (dB SPL) at 2 and 8 cm were analyzed in individuals from each species.

Table 2.13. Mean unit durations (s), standard deviations of the means, minimum and maximum unit durations (s) and sample sizes (N) in each species.

Species	**Mean (s)**	**SD**	**Min(s)**	**Max (s)**	**Number of pairs***
Amphion floridensis	0.13	0.05	0.05	0.24	29
Antheraea polyphemus	0.012	0.03	0.0003	0.17	26
Antheraea oculea	0.042	0.14	0.0002	0.72	17
Actias luna	0.005	0.01	0.0002	0.026	21

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

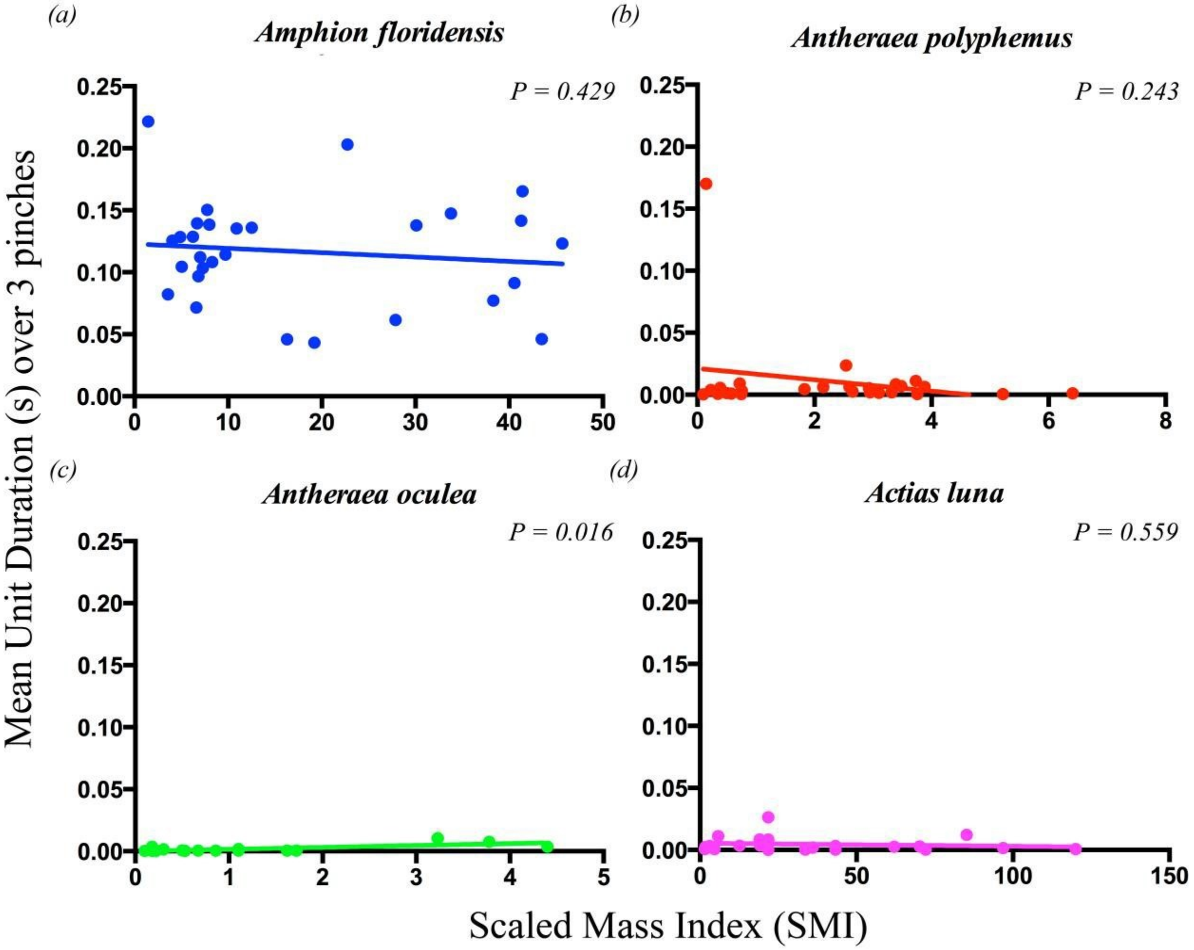


Figure 2.17. Relationship between the mean unit duration and size (SMI) in each species. See Table 2.13 for sample sizes.

Table 2.14. Mean duty cycle (%), standard deviations of the means, minimum and maximum duty cycles (%), and sample sizes (N) in each species.

Species	Mean (%)	SD	Min (%)	Max (%)	Number of pairs*
Amphion floridensis	46	1.31	0	100	14
Antheraea polyphemus	2	0.11	0	23	23
Antheraea oculea	1	0.03	0	5	13
Actias luna	1	0.06	0	11	19

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

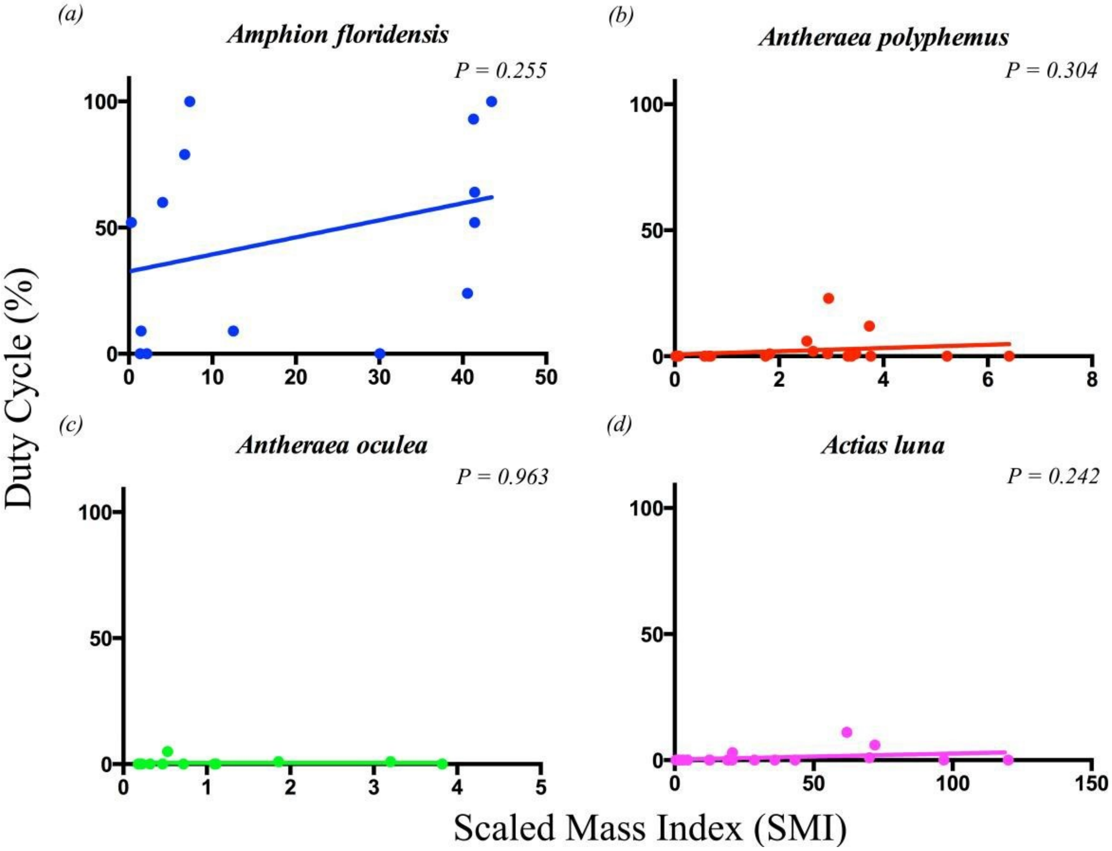


Figure 2.18. Relationship between duty cycle (% of sounds over two seconds) and size (SMI) in each species. There were no significant relationships between duty cycle and size. See Table 2.14 for sample sizes.

Overall, mean sound pressure levels at 2 cm were highest in *AF* (i.e. 91.6 dB SPL) and lowest in *AL* (i.e. 81.87 dB SPL) (Table 2.15). There were no significant correlations between sound levels at 2 cm and size (SMI) in *AF* ($R^2 = 0.3591$, $P = 0.067$, N = 10), *AP* ($R^2 = 0.1518$, $P = 0.169$, N = 14), *AO* ($R^2 = 0.0567$, $P = 0.847$, N = 3), or *AL* ($R^2 = 0.4752$, $P = 0.198$, N = 5 (Fig. 2.19). Mean sound pressure levels at 8 cm were highest in *AO* (i.e. 84.92) and lowest in *AL* (i.e. 75.38 dB SPL) (Table 2.15). There were no significant correlations between sound levels at 8 cm and size (SMI) in *AF* ($R^2= 0.2794$, $P = 0.206$, N = 7), or *AP* ($R^2 = 0.2422$, $P = 0.178$, N = 9) (Fig. 2.19). There were too few data points to analyze the correlations between sound levels at 8 cm and size (SMI) in *AO* and *AL*.

Do smaller caterpillars produce defence sounds that are in the frequency hearing range of predators?

To test Prediction 3B, we measured the relationship between dominant frequency at 2 and 8 cm and size (SMI) in individual from each species. Overall, the mean dominant frequency at 2 cm was highest in *AF* (i.e. 41 kHz) and lowest in *AO* (i.e. 22 kHz). (Table 2.16). There were no significant correlations between size (SMI) and dominant frequency at 2 cm in *AF* ($R^2 = 0.005$, $P = 0.8895$, N = 6), *AP* ($R^2 = 0.029$, $P = 0.4921$, N = 18), *AO* ($R^2 = 0.088$, $P = 0.8086$, N = 3) or *AL* ($R^2 = 0.001$, $P = 0.9530$, N = 5) (Fig. 2.20). The mean dominant frequency at 8 cm was highest in *AF* and *AO* (i.e. 8.5 kHz) and lowest in AP (i.e. 7.5 kHz) (Table 2.16) There were no significant correlations between size (SMI) and dominant frequency at 8 cm in *AF* ($R^2 = 0.005$, $P = 0.889$, N = 6), *AP* ($R^2 = 0.001$, $P = 0.902$, N = 18), *AO* ($R^2 = 0.1617$, $P = 0.734$, N= 3), or *AL* ($R^2 = 0.007$, $P = 0.896$, N = 5) (Fig. 2.20).

Table 2.15. Mean sound pressure levels (dB SPL), standard deviations of the means, minimum and maximum sound pressure levels (dB SPL) at 2 and 8 cm in each species.

Species	**2 cm**					**8 cm**				
	Mean (dB SPL)	**SD**	**Min (dB SPL)**	**Max (dB SPL)**	**Number of pairs**	**Mean (dB SPL)**	**SD**	**Min (dB SPL)**	**Max (dB SPL)**	**Number of pairs***
Amphion floridensis	91.6	5.41	82.24	100.86	10	84.56	3.94	78.56	89.72	7
Antheraea polyphemus	84.2	3.96	75.82	92.56	14	81.4	4.7	73.35	87.66	9
Antheraea oculea	86.6	4.89	82.29	91.73	12	84.92	5.92	84.44	85.44	0
Actias luna	81.87	4.2	76.03	90.73	5	75.38	0.74	68.87	83.86	2

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3)

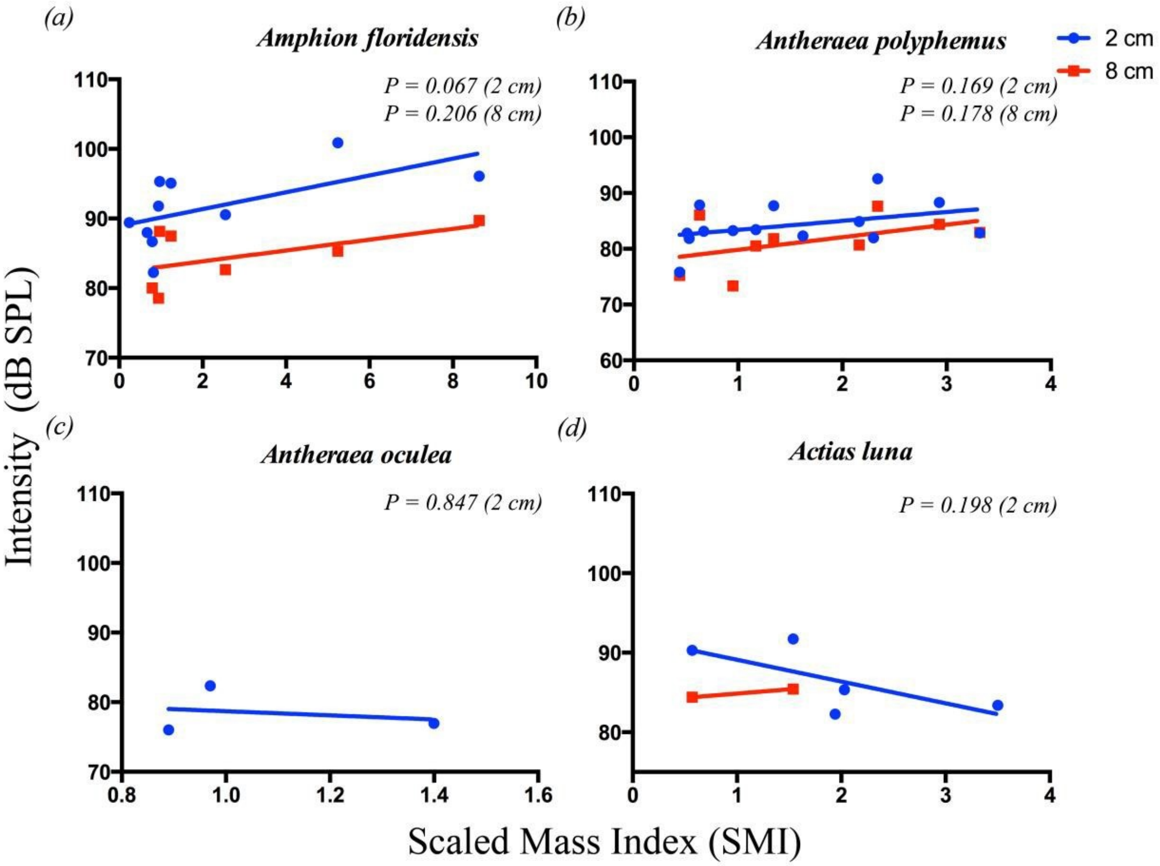


Figure 2.19. Relationship between defence sound pressure levels (dB SPL) and size (SMI) at 2 cm in each species. There were no significant relationships between sound level and size (SMI). See Table 2.15 for sample sizes.

Table 2.16. Mean dominant frequency (kHz), standard deviations of the means, minimum and maximum dominant frequencies (kHz), and sample sizes (N) at 2 and 8 cm in each species.

Species	**2 cm**					**8 cm**				
	Mean (kHz)	**SD**	**Number of pairs**	**Min (kHz)**	**Max (kHz)**	**Mean (kHz)**	**SD**	**Min (kHz)**	**Max (kHz)**	**Number of pairs***
Amphion floridensis	4.1	5.1	6	1.2	7.2	8.5	3.94	7.8	8.2	6
Antheraea polyphemus	3.2	6.8	11	1.1	5	8.2	4.7	6.3	7	11
Antheraea oculea	2.2	4.5	3	1	3.8	8.5	5.92	0.9	5.5	3
Actias luna	3.2	3.6	5	1.6	4.4	7.5	0.74	1.1	6	5

* Refers to the number of correlations between SMI and each variable in the analysis (see Table 2.3).

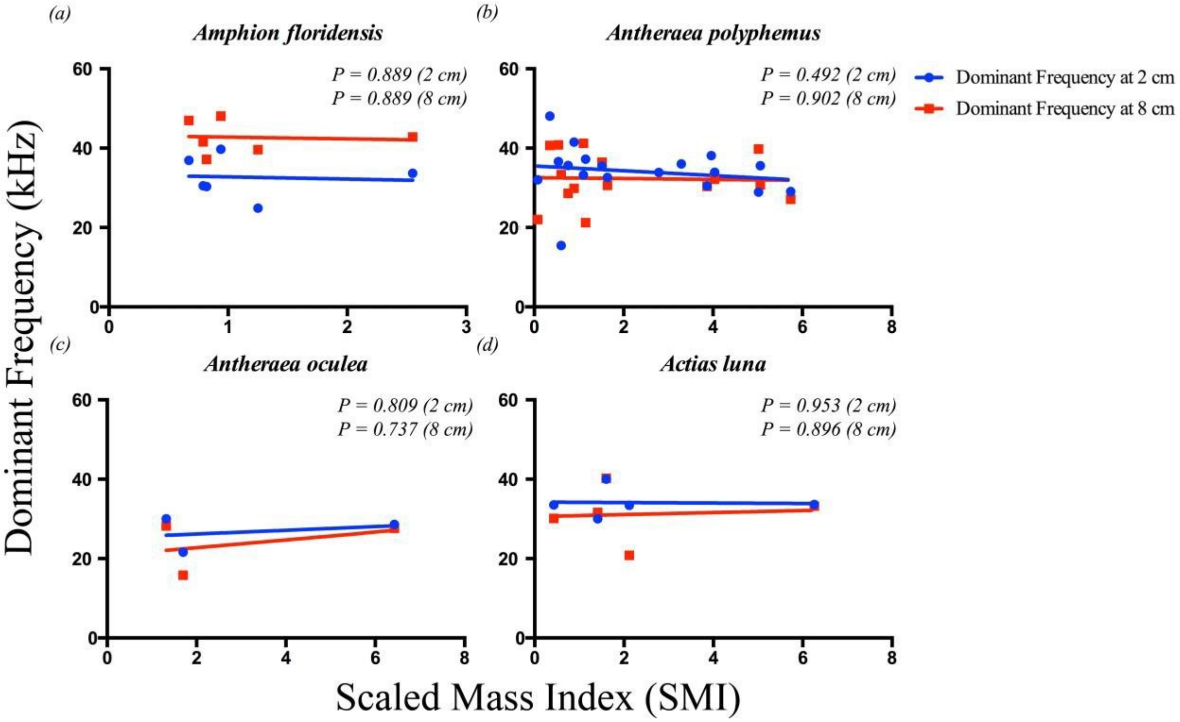


Figure 2.20. Relationship between dominant frequency (kHz) and size (SMI) at 2 cm and 8 cm in each species. There were no significant relationships between size and dominant frequencies at 2 or 8 cm. See Table 2.16 for sample sizes.

2.4 Discussion

2.4.1 Smaller Bombycoidea caterpillars do not make defence sounds

The first hypothesis tested in this study was that body size plays a role in the evolution of defence sounds in Bombycoidea caterpillars. To test this hypothesis, the development of defence sounds was followed from hatching to pupation in four Bombycoidea species, and both instar and body size was recorded throughout development. We had two predictions for this hypothesis, both of which were supported. First, we predicted that early instar caterpillars would not make defence sounds (prediction 1A). Second, we predicted that regardless of instar, defence sounds are more likely to occur in large caterpillars (prediction 1B). Overall, these results demonstrate a relationship between defence sounds and both instar and body size. The results of each prediction are discussed below.

To test prediction 1A we calculated the percentage of individuals that produced defence sounds at least once per instar. Results showed that in all species, none of the individuals tested produced defence sounds in the early instars (I, II). The possibility that early instars produced sounds that were too quiet to be detected during our tests is highly unlikely because all individuals were recorded with two microphones that together were highly sensitive across a wide range of sound frequencies. Additionally, all files were reviewed both during the trials, and following the trials on the computer. Our results disagree with a previous study (Brown et al., 2007) which reported that one second instar *A. polyphemus* produced a defence sound. In that study, instars were not followed systematically by monitoring head capsules throughout development as we have done in this study, and we proposed that in the previous study the individual was actually a third instar. In general, the percentage of individuals that produced defence sounds increased from instar III to IV, and was highest in *A. floridensis*. For instance, 100% of individuals produced defence sounds in instar IV in *A. floridensis* compared to only 12.5% in *A. luna*. Larvae from *A floridensis* rarely regurgitate after sound production (see Chapter Three), and perhaps the sounds function as startle displays, in which case it would be beneficial for all individuals to produce sounds each time they are attacked in order to deter predators right away (Edmunds, 1974). On the other hand, regurgitation usually follows mandible clicking (Brown et al., 2007), and if the sounds function as aposematic displays in *A. luna, A. oculea* or *A. polyphemus,* perhaps a lower percentage of individuals from these species produce defence sounds compared to individuals from *A. floridensis* because sound is only necessary when the caterpillars regurgitate. Overall, the percentage of individuals that produced defence sounds at least once per instar increased from instar III to V in all species.

As a follow-up analysis with regards to testing prediction 1B, the proportion of trials that resulted in sound production and how consistently sounds were produced by individuals throughout development was analyzed. In general, the proportion of trials that resulted in sound production increased throughout development in all species, and sound production was not produced consistently by individuals on each day of testing. This may have been because the caterpillars were molting, since molting impairs the ability to perform certain behaviours such as feeding (Greenlee & Harrison, 2005) and the mouthparts involved in feeding are also required for sound production (Bura, 2010; Rosi-Denadai & Yack, 2015). Another possible explanation for why individuals did not make sounds consistently is that there may be a trade-off between the energy required for molting, which is energetically costly (Slansky & Scriber, 1985), and the energy required to produce defence sounds. Regardless of how consistent sound production was amongst individuals, defence sounds were only produced by late instars, thus we suggest that the development of defence sounds may be instar-specific. Instar-specific changes may be the result of a developmental switch, and the hormonal and genetic correlates of the development defence sounds in late instars could be further studied.

To test prediction 1B we recorded the size of individuals at the onset of defence sound production. Results supported our hypothesis by demonstrating that all individuals first produced sounds when they reached the same mean mass and length (1.12 g, 26.27 mm), even though species varied in their final instar mass and length. Since all species produced defence sounds at the same relative size, we suggest that there is a relationship between the development of defence sounds and large size in caterpillars. Our results are in line with other studies that suggest large size is a factor in the evolution of certain defences (e.g. crypsis, eyespots and thrashing), because it improves the efficacy of the defence (Hossie et al., 2015; Sandre et al., 2007; Stamp, 1986). The results of this study are the first to provide evidence for a size-dependent relationship in an acoustic defence in a caterpillar, and we suggest that just as large size plays a role in the development of visual and behavioural defences, large size may play an important role in the development of defence sounds.

The results of our experiments to test the first hypothesis of this study provide indirect support for the hypothesis that body size may explain the diversity of defence sounds in other Bombycoidea species, and possibly other insect species. Thirty-three percent of the Bombycoidea species tested to date are reported to make defence sounds (Bura et al., 2016). There is a trend that larger species produce defence sounds (Bura, 2010), but this needs to be further explored by testing a larger selection of species, especially those on the smaller end of the body size spectrum (e.g. *Macroglossum stellatarum* (Sphingidae: Macroglossinae)). A relationship between defence sounds and body size has been reported in other insects such as adult adzuki beetles (*Typocopris* (Coleoptera: Geotrupidae)), Neotropical butterfly species, mantises (*Mantis religiosa* (Mantidae: Mantidae)) and cicadas (*Tibicen pronotalis* (Cicadidae: Cicadinae)) (Carisio et al., 2004; Hill, 2007; Marden & Chai, 1991; Sanborn & Phillips, 1995); however these are very few examples considering the class Insecta contains an estimated 950,000 species of insects (IUCN, 2007) and a large number of insects that produce sounds (Alexander, 1957), studies on the relationship between defence sounds and body size are lacking. Future studies that test the development of defence sounds in a wide variety of insects will be invaluable to providing further support for the hypothesis that body size is an important factor in the evolution of defence sounds.

2.4.2 Why don't small caterpillars make defence sounds?

Our second hypothesis explored a possible explanation for why small caterpillars do not make defence sounds in that they do not have enough energy to make defence sounds. To test this hypothesis, we examined the relationship between body size (SMI) and several temporal characteristics of the defence sounds. We had five different predictions, some of which were supported by evidence which showed a relationship between SMI and various acoustic characteristics including a significant increase in the mean number of units per train in *A. polyphemus,* the mean number of units within the first two seconds following the first attack in *A. polyphemus* and *A. luna,* and the mean duration of units in *A. oculea.* Overall, there were few positive relationships between body size and the temporal characteristics that we measured. This was not expected, since temporal characteristics such as the ones measured in our study are considered to be energetically costly, thus we would expect that larger caterpillars would have more energy to produce defence sounds with longer trains, unit durations, duty cycles, etc. (Peig & Green, 2009; Prestwich, 1994). In general, the temporal qualities of defence sounds are important because in order to be effectively transmited to predators, the sounds need to be sufficiently long enough to be integrated in to their sensory systems. We noted that the defence sound units produced by all of the caterpillars at the onset of sound production were already long enough to be integrated in to the sensory system of most vertebrates (e.g. ~200 ms) (Dooling & Searcy, 1985). Therefore, we suggest that the smaller caterpillars in our study already had the energy to make defence sounds that are sufficiently long enough to be detectable to vertebrate predators at the onset of sound production.

What are alternative explanations for why defence sounds do not occur in smaller caterpillars? One hypothesis is that small caterpillars are potentially capable of producing defence sounds, but because there are no selection pressures from predators, they do not rely on this secondary defence. Several studies have supported the hypothesis that smaller insects are less desirable to predators and face a lower level of predation (reviewed in Bernays, 1996; Halpin et al., 2013). However, since early instar caterpillars in our study did not produce defence sounds in response to simulated attacks, we suggest that the possibility that early instar caterpillars are capable of producing defence sounds is unlikely. Another hypothesis to explain why smaller caterpillars do not make defence sounds is that small size places restrictions on sound the intensity and spectral characteristics of the sounds which affects transmission of the signals within the environment (Carisio et al., 2004; Romer, 1993). For instance, small size restricts the intensity of the sounds that insects are able to produce, and insects are restricted to producing high-frequency sounds that degrade much faster than low frequency sounds within the environment, especially when they come in to contact with physical obstacles such as noise, wind, temperature and sounds from other animals (Bennet-Clark, 1999; Forrest, 1994; Gerhardt & Huber, 2002; Romer, 1993). Thus smaller caterpillars may not make defence sounds because they cannot produce sounds withthe intensity and spectral qualities that are required to be detected by predators. We tested this hypothesis in our final set of experiments.

2.4.3 Can predators detect caterpillar defence sounds

Our last set of experiments tested an alternative hypothesis for why smaller caterpillars do not make defence sounds, which was that defence sounds are restricted to larger caterpillars because predators cannot detect the sounds of small individuals. To test our hypothesis, we examined the relationship between body size (SMI) and the sound pressure level and dominant frequency of the defence sounds. Prediction 3A was that the sounds of small caterpillars are not loud enough for major predators (i.e. birds, bats) to be detected within a distance of 2-8 cm (i.e. the approximate distance of an attacking vertebrate predator). Our hypothesis was not supported by the results which showed no significant relationships between sound pressure level and size in any of the species. These results were unexpected, since previous research suggests that the intensity of insect sounds is related to body size (Bennet-Clark, 1999; Sanbourn & Phillips, 1995). It would be of no benefit to smaller caterpillars if they made defence sounds that are not detectable to predators, especially if the sounds function as deimatic displays which need to be loud to be effective (Blaszczky, 2003). However, the caterpillars in our study produced sounds ranging between 75.82 and 83.86 dB SPL at 2 and 8 cm. Based on these results, we suggest the caterpillars were already large enough to produce sounds that are loud enough to be heard by predators at the onset of sound production. Prediction 3B was that sounds of smaller caterpillars do not match the frequency hearing range of major predators. Again, our hypothesis was not supported by the results which did not demonstrate any significant relationships between dominant frequency and body size in any of the species. These results were also unexpected, since previous research suggest frequency is related to the size of the sound producing structure in insects (Bennet-Clark, 1999). However, it has also been suggested that the relationship between size and frequency may be hard to detect without a large range of values (Cocroft & De Luca, 2006). Thus it may be possible that the trend towards lower frequency sounds with larger body size would be more apparent if sampled from a wider range of body sizes. Nonetheless, the caterpillars in our study produced sounds ranging between 0.9 to 8.2 kHz at 2 and 8 cm, and signals in this frequency range are detectable a wide range of predators (e.g. rodents, bats, birds) (Adams & Pedersen 2000; Beason, 2004). While on average the signals contained very high frequencies, these frequencies were measured at short distances (i.e. 2 and 8 cm, the approximate distance of an attacking predator), so degradation of the high-frequency signals which occurs over long distances (Forrest, 1994; Romer, 1993) need not be a concern. Power spectra showed that the peak frequencies were as loud as 71 to 79 dB SPL at 2 cm, which is sufficiently loud enough to be detected by predators that can hear these high frequencies (Fig. 2.21). Similar to our conclusions made about the temporal and intensity analysis we performed, we suggest that the onset of defence sound production occurred when the caterpillars reached a critical size to produce sounds with frequencies that are in the hearing range of major vertebrate predators.

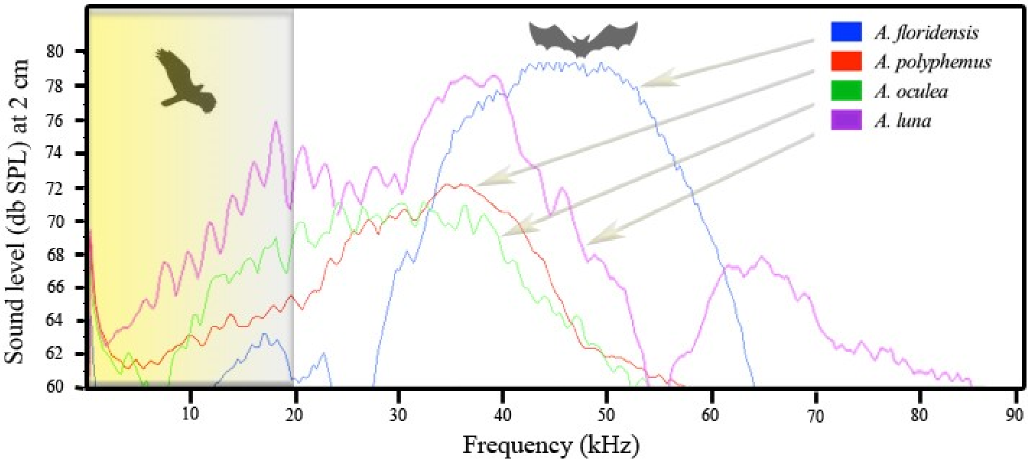


Figure 2.21. Representative power spectra of the four species tested, showing dominant frequency at 2 cm and the overlap with the hearing ranges of major predators (a) Birds (shaded area); (b) (gleaning) bats (non-shaded area).

2.5 Conclusion

Body size has been shown to play an important role in the evolution of defences in insects. However, few studies have tested the relationship between body size and acoustic defences. In this study we provide the first empirical evidence that the development of defence sounds is related to both instar and body size in caterpillars. We suggest that small/early instar caterpillars do not make defence sounds because they are not capable of making sounds that are effectively communicated to predators, and that the caterpillars begin producing sounds when they are large enough to make sounds that can be detected by predators. Future studies should further test the hypothesis on the importance and efficacy of size in the development of defence sounds using a wide range of other caterpillar and insect species on live predators.

CHAPTER THREE

Anti-Predator Defences in Sound-producing Bombycoidea Caterpillars: Ontogenetic Changes between Instars

Abstract

Instar-specific changes have been reported for a variety of defence types in insects. Chapter Two of this thesis provided evidence that sound production may be size dependent: only late instar caterpillars (III-V) of four representative Bombycoidea species produced defence sounds, and within instars sound production was linked to size. There are several possible explanations for size-and/or instar-dependent changes in acoustic defences. For example, perhaps as caterpillars grow they are pursued more by predators and rely more on secondary defences in general, the defence sounds form part of multimodal display, or they function as warning signals for a chemical defence. Alternatively, perhaps sound production is correlated to a change in the predator population (e.g. from invertebrates to vertebrates) such that defences change to target sensory systems of their changing natural enemies throughout development. In order to test such hypotheses, it is important to first document details of the defensive repertoire of the insects at each developmental stage. Therefore, the main goal of this chapter was to document and quantify the antipredator defences of the four Bombycoidea species studied in Chapter Two throughout development. It was found that as a primary defence, all of the caterpillars remained camouflaged with the host plant throughout development. A Linear Mixed Model was used to analyze changes in the frequency of secondary defences employed by the caterpillars during simulated predator attack trials in each instar. The mean frequency of secondary defences was different between some instars: (1) in *A. floridensis* the mean frequency of dropping and major thrashing significantly increased in instars III and IV; and (2) in *A. polyphemus,* the frequency of regurgitation significantly increased in instars III, IV and V. In all of the species, the frequency of defence sounds significantly increased in the later instars (III → IV). The implications of these results on future studies regarding the evolution of acoustic defences are discussed.

3.1 Introduction

In insects, antipredator defences including behavioural, chemical and morphological attributes can change throughout development (reviewed in Ananthakrishnan, 2005). There are several reported examples in different insect orders. In *Stagmatoptera biocellata* (Mantodea: Mantidae) early instars rely on crypsis, and late instars use deimatic displays (Balderrama & Maldonado, 1973; Maldonado, 1970). In *Acrythosiphon pisum* (Hemiptera: Aphidinae) early instars engage in escape behaviours, whereas late instars use defensive behaviours (Gerling, Roitberg & Mackauer, 1990). In Lepidoptera a few defence types have been reported to change throughout development. For example, in *Papilio xuthus* (Papilionidae: Papilioninae) early instars mimic bird droppings and late instars are cryptic (Futahashi & Fujiwara, 2008). In *Saucrobotys futilalis* (Crambidae: Pyraustinae) early instars are cryptic, whereas late instars develop aposematic colouration (Grant, 2007). In *Hemileuca lucina* (Saturniidae: Hemileucinae) early instars thrash in response to attack, whereas late instars evade predators by dropping from the host-plant (Cornell, 1987). While there are many studies focusing on the functions of different defence strategies in general (Lederhouse, 1990; Matthews & Matthews, 2010), few studies focus on the functional significance of ontogenetic changes in defences (Relyea, 2005; Zalucki, Clarke & Malcolm 2002).

There may be several explanations for why defences change throughout development. Some of the prominent hypotheses include the following: (1) the efficacy of a particular defence is size dependent (see Chapter Two). For example, *Stagmatoptera biocellata* (Mantidae: Stagmatopterinae) only employ deimatic displays in later development and it is suggested that larger size renders the displays more effective at scaring away birds (Maldonado, 1970); (2) selection pressures on insects increase as they become larger, either because predators prefer larger prey, or because large size makes prey more detectable, and therefore prey may have a greater need to invest in secondary defences (Edmunds, 1974; Berger et al., 2006; Halpin et al., 2013). For example, in *Saucrobotys futilalis* (Crambidae: Pyraustinae) final instar larvae develop warning displays as the benefits of crypsis decrease with increased foraging and predation rates in late instars (Grant, 2007); (3) types of predators change throughout development, such that early instars often face higher selection pressures from invertebrate predators, whereas late instars face higher selection pressure from vertebrate predators (Bernays, 1997; McClure & Despland, 2011) and therefore different defences may target different sensory systems of these different predators. For example, in *Hemileuca lucina* (Saturniidae: Hemileucinae) it is suggested that the defences of early instars (e.g. thrashing) and late instars (e.g. escape) are tailored to the changing natural enemies (i.e. from invertebrate to vertebrate predators) they encounter at different developmental stages (Cornell, 1987; Lichter-Marck, Wylde, Aaron, & Oliver, 2015; McClure & Despland, 2011). These examples illustrate how different biological and ecological factors may impact the evolution of insect defences throughout development.

In Chapter Two of this thesis I reported that only late instars (III-IV) of selected Bombycoidea caterpillars produce defence sounds. Hypotheses explaining why early instars/smaller caterpillars do not make defence sounds include the following: (1) small/early instars do not have enough energy to produce effective defence sounds that are detectable by vertebrate predators; (2) early instars face greater selection pressures from invertebrate predators (Bernays, 1997) that do not have highly developed auditory systems to detect sounds; (3) larger caterpillars develop more secondary defences due to increased selection pressures from predators (Edmunds, 1974); (4) defence sounds develop as signals to communicate the presence of another defence (e.g. chemical defence) that has developed in later instars. As a first step in testing alternative hypotheses, it is necessary to qualitatively and quantitatively document how various defences change throughout development.

The objectives of this study were to document the changes in defences exhibited by four species of Bombycoidea caterpillars that produce defence sounds as late instars: *Amphion floridensis, Antheraea polyphemus, Antheraea oculea* and *Actias luna.* Simulated predator attack trials were conducted throughout development to address the following questions: (1) What primary and secondary defences occur throughout development? (2) Does the frequency of secondary defences change with instar? (3) Do species differ in the frequency of secondary defences employed? I discuss how the findings of this descriptive ontogenetic study may be helpful in guiding future studies on the evolution of defence sounds in caterpillars.

3.2 Methods

3.2.1 Animals

Four Bombycoidea species were selected based on my previous knowledge of defence sounds in the later instars (see Chapter Two). These included 20 *Amphion floridensis* (Sphingidae: Macroglossinae), 20 *Antheraea polyphemus*, 12 *Antheraea oculea* and 25 *Actias luna* (Saturniidae: Saturniiae). Larvae were housed at an insect rearing facility at Carleton University at an average temperature of 23°C under a natural light-dark cycle. Eggs were kept in two-ounce plastic containers with holes in the lid for ventilation. Hatchlings were transferred to individual, marked 3.8 L glass mason jars and reared on their respective host plants until pupation. Host plants were replaced every 2-4 days, at which time the mason jars were also cleaned to remove frass and old plant material. Larvae of *A. floridensis* were reared on cuttings of *Vitis* (Vitaceae)*, A. oculea* on *Quercus* (Fagaceae), and *A. polyphemus* and *A. luna* on cuttings of *Betula* (Betulacaea). Instars were identified by collecting head capsules between molts (see Chapter Two*, 2.2.2 Morphological Measurements*).

3.2.2 Primary defence observations

Physical appearance and natural resting positions of larvae on their host plants were followed by photographing the caterpillars undisturbed in greenhouse light with a Canon PowerShot G7 X camera (Canon Canada Inc., Mississauga, ON). Photographs were taken every 3-4 days from the time of hatching to pupation.

3.2.3 Secondary defence observations

Simulated predator attack trials were performed on individual larvae every 3-4 days from hatching to pupation. Larvae were removed from their jar on the plant cutting held in a vial, and the vial was placed on a stand affixed with a clamp. The larva was recorded undisturbed for 30 seconds prior to attack using a Sony DCR-TRV19 camcorder (Tokyo, Japan) equipped with a Sony ECM-MS908C microphone. Attacks were then performed by lightly pinching the posterior of the abdomen five times at 5 second intervals with blunt forceps, a technique commonly used to simulate an attack by a bird (Bura et al., 2009; Grant, 2007, see also Chapter Two). To address whether the frequency of secondary defences changes with instar, I recorded the frequency of each defence (see Table 3.1) that occurred in response to each attack. If the defence was present, a score of 1 was recorded. If the defence was absent, a score of 0 was recorded. For example, if the larvae thrashed at least once during four out of the five attacks, the frequency of thrashing for would be 4. Since the larvae were tested regularly throughout development, they were tested anywhere between one to four times per instar. Some individuals were tested more than once per instar over the course of their development, so the score for any defence per individual per instar was calculated as the overall mean frequency of each defence based the frequencies recorded for all of the trials within that instar.

3.2.4 Statistical analyses

I wanted to know whether the frequency of secondary defences during simulated predator attack trials changed with instar in each species. To answer this question, I used a Linear Mixed Model (LMM) in IBM SPSS Statistics (IBM Corporation, Armonk, NY, USA) to compare instar (independent variable) on the dependent variable (mean frequency of each secondary defence during simulated predator attack trials) for each species. SPSS offers several options for modeling variance in data sets for analysis, each of which has a different level of fit for the particular data set. One way to assess the best fit of a LMM is to compare the Akaike's Information Criterion **(**AIC) generated for each model, and to choose a model with the lowest AIC value (Singer, 2002). I tested the overall significance of three models in SPSS using the AIC value (i.e. Compound symmetry, AR(1), Huynd-Felt). Since there were a total of seven defences being analyzed in our models and four to five instars, I ought to adjust my alpha level to 0.002 when using a Bonferroni correction for pairwise comparisons. Since this significance level is extremely conservative, I also report alpha levels between 0.002 and 0.05 to reduce the risk of Type II errors. I report effects at the 0.05 level with caution, as these results may be problematic, but as an exploratory component they may be nonetheless worthy of future investigation.

Table 3.1. Description of secondary defences that were quantified during each simulated predator attack trial.

Defence	Description	Measure
Regurgitation	Regurgitation is the process by which the larva expels fluid through the mouth in response to an attack or disturbance[1]	If regurgitate was excreted from the mouth after an attack, the larva was given a score of 1 for that attack.
Major Thrashing	Movement of the thorax from left to right vigorously >2 times[2]	If larva moved the thorax from left to right more than two times after an attack it was given a score of 1 for that attack.
Minor Thrashing	Movement of the thorax from left to right <2 times[2]	If the larva moved the thorax once to the left and once to the right after an attack it was given a score of 1 for that attack.
Escaping	Walking away from position before attack to escape predation.[3]	If the larva changed position after an attack it was given a score of 1.
Dropping	Dropping off the host plant.[3]	If the larva dropped from the plant after an attack, it was given a score of 1. After dropping, the larva would be replaced on the plant and attack trials continued after abdominal prolegs were re-attached to the leaf.
Curling	Movement of the anterior and posterior portions of the larva's body inward resembling the shape of the letter "C" [4]	If both the anterior and posterior portions of the body moved in to a "C" shape after an attack, the larvae were given a score of 1 for that attack.
Defence sounds	Air-borne signals produced in response to attack.[5]	If the larva produced a sound after an attack it was given a score of 1 for that attack.

References: (1) Grant, 2007; (2) Walters et al., 2001; (3) Bateman & Fleming 2015; (4) Deml & Dettner, 2002; (5) Masters, 1979.

3.3 Results

3.3.1 Growth, development, and primary defence observations

Instars were followed and identified by collecting head capsules as described in Chapter Two. *Amphion floridensis* went through four instars, and *A. polyphemus, A. oculea* and *A. luna* went through five instars (See Chapter Two, Fig. 2.3). *Amphion floridensis* went through a noticeably drastic ontogenetic colour change. Larvae were light green in instars I and II and dark brown in instars III and IV (Fig. 3.1a). During this colour change the larvae remained camouflaged with the host plant by changing their resting position from the underside of the brown branches of the plant. The remaining species were green in all instars and remained camouflaged with the background of the host plant by resting on the underside of the leaf throughout development (Fig. 3.1b-d).

3.3.2 Ontogenetic change of secondary defences

Fixed effects of Species, Instar, and Defence

I tested the hypothesis that there would be a trade-off in the frequency of secondary defences with the development of defence sounds in the late instars. My prediction was that the development of defence sounds in late instars (III, IV, V) would cause a decrease in the frequency of the secondary defences employed by the early instars (I, II). To test my prediction, I compared the relationship between two independent variables (IV) (i.e. species, instar) on our dependent variable (DV) which was the mean of the frequencies of each defence observed during simulated predator attack trials in larvae with three different Linear Mixed Models. I chose to use the model with the lowest AIC value as an indicator of best-fit (see *Methods*). The AIC statistic for each model I tested was: (1) Compound symmetry: AIC = 2687.301; (2) AR (1): AIC =

3380.469; (3) Huyndt-Felt: AIC = 3469. I therefore chose the model with the variance structure of compound symmetry based on the lowest AIC statistic. This LMM revealed that there was no significant main effect of species *F* (3, 50.04) = 1.57, *P* = 0.208 on the DV, and there was a significant main effect of instar *F* (4, 535.29) = 10.48, *P* < 0.001 and defence *F* (6, 924.56) = 26.68, *P* < 0.001 on the DV. This result supports my prediction that the mean frequency of each defence would be different in the early instars compared to the late instars. There was no significant two-way interaction between species and instar *F* (10, 591.18) = 2.24, *P* = 0.014 on the DV. There was a significant two-way interaction between species and defence *F* (18, 924.56) = 2.79, *P* < 0.001 and instar and defence F (36, 1370.39) =5.4, *P* < 0.001 on the DV. Finally, there was a significant 3-way interaction between species, instar, and defence *F* (60, 924.56) = 1.89, *P* < 0.001 on the DV, indicating that species differed in the frequency of defences employed in each instar. To understand how the various IV's affected the DV in the 3-way interaction, I split the data by species and analyzed the effects of instar and defence on the DV (mean frequency of behaviours in each instar) for each species. The results of the analysis for each species are presented below.

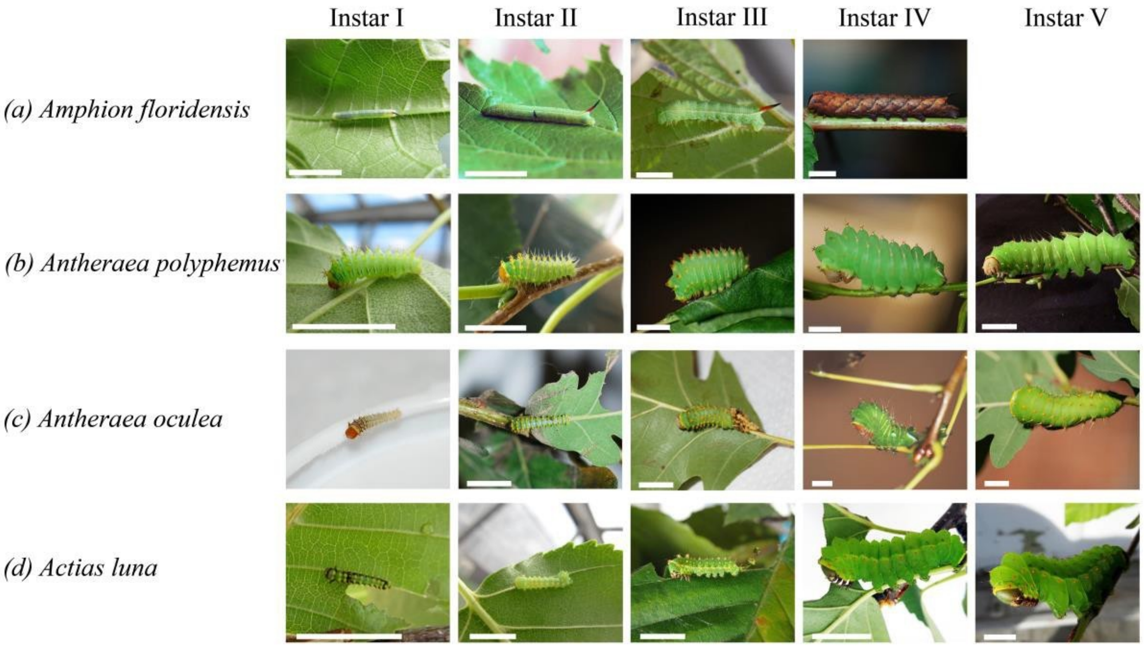


Figure 3.1. Appearance and natural resting position on host plant of larvae from each Bombycoidea species in each instar. Scale bar 10 mm. Scale bar for *A. oculea* is not available.

For each species, the simple main effects of the mean frequencies of each defence in each instar was assessed to determine whether the means were significantly different in each instar. Where there were significant main effects or two-way interactions univariate tests were performed to determine which instars were different. Where there were significant effects of the mean frequency of a secondary defence on instar, follow-up pairwise comparisons were performed to determine in which instars the frequency of defences differed. The results of the analysis for each species are presented below.

Amphion floridensis

Mean frequencies, standard deviations, and sample sizes of all defences observed in *A. floridensis* are shown in Table 3.2. There was a significant main effect of instar F (3, 247.8) = 14.63, $P < 0.001$ and defence F (6, 246.57) = 5.69, $P < 0.001$. There was also a significant two-way interaction between instar and defence F (18, 246.57) = 6.69, $P < 0.001$. Follow-up univariate tests were performed to determine which defences were involved in the two-way interaction. There was no significant effect of instar on escaping or curling (Table 3.3). There was a significant effect of instar on dropping, major thrashing and defence sounds (Table 3.3). Follow- up pairwise comparisons were performed to determine which instars were different. For dropping, instar IV had a significantly higher frequency than instar I ($P = 0.002$), instar II ($P = 0.05$) and instar III ($P = 0.018$) (Fig. 3.2b). For major thrashing, instar IV had a significantly higher frequency than instar I ($P < 0.001$), instar II ($P <0.001$), and instar III ($P <0.001$) (Fig. 3.2e). For defence sounds, instar III had a significantly higher frequency than instar I ($P < 0.001$) and instar II ($P = 0.002$) (Fig. 3.2g). Instar IV also had a significantly higher frequency of defence sounds than instar I ($P < 0.001$) and instar II ($P < 0.001$) (Fig. 3.2g).

Table 3.2. Mean frequency of defences in *Amphion floridensis* in each instar.

	Instar I		**Instar II**		**Instar III**		**Instar IV**	
Behaviour	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N
Escaping	0 ± 0	9	0 ± 0	9	0.04 ± 0.14	8	0.02 ± 0.09	9
Dropping	0 ± 0	9	0 ± 0	9	0 ± 0	8	0.19 ± 0.54	9
Curling	0.83 ± 0.66	9	0 ± 0	9	0.02 ± 0.07	8	0 ± 0	9
Major Thrashing	0 ± 0	9	0 ± 0	9	0.41 ± 0.6	8	1.47 ± 2.27	9
Minor Thrashing	0.5 ± 0.71	9	0 ± 0	9	0.03 ± 0.1	8	0 ± 0	9
Regurgitation	0.67 ± 0.66	9	1.5 ± 1.9	9	0.67 ± 1.04	8	0.04 ± 0.18	9
Defence sounds	0 ± 0	9	0±0	9	1.33 ± 1.19	8	3.23 ± 1.75	9

Table 3.3. Univariate tests of the simple main effects of instar on defence in *A. floridensis*.

Defence	**Number df**	**Denominator df**	**F**	**Sig.**
Escaping	3	246.573	0.345	0.793
Dropping	3	246.573	2.945	0.033*
Curling	3	246.573	1.978	0.117
Major thrashing	3	246.573	12.827	0.000***
Minor thrashing	3	246.573	0.694	0.556
Regurgitation	3	246.573	2.342	0.073
Sound	3	246.573	48.436	0.000***

* $\alpha < 0.05$
*** $\alpha < 0.001$

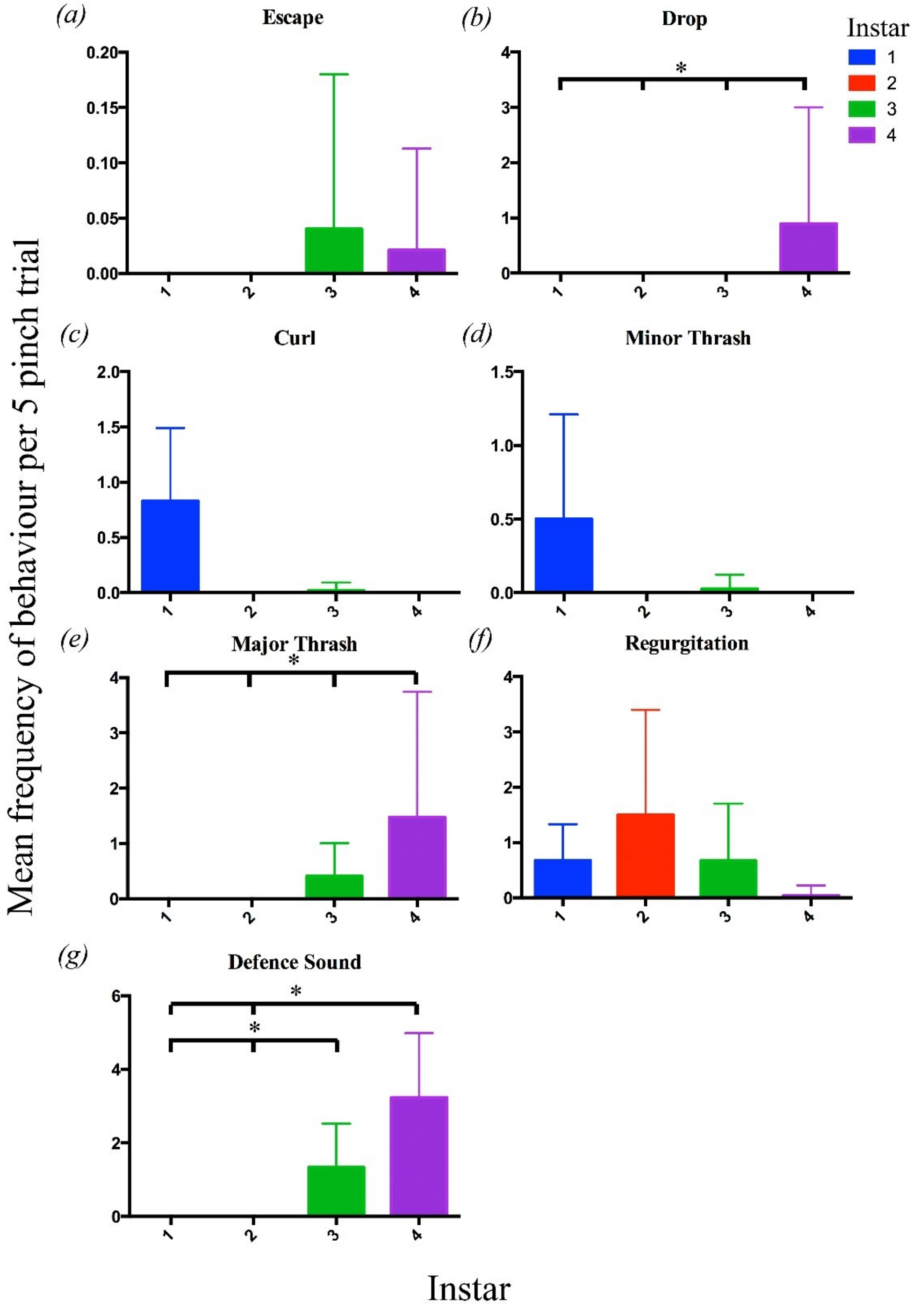


Figure 3.2. Mean frequency of defences observed during simulated predator attack trials in each instar in *Amphion floridensis.*

Antheraea polyphemus

Mean frequencies, standard deviations, and sample sizes of all defences observed in *A. polyphemus* are shown in Table 3.4. There was a significant main effect of instar F (4, 399.77) = 9.4, $P < 0.001$ and defence F (6, 401.61) = 30.81, $P < 0.001$. There was also a significant two-way interaction between instar and defence F (24, 401.61) = 5.4, $P < 0.001$. Follow-up univariate tests were performed to determine which behaviours were involved in the two-way interaction. There was no significant effect of instar on escaping, dropping, curling, major thrashing, or minor thrashing (Table 3.5). There was a significant effect if instar on regurgitation and defence sounds (Table 3.5). Follow-up pairwise comparisons were performed to see which instars were different. For regurgitation, instar I had a significantly higher frequency than instar IV (P = 0.008) and instar V (P = 0.02) (Fig. 3.3f). Instar II also had a significantly higher frequency of regurgitation than instar IV (P = 0.004) and instar V (P = 0.01) (Fig. 3.3f). For defence sounds, instar I had a significantly lower frequency of defence sounds than instar III ($P < 0.001$), instar IV ($P <0.001$), and instar V ($P < 0.001$) (Fig. 3.3g). Instar II also had a significantly lower frequency of defence sounds than instar III ($P < 0.001$), instar IV ($P <0.001$), and instar V ($P <0.001$) (Fig. 3.3g).

Table 3.4. Mean frequency of defences in *Antheraea polyphemus* in each instar.

Behaviour	Instar I		Instar II		Instar III		Instar IV		Instar V	
	Mean ± SE	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**
Escaping	0 ± 0	5	0 ± 0	8	0.13 ± 0.4	10	0 ± 0	5	0 ± 0	10
Dropping	0 ± 0	5	0 ± 0	8	0 ± 0	10	0 ± 0	5	0 ± 0	10
Curling	0 ± 0	5	0 ± 0	8	0 ± 0	10	0 ± 0	5	0 ± 0	10
Major Thrashing	0 ± 0	5	0 ± 0	8	0.08 ± 0.34	10	0.22 ± 0.75	5	0.29 ± 0.69	10
Minor Thrashing	0 ± 0	5	0 ± 0	8	0.08 ± 0.25	10	0.03 ± 0.13	5	0.16 ± 0.41	10
Regurgitation	0 ± 0	5	0 ± 0	8	0.34 ± 0.65	10	0.78 ± 1.11	5	0.68 ± 0.73	10
Defence sounds	0 ± 0	5	0 ± 0	8	1.70 ± 1.47	10	2.53 ± 1.78	5	2.87 ± 1.22	10

Table 3.5. Univariate tests of the simple main effects of instar on defence in *A. polyphemus.*

Defence	Number df	Denominator	F	Sig.
Escaping	4	401.613	0.18	0.948
Dropping	4	401.613	0.000	1.000
Curling	4	401.613	0.000	1.000
Major thrashing	4	401.613	0.6	0.666
Minor thrashing	4	401.613	0.17	0.952
Regurgitation	4	401.613	3.96	0.004**
Sound	4	401.613	50.29	0.000***

** $\alpha < 0.005$
*** $\alpha < 0.001$

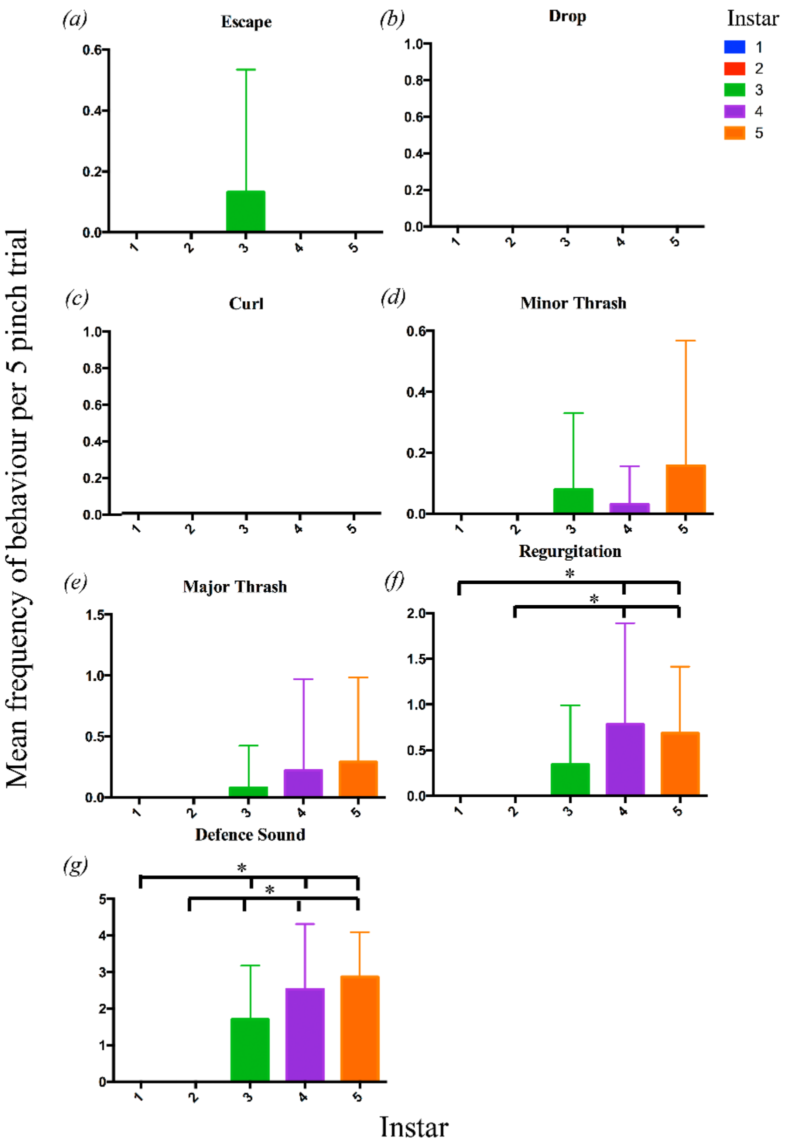


Figure 3.3. Mean frequency of defences during simulated predator attack trials in each instar of *Antheraea polyphemus.*

Antheraea oculea

Mean frequencies, standard deviations and sample sizes of all defences observed in *A. oculea* are shown in Table 3.6. There was a significant main effect of instar F (3, 22.86) = 4.18, $P = 0.017$ and defence F (6, 100.01) = 5.14, $P < 0.001$. Overall, there was no significant two-way interaction between instar and defence (18, 100.01) = 1.32, $P = 0.193$. There was no significant effect of instar on escape, dropping, curling, major thrashing, minor thrashing or regurgitation (Table 3.7). There was a significant main effect of instar on defence sounds (Table 3.7). Follow- up pairwise comparisons were performed to determine which instars were different, which revealed that instar II had a significantly lower frequency of defence sounds than instar IV ($P = 0.003$) and instar V ($P = 0.004$). Instar III also had a significantly lower frequency of sound production than instar IV ($P = 0.001$) and instar V ($P = 0.003$) (Fig. 3.4g).

Actias luna

Mean frequencies, standard deviations, and sample sizes of all defences observed in *A. luna* are shown in Table 3.8. There was no significant main effect of instar F (4, 178.34) = 1.22, $P = 0.304$. There was a significant main effect of defence F (6, 204.89) = 2.45, $P = 0.026$. There was no significant two-way interaction between instar and defence F (24, 204.889) = 1.47, $P = 0.079$. Follow-up univariate tests were performed to determine which defences were involved in the main effect. There was no significant effect of instar on escaping, dropping, curling, major thrashing or minor thrashing (Table 3.9). There was a significant main effect of instar on defence sounds (Table 3.9). Follow-up pairwise comparisons revealed that instar IV had a significantly higher frequency of sound production than instar I ($P < 0.001$), instar II ($P = 0.004$) and instar III ($P < 0.001$) (Fig. 3.5g).

Table 3.6. Mean frequency of defences in *Antheraea oculea* in each instar.

	Instar I		Instar II		Instar III		Instar IV		Instar V	
Behaviour	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**
Escaping	NA	NA	0 ± 0	10	0.13 ± 0.35	9	0.00	9	0 ± 0	2
Dropping	NA	NA	0 ± 0	10	0.13 ± 0.35	9	0.00	9	0 ± 0	2
Curling	NA	NA	0 ± 0	10	0.00	9	0.00	9	0 ± 0	2
Major Thrashing	NA	NA	0 ± 0	10	0.13 ± 0.35	9	0.67 ± 1.63	9	0 ± 0	2
Minor Thrashing	NA	NA	0 ± 0	10	0.00	9	1.04 ± 0.9	9	0 ± 0	2
Regurgitation	NA	NA	0 ± 0	10	0.88 ± 0.83	9	1 ± 0.71	9	2 ± 1.41	2
Defence sounds	NA	NA	0 ± 0	10	1.13 ± 1.81	9	2.58 ± 1.28	9	2.63 ± 1.89	2

Table 3.7. Univariate tests of the simple main effects of instar on defence in *A. oculea.*

Defence	**Number df**	**Denominator df**	**F**	**Sig.**
Escaping	3	100.014	0.03	0.993
Dropping	3	100.014	0.03	0.993
Curling	3	100.014	0.001	1.000
Major thrashing	3	100.014	0.81	0.491
Minor thrashing	3	100.014	2.37	0.073
Regurgitation	3	100.014	2.57	0.056
Sound	3	100.014	6.98	0.000***

*** $\alpha < 0.001$

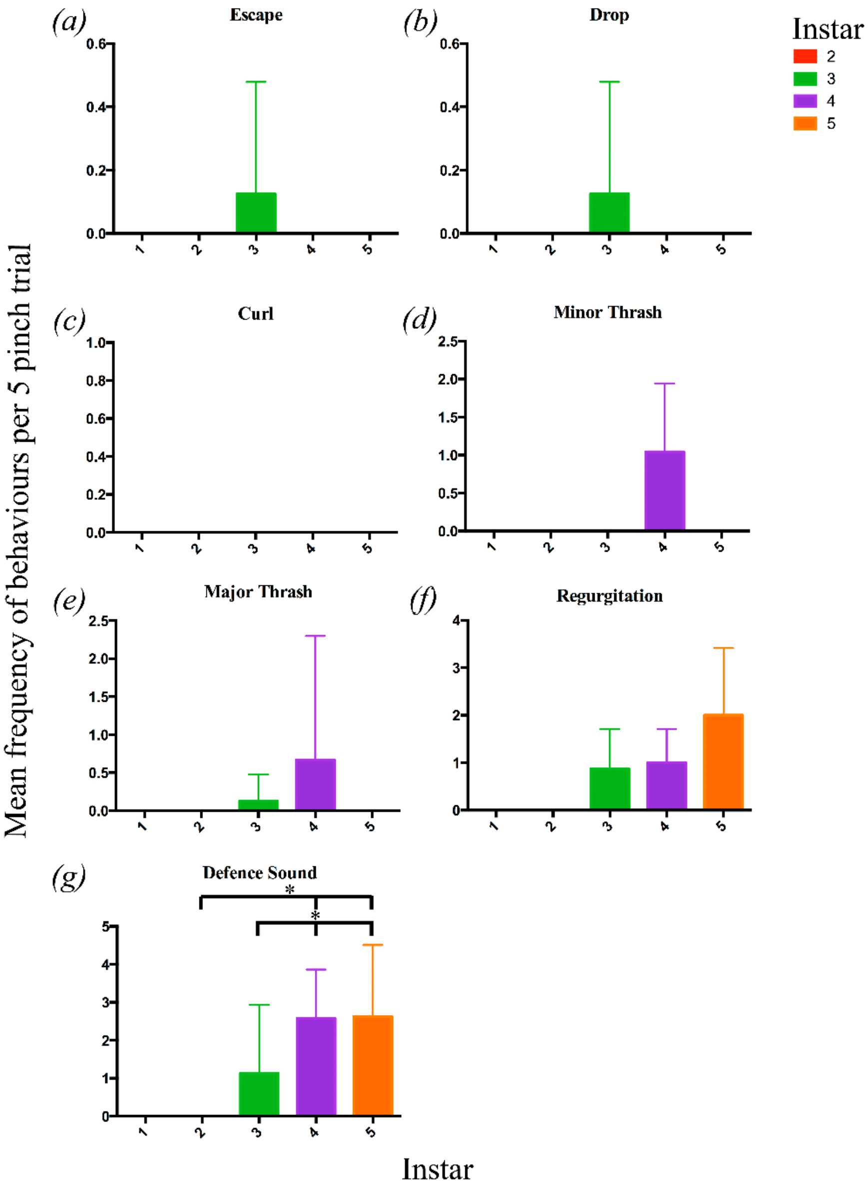


Figure 3.4. Mean frequency of defences during simulated predator attack trials in each instar of *Antheraea oculea.*

Table 3.8. Mean frequency of defences in *Actias luna* in each instar.

Behaviour	Instar I		Instar II		Instar III		Instar IV		Instar V	
	Mean ± SE	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**
Escaping	0 ± 0	25	0 ± 0	10	0.13 ± 0.42	9	0 ± 0	8	0 ± 0	7
Dropping	0 ± 0	25	0 ± 0	10	0 ± 0	9	0 ± 0	8	0 ± 0	7
Curling	0 ± 0	25	0 ± 0	10	0 ± 0	9	0 ± 0	8	0 ± 0	7
Major Thrashing	0 ± 0	25	0 ± 0	10	0.70 ± 1.49	9	0.21 ± 0.39	8	0.07 ± 0.19	7
Minor Thrashing	0 ± 0	25	0 ± 0	10	0.50 ± 1.27	9	0.41 ± 0.53	8	0.36 ± 0.48	7
Regurgitation	0 ± 0	25	0 ± 0	10	0 ± 0	9	0.21 ± 0.39	8	0.19 ± 0.49	7
Defence Sound	0 ± 0	25	0 ± 0	10	0.15 ± 0.34	9	0.57 ± 1.13	8	1.24 ± 1.42	7

Table 3.9. Univariate tests of the simple main effects of instar on defence in *A. luna*.

Defence	**Number df**	**Denominator df**	**F**	**Sig.**
Escaping	4	204.889	0.12	0.974
Dropping	4	204.889	0.05	0.996
Curling	4	204.889	0.05	0.996
Major thrashing	4	204.889	2.13	0.077
Minor thrashing	4	204.889	0.83	0.504
Regurgitation	4	204.889	0.142	0.996
Sound	4	204.889	5.2	0.000***

*** $\alpha < 0.001$

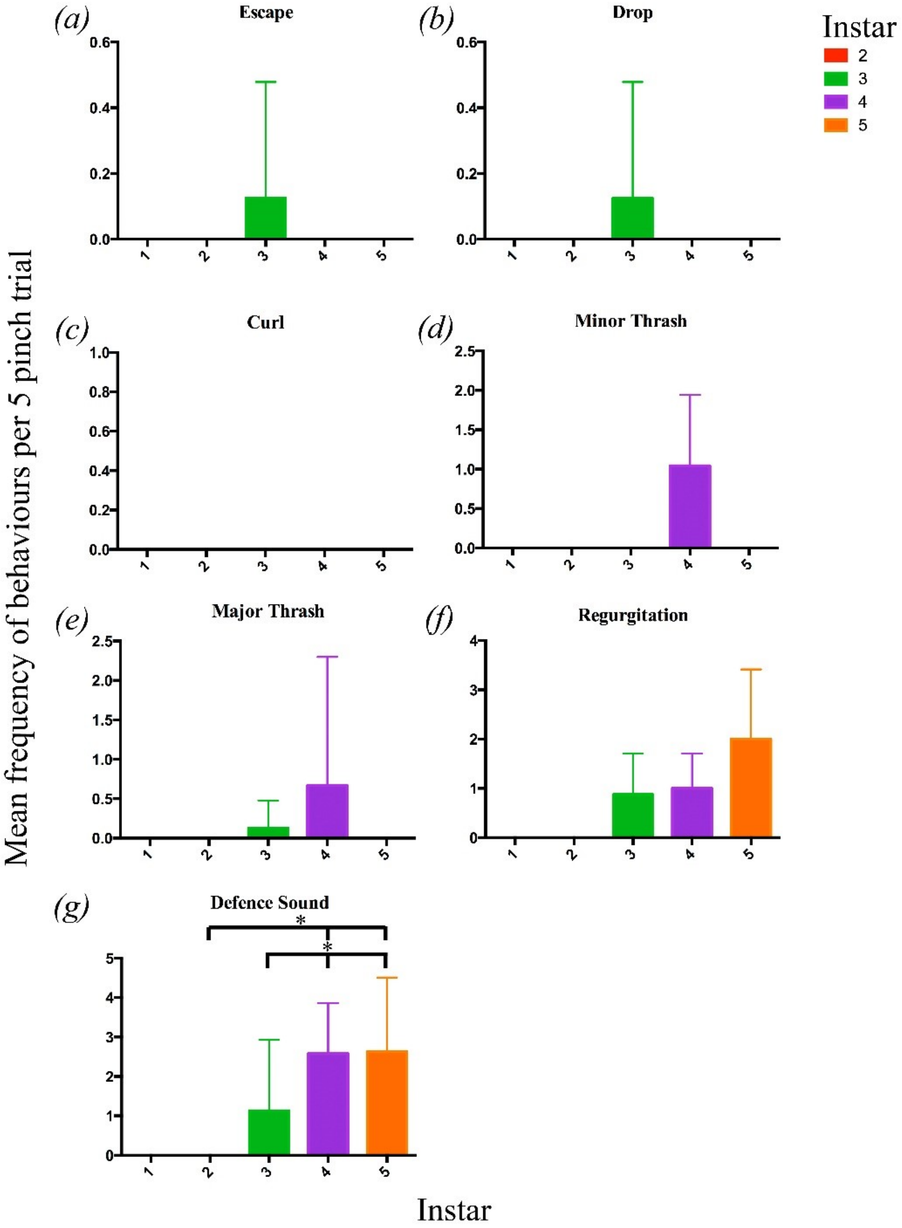


Figure 3.5. Mean frequency of defences during simulated predator attack trials each instar of *Actias luna.*

3.4 Discussion

The main goal of this study was to follow the various antipredator defences employed by sound-producing Bombycoidea caterpillars throughout development. The results of this study provide the necessary background research for future testing of hypotheses regarding why some Bombycoidea caterpillars produce defence sounds while others do not (e.g. multimodal defence hypothesis, trade-off hypothesis, aposematism) by documenting the other defences that may be involved in the antipredator displays and/or in a trade-off scenario. In general, all of the species appeared to remain camouflaged as their primary defence throughout development, and the mean frequency of some secondary defences (i.e. dropping, thrashing, regurgitation, defence sounds) increased in the later instars (e.g. instars III, IV, and V). The results for each species in reference to their significance for future hypotheses testing are discussed below.

3.4.1 Primary defences throughout development

While crypsis was not formally studied here, all four species appeared to blend in to the background on their host-plants during all stages of development. This result corroborates with previous literature that describes the species as cryptic in their larval form (Brown et al., 2007; Hall, 2012; Frank, 2015; Stamp & Casey, 1993). *Amphion floridensis* went through a dramatic change in colour from green to dark brown from instar III to IV. Some animal resemble the background by changing their behaviour in order to match its appearance (Endler, 1984). In the case of *A. floridensis,* the larvae shifted their natural resting position from the plant's leaf to its branches which were also dark brown. This type of colour change is exhibited in other hawkmoth species such as *Agrius convolvuli* (Sphingidae: Sphinginae) and *Errinyis ello* (Sphingidae: Macroglossinae) (Curio, 1965). It is suggested that species that undergo this type of colour change feed at night on the leaves so as not to appear conspicuous against the green leaves to predators that hunt by vision during the day (Curio, 1965). In general, crypsis is a major strategy for many insects (Gullan & Cranston, 2010) and indeed many caterpillars (Wicksten, 1990) because it helps to increase their chance of survival by preventing predators from finding them (Stevens & Merliata, 2011). However, studies have shown that the efficacy of crypsis becomes impaired when insects get larger and become more visible to predators (Berger et al., 2000; Bernays, 1997). If crypsis fails, some insects will employ secondary defences as a backup line of defence against predators (Brakefield, 2009; Edmunds, 1985; Ruxton et al., 2001). Since Bombycoidea caterpillars are some of the largest in the world (Heppner, 2008), perhaps defence sounds develop because protection via crypsis becomes impaired in later development. However, since crypsis was not formally evaluated in this study, future experiments that include a careful analysis crypsis, including UV assessment of the prey appearance and the visual perception of each predator are required to experimentally confirm the apparent crypsis observed in these species.

3.4.2 Secondary defences throughout development

Overall, the caterpillars exhibited 7 different secondary defences throughout development. In some species, the mean frequency of several of these defences (i.e. dropping, major thrashing, regurgitation and defence sounds) significantly increased in the later instars. As discussed above, the need for an additional line of defence against predators in later development may explain why the frequency of major thrashing, dropping, regurgitation and defence sounds increased. Alternatively, large size in later development may render some defences more effective (Griethuijsen, Banks, & Trimmer, 2013; Hossie et al., 2015; Marden & Chai, 1991). Since species differed in the frequency of secondary defences employed in each instar, ontogenetic changes in secondary defences between instars in each species are discussed separately below.

In *A. floridensis*, there was a significant increase in the frequency of major thrashing, dropping and defence sounds in the late instars. The mean frequency of major thrashing was significantly higher in the final instar IV compared to the remaining instars I, II and III. Thrashing may have increased in late instars because larger larvae have a wider range of motion for thrashing (Griethuijsen et al., 2013). In Chapter Two, I showed that late instar larvae from *A. floridensis* are significantly longer than *A. polyphemus, A. oculea*, and *A. luna* (see Fig. 2.9). Major thrashing may have increased in *A. floridensis* because they are sufficiently long enough to deflect attacks from predators directed at their body, whereas the other species may not be long enough to protect themselves against predators by thrashing due to their shorter body lengths. There was also a significant increase in the frequency of dropping in the final instar IV compared to the remaining instars I, II and III. While some researchers argue that dropping is costlier for large insects that may be too heavy to drop from silk strands (Sugiura & Yamazaki, 2006), it is suggested that dropping may be an effective defence in late instars because they may have an easier time relocating to the host plant (Dethier, 1954). Thus dropping may have increased in the late instars because it is less costly for the caterpillars who are capable of relocating their host- plant after falling to the ground. Finally, there was a significant increase in the mean frequency of defence sounds in instars III and IV. Since major thrashing, dropping and defence sounds all concurrently increased in the later instars in *A. floridensis*, it may be possible that the combination of secondary defences function as a multimodal display. For instance, movement combined with sound may enhance the effect of an antipredator display (Rowe & Guildford, 1999; Rowe & Halpin, 2013). If defence sounds are part of a multimodal display in larvae from *A. floridensis,* they should concurrently increase in frequency with the other modes of the display. Future tests to determine the combined effects of the defences on live predators would provide support for the multimodal display hypothesis.

In *A. polyphemus*, there was a significant increase in the frequency of regurgitation in instars IV and V compared to instars I and II. The frequency of regurgitation may have increased in later instars because it can take time to sequester chemical compounds (Hagg et al., 2014). Alternatively, regurgitation may have increased in later instars because the sounds function as a form of acoustic aposematism which has been previously proposed and tested in *A. polyphemus* and *M. sexta* (Bura et al., 2012; Brown et al., 2007).

In *A. oculea* and *A. luna* no secondary defences were found to change over the course of development aside from defence sounds which increased in the late instars. This was unexpected because these species belong to the same family as *A. polyphemus* and an increase in regurgitation was expected. However, a limitation of this study was that the quantity of regurgitate produced by the caterpillars was not measured, and it may be possible that the caterpillars regurgitated more often than I was able to observe between attacks because there was a build-up of regurgitate that remained on the mouthparts. Future studies that quantify the regurgitate produced by *A. oculea* and *A. luna* would aid in determining whether the amount, rather than just the frequency, of regurgitation increases in later instars. Alternatively, the sounds alone may be a sufficient defence against predators. For example if the sounds function to startle predators, no other additional secondary defences may be necessary to deter them. Future studies that test the effects of the defence sounds in *A. oculea* and *A. luna* on live predators would provide insights in to their functions.

3.5 Conclusion

Evolutionary adaptations arise from ontogenetic changes, and play an essential role in understanding the form and function of evolved traits (Adams & Pedersen, 2000; Tinbergen, 1963; Zelditch, Swiderski, & Sheets, 2012). Here, I followed the ontogenetic development of antipredator defences in sound-producing Bombycoidea caterpillars. The results of this experiment show that sound producing caterpillars employ a variety of antipredator defences throughout development. In some species the mean frequency of secondary defences increased in later development, concurrent with the development of defence sounds. The findings of this study provide some useful ground work for future testing of hypotheses regarding the evolution of defence sounds in caterpillars, including the hypothesis that defence sounds are part of a multimodal antipredator display, or that they communicate the presence of an additional secondary defence (e.g. acoustic aposematism).

CHAPTER FOUR

Why do Caterpillars Whistle at Birds? Insect Defence Sounds Startle Avian Predators

Abstract

Many insects produce sounds when attacked by a predator, yet the functions of these signals are poorly understood. It is debated whether such sounds function as startle, warning or alarm signals, or merely serve to augment other defences. Direct evidence is limited owing to difficulties in disentangling the effects of sounds from other defences that often occur simultaneously in live insects. We conducted an experiment to test whether an insect sound can function as a deimatic (i.e. startle) display. Whistles of walnut sphinx caterpillars (*Amorpha juglandis*) were presented to a predator, red-winged blackbirds (*Agelaius phoeniceus*), when birds activated a sensor while feeding on mealworms (*Tenebrio molitor*). Birds exposed to whistles played back at natural sound levels exhibited significantly higher startle scores (by flying away, flinching, and hopping) and took longer to return to the feeding dish than during control conditions where no sounds were played. Birds habituated to sounds during a one-hour session, but after two days the startling effects were restored. Our results provide the first empirical evidence that an insect sound alone can function as a deimatic display in an avian predator. We discuss how whistles might be particularly effective 'acoustic eye spots' on avian predators.

4.1 Introduction

Defence sounds, variously named distress, alarm, warning or disturbance signals, depending on their purported functions, are widespread throughout the class Insecta (Alexander, 1967; Ewing, 1989; Masters, 1980). These acoustic signals, which can be transmitted as air- or solid-borne vibrations, are made in response to being handled or otherwise disturbed. They occur in species ranging in size from tiny beetles (Lewis & Cane, 1990) to giant wetas (Field, 1980) and are generated by a variety of mechanisms including stridulation, forced air, percussion and tymbalation (Ewing, 1989). Despite their prevalence and diversity, surprisingly little is known about the effects of these sounds on predators, and this subject has generated ongoing debate (Conner, 2014; Rowe & Halpin, 2013; Siddall & Marples, 2011). Proposed functions can be broadly categorized according to the target audience of the signals; those directed at conspecifics and some heterospecifics may function as alarm signals to warn of impending danger or to recruit help (Cocroft, 1999; Hager & Kirchner, 2013). Alternatively, signals may be directed at predators, where proposed functions include but are not limited to, acoustic aposematism, startle, enhancement of visual signals, sonar jamming, and mimicry of something dangerous (Conner 2014; Masters, 1980; Rowe & Halpin, 2013). Our understanding of the protective value of defence sounds is limited because live insects may have multiple components to their displays, making it difficult to isolate the effects of the sounds alone (Rowe & Halpin, 2013; Siddall & Marples, 2011). Also, a defence sound may have overlapping functions; for example, in a live insect that is chemically defended, a sound could function as both an aposematic and a deimatic display (Ruxton et al., 2004; Skelhorn et al., 2016). In this study we test the hypothesis that an insect sound can function as a deimatic display, by isolating the effects of sound from the insect using a playback system. Deimatic displays have long been defined as a type of antipredator defence that functions by frightening or startling a predator (Edmunds, 1974; Ruxton et al., 2004; Sargent, 1990; Skelhorn et al., 2016). Classically cited examples include rapid eyespot displays on the hind wings of moths, or the sudden appearance of high contrast markings in cuttlefish (reviewed in Edmunds, 1974). Three criteria are commonly used to assess the startling effects of a stimulus included in a deimatic display: (i) it evokes a startle response (e.g. fear, surprise, confusion) in a predator; (ii) it causes hesitation, resulting in a longer time to return to the prey; and (iii) it has a transitory effect on the predator, whose response should habituate over repeated exposure to the stimulus (Pilz & Schnitzler, 1996; Pomeroy & Heppner, 1977; Ruxton et al., 2004; Sargent, 1990; Skelhorn et al., 2016). Many studies have proposed that insect sounds function as deimatic displays against vertebrate predators including bats (Bates & Fenton, 1988; Mohl & Miller, 1976), rodents (Masters, 1979; Oloffson, Jakobsson & Wiklund, 2012a; Smith & Langley, 1978), reptiles (Sandow & Bailey, 1978) and birds (Maldonado, 1970). However, few studies have empirically measured the startle response in predators (Umbers & Mappes, 2016), and a caveat must be placed on conclusions drawn from characteristics of the display alone (Skelhorn et al., 2016), or when live prey are used for previously stated reasons. To date, the deimatic function of insect defence sounds has been not been subject to rigorous experimental testing.

There is current debate on what comprises a deimatic display, and whether in fact it is even a distinct form of defence (Rowe & Halpin, 2013; Skelhorn et al., 2016; Umbers, Lehtonen & Mappes., 2015; Umbers & Mappes, 2016). A deimatic function cannot be confirmed based solely on display characteristics (e.g. being evoked by a predator, or the sudden and conspicuous nature of the components), because these features can also function in aposematism or retaliation (Ruxton et al., 2004; Skelhorn et al., 2016). Also, defensive displays often have multiple components, and conceivably each component could have a different function (Skelhorn et al., 2016). Skelhorn et al., (2016) argue that instead of focusing on the display characteristics of the prey, we need to ‘ask their predators’. Deimatic displays, they argue, should be defined based on the display’s ability to exploit classic fear responses in predators, which can be empirically measured as a startle reflex; and ideally, such tests should be conducted using ecologically relevant predators. At present, they argue, there is no direct evidence that deimatic displays elicit fear responses in predators (Skelhorn et al., 2016). Furthermore, they advocate that it is particularly important to know the mechanism through which each component of the display operates. Umbers & Mappes (2016) concur that a predator’s startle reflex should be elicited, but caution that this is difficult to measure directly. Our experiment addressed these concerns by empirically measuring startle responses elicited by one stimulus component- sound- in an ecologically relevant predator.

Our goal was to test the hypothesis that caterpillar whistles function as deimatic displays. The walnut sphinx caterpillar *(Amorpha juglandis)* (Sphingidae) (Fig. 1a) is a cryptically coloured species native to North America that when attacked, generates whistles from its spiracles (Bura et al., 2011). Whistles are short in duration (~450 ms), multiharmonic with peaks from 9-20 kHz, and relatively loud (69-82 dB SPL at 5 cm) (Bura et al., 2011) (Fig. 1b-c). Previously it was proposed that whistles startle predators based on characteristics of the sounds (sudden, short duration and loud), the lack of an obvious associated chemical defence, and because in predatory trials with captive yellow warblers (*Dendroica petechia,* Parulidae), birds attacking live caterpillars jumped back or dove away (Bura et al., 2011). While these results support the hypothesis that whistles startle birds, the results are inconclusive, because responses of the three birds were not quantified or compared to controls, and the effects of sounds were confounded by the use of live caterpillars that also thrashed in response to attack (Bura et al., 2011) which may in itself have a startling effect. Here we test that whistles alone elicit a startle response in a native predator of Lepidoptera, the red-winged blackbird (*Agelaius phoeniceus,* Icteridae) (Robertson, 1973). To isolate the effects of the sound we used a sensor programmed to trigger sound playbacks when birds attacked mealworms. Following the criteria for deimatic displays described above, we predicted: (i) birds feeding on mealworms paired with sounds played at natural levels (60, 70 dB SPL) would exhibit higher startle scores compared to controls where no sounds were played; (ii) birds exposed to sound while feeding on mealworms would take longer to re-approach mealworms a second time compared to controls; (iii) birds would habituate to sounds upon repeated exposure within a trial.

4.2 Materials and Methods

4.2.1 Animals and housing conditions

Twelve wild adult male red-winged blackbirds *(Agelaius phoeniceus*, Icteridae*)* were originally captured as controls for a previous experiment under a Canadian Wildlife Service permit to capture migratory birds (10771) and a Queen's University Animal Care Committee (UACC) protocol (2013-027) and subsequently used in our experiment under a UACC protocol (2014-1487) (Appendix B, Data B1). All experiments were conducted at the Queen's University Biological Station (QUBS) near Chaffey’s Lock, Ontario, Canada (44°33’55.34” N, 76°19’26.59"W) during June and July 2014 where birds were housed singly in individual flight cages in an outdoor aviary. The aviary consisted of 30 large (6 x 2.5 x 2.5m) cages arranged in two rows with an access hallway down the middle.

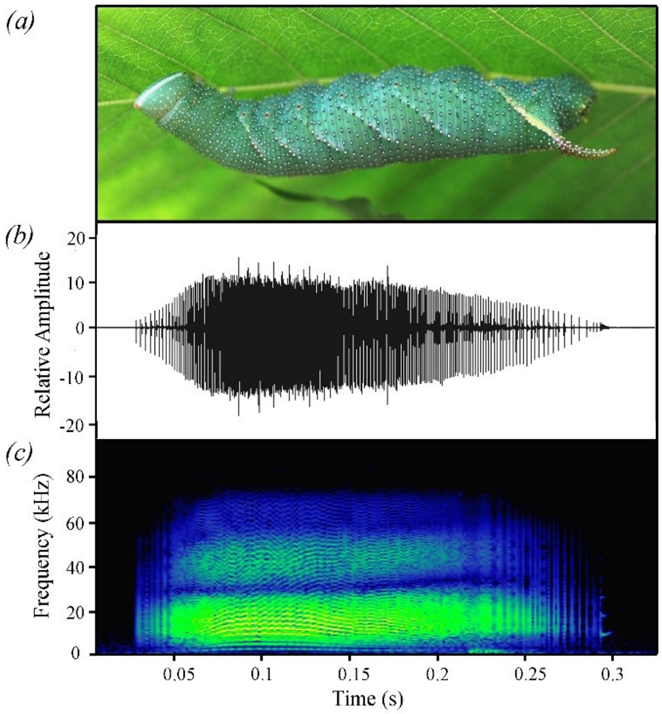


Figure 4.1. (a) A late instar larva of the walnut sphinx moth (*Amorpha juglandis)* in its natural resting position on the underside of a leaf on its host plant. (b-c) Sound waveform and corresponding spectrogram of a single defensive whistle.

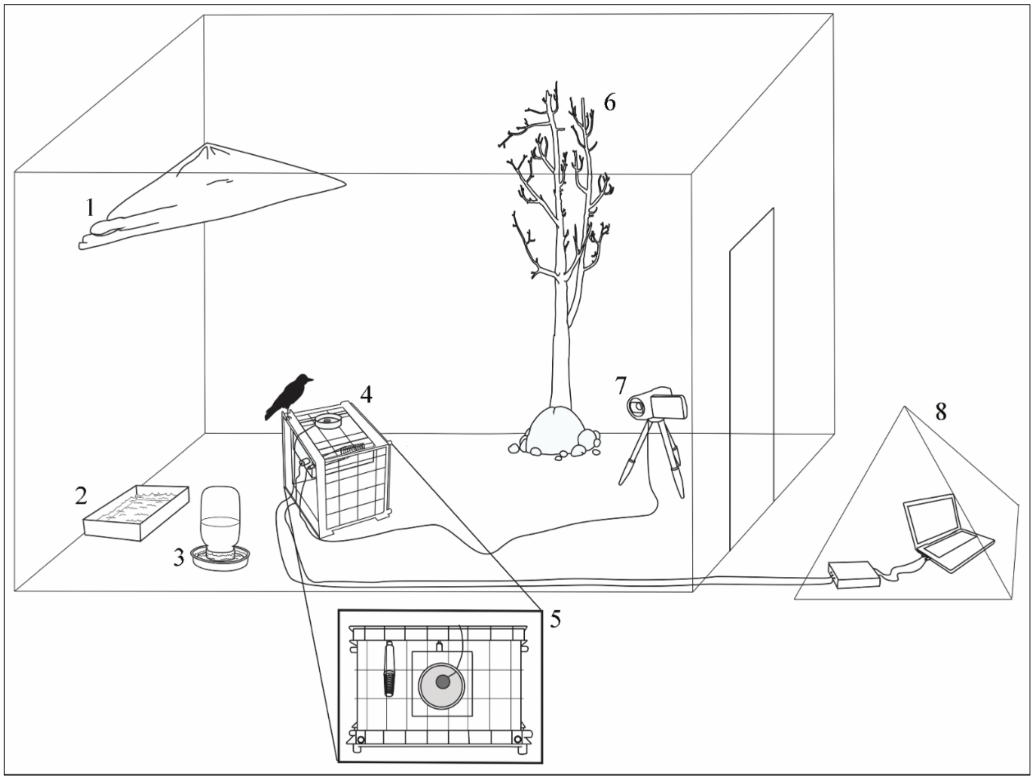


Figure 4.2. Schematic representation of the experimental set up, showing the aviary cage and equipment used during control and experimental phases of the study: (1) tarp (2) bath (3) water dish (4) platform (5) sensor feeding dish (6) branches (7) video camera (8) laptop and tent. The feeding platform was replaced with an identical feeding platform with a sound activated sensor and speaker connected to a laptop to relay the sounds, and a video camera was placed inside the cage and turned on in the experimental phases.

Each cage was equipped with tree branches for perching, a sheltered area, shallow water bath, water dish, and a feeding platform comprising a plastic crate (60 x 60 x 30 cm) with a Petri dish (9 x 1.5 cm) affixed to the top (Fig. 2). Birds were offered a variety of foods daily including poultry starter, dragonflies, romaine lettuce, corn, sunflower seeds, wild bird seed, thistle-based seed, eggs, strawberries, apples, kiwis, poultry starter, cuttlebone, and oyster shells. When experiments were completed, birds were observed for a minimum of two days to confirm that there were no signs of stress or illness, after which the birds were released at QUBS in a field ~ 1 km from the aviaries during good weather conditions.

4.2.2 Experimental set up

Each bird experienced a training phase, control phase, and one or two experimental phases (see *4.2.4 Experimental Design*) and all phases were conducted within the bird's individual cage. During the training phase, only the equipment described above was present in the cage. During the control and experimental phases, the feeding platform used for training was replaced with an identical platform except that it contained a modified Petri dish with a piezoelectric sensor and speaker (Fig. 2; see *4.2.3 Sound Playback*). The sensor was connected to a Sony PCG 7185L personal computer (Sony; Tokyo, Japan) located in the aviary access hallway under a small tent ~1 m outside the cage door and ~2 m from the feeding platform, and the laptop in turn was connected to playback electronics (Fig. 2; see *4.2.3 Sound Playback*). A Sony camcorder (DCRTRV19) on a tripod (60 cm high) was placed inside the cage ~1 m from the aviary door and ~1 m from the feeding platform (Fig. 2). A Sony ECM-MS908C microphone connected to the camcorder was placed 10 cm from the playback speaker to monitor sound events for subsequent video analyses (Fig. 2). The set-ups used for the control and experimental phases were identical, except that in the control phase the sensor was disabled.

4.2.3 Sound playback

The playback stimulus was obtained from a previous study whereby defence sounds were elicited from a walnut sphinx caterpillar by administering a light pinch with forceps to its abdomen (Bura et al., 2011). Sounds were recorded using a Brüel & Kjaer (B&K; Naerum, Denmark) ¼ inch microphone (type 4943), amplified with a B&K Nexus conditioning amplifier (type 2690), and recorded to a Fostex FR-2 Field Recorder (Gardena, CA, USA) at a sampling rate of 192 kHz. The sound used for playback experiments was typical of a late instar caterpillar (Bura et al., 2011), comprising a single whistle with a dominant frequency of 9 kHz (bandwidth 14 kHz at -10 dB from peak) and a duration of 410 ms.

Sounds were played when contact was made with a piezoelectric sensor housed within a custom-made feeding dish unit (Fig. 2). The unit comprised a circular black Delrin® plastic receptacle (9.5 cm diameter x 3.5 cm high) with upper and lower compartments. The upper compartment held a clear plastic Petri dish (9 cm diameter x 1.6 cm high). The lower compartment was located inside the unit below the Petri dish, and contained the sensor's circuitry. The sensor was made of a Murata 7BB27-4L0 piezoelectric diaphragm (sensitivity 5 V/microstrain) (Murata Manufacturing Co., Ltd, Nagaokakyo, Kyoto) and a circuit with the output amplified to trigger on a deflection of 0.03 μm. The unit was powered by a USB adaptor connected to the laptop. The diaphragm was placed in the upper receptacle directly beneath the Petri dish so that contact made by the bird feeding on mealworms would trigger the playback. Sounds were broadcast by an Avisoft ScanSpeak (Avisoft Bioacoustics, Glienicke, Germany) (3-120 kHz) speaker concealed beneath the feeding dish (Fig. 2). The sensor dish and speakers were connected to an Avisoft playback system that included an Ultrasound Gate (USG) player 116, and Recorder USGH software.

Sounds were played at two levels, 70 and 60 dB SPL, in experimental phases one and two respectively (see *4.2.4 Experimental Design*) in order to assess the bird's response to a range of naturally occurring intensities of the walnut sphinx's whistle. Sound levels were measured 22 cm from the speaker, within range of the bird's head at the dish, using a B&K sound level meter (type 2239). To ensure sound characteristics represented those of the original recordings, sounds were re-recorded using the B&K microphone and recording chain (see above) and analyzed using RavenPro Bioacoustics Research program 1.4 (Cornell Laboratory of Ornithology, Ithaca, NY, USA).

4.2.4 Experimental design

Each bird experienced up to four phases: a training phase, a control phase (no sound), experimental phase one (sounds were played at 70 dB SPL), and experimental phase two (sounds were played at 60 dB SPL). The stimuli were presented in separate phases to ensure measurement of habituation of the startle response over time was possible (see *4.2.6 Statistical Analyses*). The purpose of the training phase was to familiarize the birds with consuming mealworms from a Petri dish on a feeding platform. Each bird was provided with six mealworms each day for four consecutive days. The dishes were checked after one hour for the number of mealworms consumed. A bird was considered to have been successfully trained if it removed any of the mealworms from the dish each day. Birds that successfully completed the training phase (11/12) served as subjects in the control phase two days later. During the control phase, birds fed on mealworms from the disconnected sensor feeding dish. One hour prior to testing, all food was removed to increase the bird's motivation to eat. Equipment was then placed in the bird's cage and six mealworms were placed in the feeding dish. Behaviours were video recorded for up to one hour, or until all mealworms were consumed, after which time the equipment was removed from the cage.

Two days later, all birds that completed the control phase (9/11) were given the opportunity to participate in experimental phase one, where all conditions were the same as for the control phase except the sensor dish was activated, triggering sound playbacks at 70 dB SPL when the bird fed from the dish. Two days following phase one, all birds that completed the control phase (9/11) were given the opportunity to participate in experimental phase two. In phase two all conditions were the same as for phase one except the sound volume was decreased to 60 dB SPL. Some birds successfully participated in both experimental phases while others only participated in phase one (see 4.3 Results).

4.2.5 Behavioural assessment

The following measurements were obtained from video recordings of the control and experimental phases: (i) Startle behaviours were quantified for each bird by counting the most intense response that occurred within 500 ms following each contact with the feeding dish, and then generating a startle score (e.g. fly away was given a score of 2, so it was considered more intense than the behaviours that were given a score of 1). Behaviours were recorded blind (the sound was not turned on while recording the behaviours) and startle scores were assigned to each behaviour were based on previous grading systems for avian startle responses, where behaviours that caused predators to retreat from the prey (i.e. fly away) were given a higher score (Olofsson et al., 2012b; Schlenoff, 1985) (Table 1); (ii) Latency to return to the feeding dish was measured by noting the times between up to five consecutive contacts with the feeding dish. All behavioural analyses were conducted using JWatcher 0.9 (D.T. Blumstein et al.

UCLA & Macquarie University).

4.2.6 Statistical analyses

To test whether birds startled in response to the sound within 500 ms after first contact with the feeding dish, the startle score of each bird in its control phase was compared with its startle score in the experimental phases that they participated in using a Fisher-Pitman permutation test for paired data. As a directional hypothesis was being tested, a one-tailed test was used (Ruxton & Neuhauser, 2010). A non-parametric test was chosen because the sample size was small (Ryan, 2013). To test whether birds took longer to return to the dish following their first exposure to sound (in experimental phases one and two) compared to no sound (control), the latencies between first and second contact with the dish were log transformed and then compared for each bird using a Fisher-Pitman permutation test for paired data.

To test whether birds habituated to the sounds (or lack thereof) within a phase, we measured the change in startle scores over up to six consecutive contacts with the feeding dish. To quantify habituation, we measured the slope of the least squares line expressing the bird’s startle score as a function of the discrete time of dish contact, where the first dish contact is a time of 1, the second dish contact is a time of 2, and so on. Under the assumption of no habituation, a bird's startle scores are exchangeable and on average the slope of the startle score against time is zero. To test the significance of the mean slope of the startle score against time, we used a permutation test with within-bird resampling. Since the number of permutations is too large, we used a Monte-Carlo simulation with 5000 samples to estimate the p-value (Motulsky & Christopoulos, 2004). By using 5000 samples, the maximum error of the estimate will be less than 1.5% at a 95% confidence level. As an additional measure of habituation, we assessed whether birds took less time to return to the feeding dish over time by measuring latencies between up to five contacts with the feeding dish within a phase. The latencies were log transformed and then we measured the slope of least square line of the latencies. To test the significance of the mean slope against time, we used the same tests as described above.

We also examined how long it took birds to consume mealworms over the course of six contacts with the feeding dish, and how the types of startle behaviours changed during this period. We measured the time difference between the first and sixth contact with the dish for each bird in each experimental phase and compared this to the difference in the control phase using a two- tailed Fisher Pitman permutation test for paired measurements. Changes in startle behaviours over time were documented by charting the proportion of each behaviour type that contributed to the mean startle score for each dish contact.

To determine whether birds that had previously experienced the sound in experimental phase one remained habituated to the stimulus in experimental phase two, and to test whether birds responded differently to sound levels in experimental phases one (70 dB SPL) and two (60 dB SPL), we conducted two separate types of analyses. First, we compared startle scores (up to 500 ms after first exposure to sound) and latencies (between first and second contact with dish) of birds that had participated in both phases using a two-tailed paired samples Fisher-Pitman permutation test.

Table 4.1. Avian startle behaviours and scoring.

Behaviour	**Description of behaviour**	**Score**
No reaction	No discernible reaction to consuming mealworm with or without sound	0
Shoulder Flinch	Flinching one or both wings, wings do not extend.	1
Wing flap	Full extension of one or both wings without flying away from platform	1
Ruffles feathers	Shaking/trembling feathers raised up from skin	1
Body flinch	A whole body sudden short movement	1
Startle Hop	A sudden upward hop where both feet go up in the air and back down to the ground	1
Fly away	Flying off platform, flapping both wings	2

Table 4.1. A list of avian startle behaviours recorded, definitions of the behaviours and how each behaviour was scored for analysis. Avian Startle Scores were assigned to behaviours based on the following criteria: A score of 0 was given for no discernible reaction to prey; a score of 1 was given for behaviours that caused hesitation to consume prey; and a score of 2 was given for behaviours that caused the predator to retreat away from prey. Adapted from Olofsson et al., 2012b; Schlenoff, 1985.

We also compared the startle scores (up to 500 ms after first exposure to sound) and latencies (between first and second contact with dish) of all birds regardless of whether they had participated in both phases, using a combined paired and two-sample data permutation test (Einsporn & Habtzghi, 2013). For the second analysis to combine the paired and unpaired observations, the test statistic generated was a weighted combination of the mean differences of the unpaired and paired observations. All statistical analyses were conducted using the R Project 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

4.3 Results

4.3.1 Participation in experimental phases

Eleven of the original 12 birds successfully completed the training phase, but only nine (Birds 2,4,5,8,9,11,18,21,30) participated in the control phase. All nine birds were given the opportunity to participate in each of the following two experimental phases. Of these nine birds, six participated in phase one (Birds 2,9,11,18,21,30), and eight in phase two (Birds 2,4,5,8,9,18,21,30). All behaviours identified *a priori* as startle responses (Fig. 4.3, Table 4.1) were observed over the course of this study.

4.3.2 Do sounds startle birds?

Startle scores measured within 500 ms of first contact with the dish were significantly higher in both experimental phases (with sound) than in control phases (no sound) (Phase one: X ± SE = 1.83 ± 0.41, N = 6; Control: X ± SE = 0.33 ± 0.82, N = 9, Fisher Pitman test: t6 = -4.39, P = 0.016), (Phase two: X ± SE = 1.63 ± 0.74, N = 8; Control: X ± SE = 0.5 ± 0.93, N = 8; Fisher Pitman test: t8 = 3.21, P = 0.027) (Fig. 4.4). Of the startle behaviours listed *a priori*

(Table 4.1), fly away was the most common upon first contact with the dish in all phases (Appendix B. Fig. B1). Latencies to return to feed following first contact with the feeding dish were higher for experimental conditions compared to controls but these differences were not significant (Phase one: $X \pm SE = 673 \pm 1154$ s, $N = 6$; Control: $X \pm SE = 3.6 \pm 5.83$ s, $N = 9$; Fisher Pitman test: $t6 = -2.8$, $P = 0.147$), (Phase two: $X \pm SE = 36.63 \pm 60.94$ s, $N = 8$; Control: $X \pm SE = 5.36 \pm 7$ s, $N = 8$; Fisher Pitman test: $t8 = -1.14$, $P = 0.281$).

4.3.3 Do birds habituate to sounds within a phase?

Habituation was assessed by evaluating the changes in startle scores, and latencies to return to the dish across six consecutive contacts within a phase. Our results show that startle scores for both experimental phases decreased over time; in experimental phases one and two the slopes across all birds were -0.23 and -0.18 respectively. While startle scores decreased over time, this change was not statistically significant for experimental phase one ($P = 0.11$), but was for phase two ($P = 0.003$) (Fig. 5 a-b). In the control condition, the startle scores also decreased significantly over time ($P = 0.005$), although the slope of the line (Slope= -0.12) was small relative to the experimental phases (Fig. 4.5c). The change in latencies between successive contacts with the dish decreased over five contacts with the feeding dish for both experimental phases (Appendix B, Fig. B2). In phase one this decrease was significant (Slope= -1.07, $P = 0.0002$). In experimental phase two there was a negative but non-significant decrease in latency over time (Slope= -0.63, $P = 0.069$). As expected, in the control phase there was no significant positive or negative change in latency over time (Slope = -0.01, $P = 0.491$) (Appendix B, Fig. B2).

(a) Fly Away

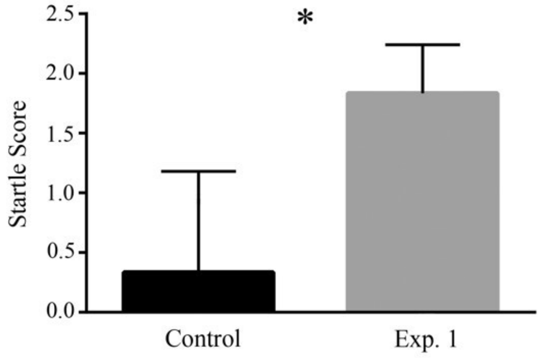
(b) Wing flap

(c) Startle hop

(d) Shoulder flinch

Figure 4.3. The four most frequent startle behaviours occurring 500 ms after exposure to the sounds in experimental phases one and two were (a) fly away (b) wing flap (c) startle hop and (d) shoulder flinch. The numbers indicate consecutive screen captures of the first to fourth positions of the bird during each response.

(a) Exp. 1 (70 dB SPL)



(b) Exp. 2 (60 dB SPL)

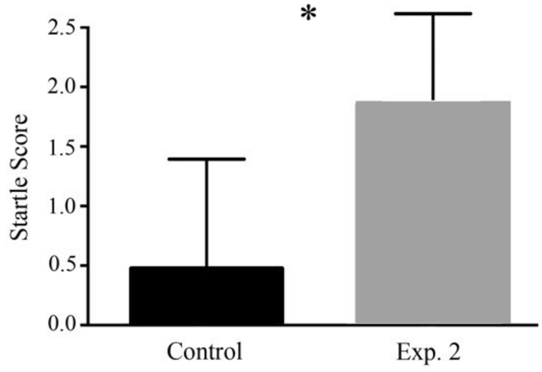
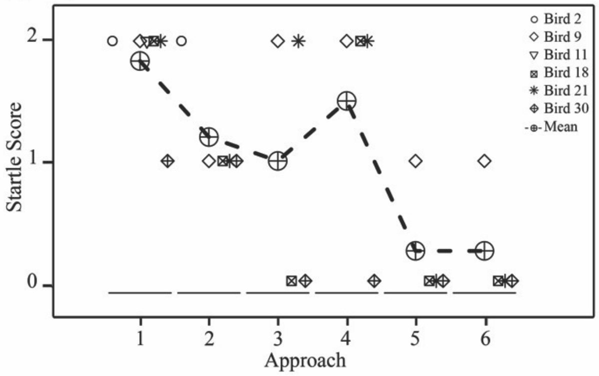
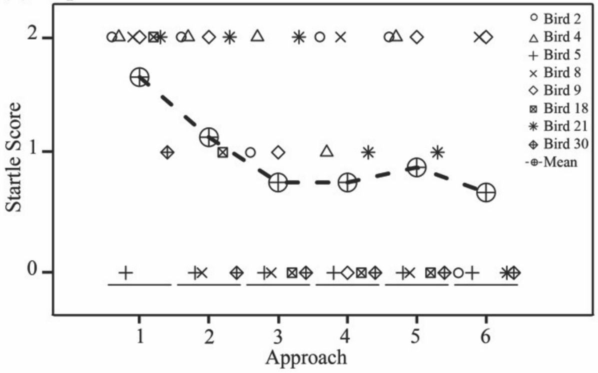


Figure 4.4. Birds exposed to playbacks of walnut sphinx whistles show significantly higher mean startle scores up to 500 ms after first contact with the feeding dish than in their respective control conditions in (a) experimental phase one (Phase one: X ± SE = 1.83 ± 0.41, $N = 6$; Control: X + SE = 0.33 ± 0.82, $N = 6$; $P = 0.016$); and (b) experimental phase two (Phase two: X ± SE = 1.63 ± 0.74, $N = 8$; Control: X + SE = 0.5 ± 0.93, $N = 8$; $P = 0.027$).

(a) Exp. 1



(b) Exp. 2



(c) Control

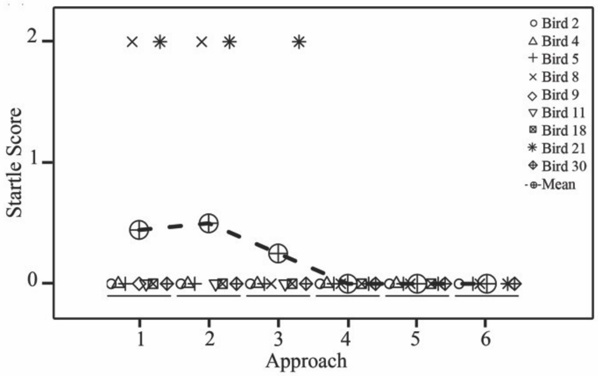


Figure 4.5. Change in startle score over the course of 6 contacts with the feeding dish amongst individuals in (a) control phase (b) experimental phase one (c) experimental phase two. The dotted line represents the overall mean within each phase.

Birds took longer to consume all mealworms during the course of the trial in both experimental phases compared with the control. This time difference was significantly higher in experimental phase two (Phase two: X + SE= 378.57 ± 524.6 s, $N = 8$; Control: X + SE = 9.99 ± 8.88 s, $N = 8$; Fisher-Pitman test: $t8 = -1.77$, $P = 0.03$), but not for experimental phase one (Phase one: X ± SE = 531.75 ± 579.41 s, $N = 6$; Control: X + SE = 9.18 ± 10.1 s, $N = 6$; Fisher Pitman test: $t6 = 1.78$, $P = 0.13$) (Appendix B, Table B2).

The types of startle behaviours changed over the course of all phases. Upon first contact with the dish within a phase, flying away comprised the highest proportion of all behaviours observed: phase one startle score = 1.4 (83%), phase two = 1.25 (66%), control = 0.33 (100%). However, as the number of contacts with the dish increased, a greater variety of behaviours was observed (i.e. wing flapping, hopping, flinching, ruffling feathers) (Appendix B Fig. B1, Table B1).

4.3.4 Do birds respond differently between experimental phases?

There were no significant differences between experimental phases with respect to the birds' startle scores or latencies to return to feed upon first approach, regardless of experience or sound level. Startle scores for birds that participated in both phases did not differ between experimental phase one (X ± SE = 1.8 ± 0.44, $N = 6$) and phase two (X ± SE = 1.8 ± 0, $N = 8$) (X Difference = -0.47; Fisher-Pitman test: $t4= -1.63$, $P = 1$), nor did latencies differ between phase one (X ± SE = 226.86 ± 413.48 s, $N = 6$) and phase two (X ± SE =17.86 ± 20.54 s, $N = 8$) (X Difference = 209; Fisher Pitman test: $t4 = 1.45$, $P = 0.302$). These results suggest that even though birds habituated in phase one, they startled to the sound when reintroduced in phase two, and startle scores and latencies did not differ by sound level. When considering all birds combined, regardless of their previous experience, startle scores (Combined Paired Permutation Test: Difference^{w} = 0; $P = 1$) and latencies between first and second contact with the feeding dish (Combined Paired Permutation Test: Difference^{w} = 921.1, $P = 0.08$) were also not found to be significantly different between phases one and two. This result suggests that startle scores and latencies were the same at the two sound levels regardless of experience.

4.4 Discussion

4.4.1 Are caterpillar whistles deimatic displays?

Our results show that caterpillar whistles evoke startle reflexes in an avian predator. Captive red-winged blackbirds exhibited significantly higher startle scores when exposed to whistles played at naturally occurring sound levels (60-70 dB SPL) compared to control conditions (no sound). Birds responded by flying away from the platform, ruffling their feathers and flinching, all direct measures of the avian startle reflex (Morris, 1956; Olofsson et al., 2012b; Pomeroy & Heppner, 1977). While these measures of startle in avian predators are indicators of fear (Hebb, 1946), supporting Skelhorn et al.'s (2015) argument that deimatic displays should be defined by their ability to evoke fear responses, we concur with Umbers & Mappes (2016) that fear responses are physiological states that are difficult to measure in predators, particularly in the field. As a second measure of startle we predicted that birds would take longer to re-approach the feeding dish following exposure to sound. Latencies were higher in both experimental phases compared to the control, but these differences were not significant. We propose that the lack of a significant difference may be due to the small sample size or the semi-natural conditions used in our aviary experiments, since the foraging strategy that animals use in a lab setting is not necessarily the same the one they would use the field (Shettleworth, 1989). Therefore, habituation may be more likely in the lab where animals are accustomed to being fed rather than having to forage for food on their own in the wild. As well, we noticed inter-individual variability in latencies, with some birds continuing to return to the dish promptly after being startled (<50 s), while others took longer (>2500s) perhaps due to personality differences. Perhaps these birds were less reluctant to return to eat because of their familiarity with the feeding conditions, compared to what they might experience in the wild when presented with many novel foods. Also, unlike wild birds, they had nowhere else to find food.

Predators are expected to habituate to a deimatic display over time (Pomeroy & Heppner, 1977; Sargent, 1990) particularly in the lab where there is repeated exposure to startle stimuli (Ingalls, 1993; Sargent, 1990). This measure allows one to differentiate between alternative hypotheses such as aposematism, or that the sounds themselves are harmful or aversive. Our results showed that predators habituated to the sounds based on a decrease in startle score following repeated exposure. The slope was more gradual in experiment one (not significant) and steeper in experimental phase two (significant). In the control phase, two birds flew away upon first pecking at the dish. We observed that the birds that flew away in the control phase were skittish in all phases when feeding, and perhaps were more cautious when feeding, causing them to fly away. Due to the fact that the rest of the birds did not startle at all in the control phase, the difference in response in these two birds was pronounced resulting in a decrease in startle score (significant), although the slope was much smaller than in both experimental phases. As a second indicator of habituation within a trial, we found that latencies to return to the dish decreased over time in both experimental phases, although not significantly so in experimental phase two. As predicted, there was no significant positive or negative change in latency over time in the control phase where no sound stimulus was present. Collectively these results demonstrate that within a given trial, birds habituated to the stimulus, indicating that the sound itself was not aversive to the predator.

Birds that habituated to the sounds in experiment one became dishabituated to these sounds two days later, underscoring the importance of novelty for a deimatic display to be effective (Sargent, 1990). It is likely that encounters with these caterpillars in the wild would be infrequent, because they are cryptically coloured, and occur solitarily on their host plants (Bura, 2010). Even birds that have evoked a deimatic display in a caterpillar are unlikely to take the risk to return to the potentially dangerous source (Janzen, Hallwachs & Burns., 2010). Therefore, re-sensitization of the startle response after a few days is likely to occur in nature as well as in the captive condition.

4.4.2 How do whistles protect caterpillars?

Whistles played at natural levels caused birds in an aviary to startle, but by which mechanisms might these sounds protect caterpillars in the wild? It has been postulated that deimatic displays protect prey in a number of different ways: the predator might drop the prey, give the prey time to escape, or be sufficiently frightened that it abandons the prospective prey altogether (Edmunds, 1974; Sargent, 1990), although there is little empirical support for any such mechanisms. The primary defence of the walnut sphinx is crypsis, and only when disturbed does it produce whistles (Bura et al., 2011). Following attack, caterpillars hang on to the host plant tightly with their terminal abdominal prolegs, and sometimes thrash their anterior body segments while producing sound. However, sound production occurs with or without the accompaniment of thrashing. In a previous study involving both simulated and live predator attacks, caterpillars did not attempt to escape, similar to the responses of most other Bombycoidea caterpillars tested to date (Brown et al, 2007; Bura et al., 2009; Bura, 2010; Bura et al., 2011; Bura et al., 2012). The large body size of these larvae might make it difficult to drop from silk strands, which are likely to snap, and dropping to the ground is costly (Bura, 2010; Sugiura & Yamazaki, 2006). Therefore, the main protective value of the deimatic display in this species is not likely to lead to prey escape. We also rule out the likelihood that sounds provoke the predator to drop the prey, because caterpillars hold steadfast to their host plant while producing sound. Instead, we argue that whistles are effective because they elicit an innate startle response in the predator, causing it to abandon the prey. To a foraging bird in the wild, further pursuit of a potentially lethal threat may not be worthwhile, particularly when the bird is capable of moving on in search of more profitable prey (Janzen et al., 2010). While the birds in our experiments startled to sound alone, we expect that if exposed to live caterpillars, the effects of thrashing would have a synergistic effect as a multi-component display. Also, we cannot rule out the possibility that walnut sphinx caterpillars are chemically defended, and that whistles could function as both deimatic and aposematic displays. However, we argue against aposematism because chemicals are not expelled during sound production. All things considered, we argue that the primary function of caterpillar whistles is to frighten predators.

4.4.3 Why do whistles startle birds?

Deimatic displays are proposed to evoke a startle or fear response in a predator by the following non mutually exclusive mechanisms: (i) by mimicking the appearance of the predator's own predator, (ii) by exploiting the predator's innate startle reflexes, or (iii) by causing the predator to mistaken the prey for something else that could inflict harm (Skelhorn et al., 2016). We offer an alternative hypothesis: that the whistles mimic a bird alarm call.

Alarm calls in passerine birds are widespread and function to alert conspecifics or heterospecifics to the presence of danger (Caro, 2005). Such calls elicit behaviours not unlike startle reflexes; in birds, these including escape, diving for cover and increased vigilance (Fallow, Pitcher & Magrath, 2013; Griesser, 2013; Marler, 1955). Alarm calls across different bird species have common features including short duration and high frequency (Fallow, Gardner & Magrath, 2011). For example, the *seet* call of the American Robin is 1.8 s and 9.5 kHz (see Vanderhoff & Eason, 2009). The whistles of the walnut sphinx are also of short duration (2.6 s), and have the same dominant frequency (9.5 kHz) (Bura et al., 2011). Both the red-winged blackbirds in our experiments and the yellow warblers from a previous experiment (Bura et al., 2011) exhibited flight responses and diving behaviours to the caterpillar sound. Therefore, it is conceivable that caterpillars might mimic bird alarm calls. This intriguing hypothesis could be tested by comparing the responses of birds to bird alarm calls with those of walnut sphinx whistles. Even then, it may be difficult to disentangle the 'deimatic' from the 'alarm call mimicry' hypotheses, because the latter response may have evolved from the same neural circuitry involved in the innate startle reflex (Hollen & Radford, 2009).

4.5 Conclusion

Defence sounds have been reported in almost every insect order (Alexander, 1967). Onomatopoeically called hisses, clicks, buzzes, chirps, rattles, taps, knocks, whistles, squawks and squeaks, among other things (Alexander, 1967; Ewing, 1989; Masters, 1980; Rowe & Halpin, 2013) defence sounds can differ significantly in their temporal, spectral and amplitude characteristics. Yet, there has been little rigorous experimental investigation of the functions of these signals on predators. Currently, studies are mostly restricted to the unique relationship between tiger moths and their echolocating bat predators (Conner & Corcoran, 2012; Conner, 2014). It has been recently argued that bio-acousticians fall behind visual ecologists in understanding the complexity between predator-prey interactions involving acoustic defence signals (Conner, 2014). Our study provides the first empirical evidence that an insect sound alone can evoke a fear response in an avian predator; thus functioning as an acoustic ‘eyespot’. Caterpillar whistles are but one type of defence sound reported for Bombycoidea caterpillars (Brown et al., 2007; Bura, 2010; Bura et al., 2011; Conner, 2014; Ewing, 1989). It is proposed that in caterpillars, different sound types, including whistles, clicks, chirps and vocalizations, convey different messages to predators, with some functioning better to reinforcing learning, while others are more suited to frightening predators (Bura, 2010). We propose that Bombycoidea caterpillars are excellent models for studying the functions of defence sounds, because the sounds are less likely to serve overlapping functions in social or sexual contexts as they would in adult insects such as tiger moths. Future studies should focus on the selection pressures for different defensive ‘vocabularies’ by considering the effects of phylogeny as well as predator psychology.

CHAPTER FIVE

General Conclusions and Future Directions

5.1 In brief

Defence sounds in insects have been understudied despite being taxonomically widespread and highly diverse in terms of their mechanisms and signal characteristics (Alexander, 1957; Conner, 2014). There remain important outstanding questions regarding the functions and evolution of these sounds. This doctoral thesis focused on testing hypotheses to explain why defence sounds have evolved in some insects and not others, and how they function to protect prey against predators using caterpillars as a model system. Chapter One introduces the literature on insect defence sounds and summarizes what is known to date about sound production in Bombycoidea (silk and hawkmoth) caterpillars. Chapter Two provides support for the hypothesis that body size plays a role in the evolution of defence sounds by conducting a developmental study in four Bombycoidea species. Chapter Three reports on the ontogenetic changes in primary and secondary defences in sound producing Bombycoidea caterpillars, laying the groundwork for future testing of alternative hypotheses regarding why some caterpillar species produce defence sounds while others do not and their functions. Chapter Four tests the startle response of avian predators to the defensive whistles of the walnut sphinx caterpillar, *Amorpha juglandis*, providing support for the startle hypothesis. The studies conducted in this thesis help to provide a more comprehensive understanding of the adaptive significance of acoustic defences in insects, and contribute to our understanding of the role of sounds in the defence systems of larval insects.

5.2 Thesis Contributions and Future Directions

5.2.1 Chapter 2: What makes a caterpillar tick?

Not all insect species use defence sounds, but explanations for the species diversity in this trait remain untested. In Bombycoidea caterpillars some species produce sounds when attacked while others do not (Bura et al., 2016). It has been argued that the diversity of defence sounds found amongst Bombycoidea species "rivals that found within most groups of adult insects," occurring in five unrelated subfamilies from Sphingidae and Saturniidae (Bura et al., 2016). In Chapter Two I tested the hypothesis that size plays a role in determining whether defence sounds occur by conducting a developmental study in four caterpillar species from two families, Saturniidae and Sphingidae, that produce sounds as late instars. I predicted that early instar/small caterpillars would not make defence sounds. I measured the occurrence of defence sounds and their association with instar, mass, and length. I found that early instar caterpillars (instars I, II) did not make defence sounds, but that they were present in all other instars (instars III→V). I also found that regardless of instar, all individuals first began to make defence sounds at the same relative mass and length, despite the fact that overall the species differed in their final instar sizes. I concluded that the development of defence sounds was dependent on both body size and instar.

In the second half of my experiments in Chapter Two, I tested two hypotheses to explain why defence sounds do not occur in small caterpillars. The first hypothesis was that smaller caterpillars do not have enough energy to make defence sounds. I predicted that smaller caterpillars would signal less than larger caterpillars, and produce shorter signals units, trains and lower duty cycles. To test my predictions, I analyzed the relationship between these temporal characteristics and a measure of body size (SMI). Results provided only partial support for this hypothesis, as not all relationships were significant. Based on my findings, I concluded that the caterpillars made sounds that are effective communication signals because they were all sufficiently long enough to be integrated in to the sensory system of major vertebrate predators from the onset of development. The second hypothesis was that defence sounds are limited to larger caterpillars because predators cannot detect the sounds of small caterpillars. I predicted that smaller caterpillars would not produce defence sounds that are loud enough to be heard by an attacking predator, and that the defence sounds of small caterpillars do not match the frequency hearing range of predators. To test my predictions, I analyzed the relationship between the sound pressure levels and dominant frequency of the defence sounds and body size (SMI). I found that sound pressure levels and dominant frequencies did not increase with body size in caterpillars that produced sounds. The defence sounds of small caterpillars were already loud enough to be heard at an attacking distance of 2-8 cm, and in the frequency hearing range of major predators at the onset of sound production. I concluded that the caterpillars need to reach a critical size to produce a signal that is loud enough to be heard by predators, and within the correct frequency range to be detected by them.

The results of the spectral analysis of the defence sounds demonstrated that the signals extended in to the ultrasonic range. Future testing of the defence sounds on predators that can hear ultrasonic frequencies, such as gleaning bats and rodents (Thomas & Jalili, 2004; Wagner et al., 2011; Wilson & Barclay, 2006) would open up new avenues of insight concerning the function of defence sounds in predator-prey interactions. For instance, one of the key characteristics of typical acoustic startle stimuli is high intensity, which will be affected by the frequency of the signal and the hearing range of the receiver (Olson, 1972). If the defence sounds have a startle function, they may be particularly effective at startling predators that are sensitive to the high concentration of energy within the ultrasonic range, such as bats. However, to date studies have not tested the effects of these sounds on bat predators.

Overall, findings from Chapter Two provided support for the hypothesis that size is an important factor in the evolution of defence sounds in insects, however this hypothesis should be further tested using more species. With the exception of a few species, the Bombycoidea species that have been tested for defence sounds to date have been large, and future studies should target species that are on the smaller side of the body size spectrum. A multitude of other insect species also produce defence sounds, yet only a handful of studies have examined the relationship between defence sounds and size in other insects. Future testing of the relationship between defence sounds and size in more species will be important to provide broader support for the hypothesis that body size is plays a role in the evolution of defence sounds in insects.

5.2.2 Chapter Three: Ontogenetic changes in primary and secondary defences in Bombycoidea caterpillars

In Chapter Three I followed the ontogenetic changes in antipredator defences of sound producing Bombycoidea caterpillars throughout development. I measured the frequency of secondary defences during simulated predator attack trials in four Bombycoidea species, and tested whether the frequency of defences changed with instar. Results demonstrated that in some species, the frequency of several defences (e.g. dropping, major thrashing, regurgitation) increased in later instars. By showing that these defences increased in late instars my findings from Chapter Three lay provide direction for future testing of hypotheses regarding the function of defence sounds in caterpillars. For instance, since several other secondary defences are present in late instar caterpillars, it is possible that defence sounds function as part of a multimodal display or as a non-visual warning signal. Future experiments that compare the effect of defence sounds alone to the combined effect of defence sounds accompanied with other secondary defences on live predators could provide support for these hypotheses.

5.2.3 Chapter Four: Why do caterpillars whistle at birds?

In Chapter Four I tested the startle response of wild-caught avian predators to playback recordings of the defensive whistle of *Amorpha juglandis*. I tested the hypothesis that an insect defence sound can function as a startle display. I predicted that the birds would exhibit more startle behaviours (i.e. a higher startle score) while feeding on mealworms paired with playbacks than in the control conditions where no sound was played. I also predicted that upon repeated exposure to whistles the birds would to habituate to the effects of the sounds, resulting in a decreased number of startle behaviours (i.e. lower startle score) and latencies to return to the feeding dish. Results showed that the birds exhibited significantly more startle behaviours (flying away, hopping, ruffling feathers and flinching) when consuming mealworms paired with the defence sound, and that they habituated to sounds over a one-hour period. When the experiment was repeated two days later on the same individuals, the startle response returned. I concluded that sounds of *Amorpha juglandis* function as an effective startle display against bird predators. This study was the first to show that the isolated effect of a caterpillar defence sound can startle an avian predator. By using a biologically relevant stimulus to activate the startle reflex, a biological reaction that is present in all vertebrates, this chapter also demonstrated how the acoustic startle reflex may offer protection to predators from potentially harmful prey.

The experiments conducted in Chapter Four took place in outdoor aviaries, which placed several restrictions on the inferences that can be made regarding the survival value of the startle displays. First, key elements of startle displays are surprise and novelty (Sargent, 1990), which are to some extent removed in a lab setting where animals are accustomed to being fed by researchers as opposed to foraging for their own food in the wild. The Bombycoidea caterpillars used in my study were cryptic and rested on leaves, and in a natural setting, avian predators would have to forage amongst trees to find them. Future experiments that can determine whether the sudden onset of defence sounds from a cryptic caterpillar hidden amongst the leaves would enhance the startle response of the birds compared to the responses observed in the experiments in Chapter Four would provide further support for the startle hypothesis. Second, it is argued that startle displays provide prey that cannot quickly flee (e.g. caterpillars) with protection because a bird that is startled away from potentially harmful prey would not likely return to feed on it in the wild (Janzen et al., 2010). To test this hypothesis, it is necessary to measure the predators' escape and habituation responses, and to date this has not been conducted (Götz & Janik, 2013; Ratcliffe & Fullard, 2005). During my experiments in Chapter Four it was not possible for birds to move on to new foraging opportunities after being scared away by the defence sounds because the birds were kept in enclosed aviaries. I attempted to overcome this limitation by conducting experiments at an outdoor feeder located in Gatineau, QC to observe the response of free-range wild birds to the defence sounds of the caterpillars (Appendix C). I found that some of the birds exhibited startle behaviours towards the defence sounds, but it was difficult to determine if the same bird returned to the feeder or habituated to the sounds. Due to the nature of startle displays and startle responses, experimental testing of the startle hypothesis in the wild is limited, and the results presented in Appendix C portray these limitations.

Future studies that can test the startle, escape and habituation responses of predators in the field would provide invaluable support for the startle hypothesis. These studies prove to be difficult, but are possible with the proper equipment, planning and time. Wild birds require a band with a tracker that can be placed by the feeding dish to determine if the same bird returns to the prey after being startled to measure habituation. Audio-visual equipment that is small enough to be placed inconspicuously within a tree and capable of recording the response of the bird and the defence sounds (in order to confirm that the stimulus was present) would allow us to capture the startle response of avian predators in a natural setting. A small camera placed directly above the feeder could record whether the prey was attacked or not. Since live caterpillars may walk away from the feeding dish, it may be possible to use a playback system similar to the one I used in Chapter Four, however, tiny, custom made high-frequency speakers would be required in order to playback the sounds at their naturally occurring frequency range. These are just some of the limitations (aside from provincial regulations that limit feeding experiments in the field) that I encountered when designing my experiments in Chapter Four, and why ultimately I pursued experiments in the aviaries as opposed to the field.

An alternative and intriguing hypothesis which has been put forward to explain how visual startle displays function to protect prey is that the sudden change in appearance causes a startle reaction in predators which disrupts the predators' ability to form a search image (i.e. memory of what a prey looks like) (Hanlon, 1999). Since memory is essential for learning a search image (Dukas & Kamil, 2001), this hypothesis may potentially apply to defence sounds because acoustic startle stimuli can cause glucocorticoids to be released in the brain, impairing learning and memory (McEwen & Sapolsky, 1995). If a bird who is startled by a defence sound responds by flying away and does not have a chance to form a memory of the cryptic caterpillar amongst the leaf, the display may offer protection to prey by making it difficult for the predator to find. This hypothesis would explain how startle displays have a survival value despite the fast habituation of the startle response that often occurs in avian predators (Bates and Fenton, 1988, this thesis). To test this hypothesis, future studies could evaluate how well predators form search images of prey in the presence and absence of insect defence sounds.

5.3 Final Remarks

Defences in insects are rich in diversity, and defence sounds are no exception (Conner, 2014; Ruxton et al., 2005). In this thesis I showed how body size plays a role in the evolution of defence sounds, and that a caterpillar defence sound functions to startle avian predators. Most predators hunt using multiple modalities, and the role of the acoustic senses should not be overlooked when considering that many prey defences are multimodal (Conner, 2014; Rowe, 2013). Few studies have examined the effect of acoustic defences on predator psychology (Conner, 2014), thus the studies conducted in this thesis contribute to our understanding of this once understudied area of insect defences. Prey defences are powerful forces acting on the biodiversity of the planet, and our understanding of how they function in predator-prey interactions is increasing in importance as a growing body of literature suggests they play a crucial role in determining how ecosystems respond to environmental change (Abrahams et al., 2007; Allen et al., 2015; Barton, 2014; Smee, 2010; Tseng & O'Connor, 2015). It is my hope that this thesis will inspire others to open not only their eyes, but their ears to the beautiful world of insects and the important roles they play in nature.

APPENDIX A: The Defences of non-sound producing Bombycoidea caterpillars tested

Introduction

In Chapter Two I tested the hypothesis that body size plays an important role in the development of defence sounds by conducting a developmental study to assess the size/instar at which defence sounds were produced in Bombycoidea caterpillars. During my two field seasons, I tested as many Bombycoidea species as I could obtain to identify species that produce defence sounds to include in the developmental study. Eleven species were tested in total, and of those, seven species did not produce defence sounds (See Table 2.2). These species were therefore excluded from analyses conducted in Chapter Two, and the defences of the larvae are presented here.

Methods

Seven Bombycoidea species were chosen based on availability from Akito Kawahara at the University of Florida, FL, USA, during the months of May to October 2013 and July to August 2014 (Fig. A1). Photographs were taken of the larvae throughout development to observe their physical appearance including the appearance of any visual and/or morphological defences (e.g. eyespots, spines). Simulated predator attack trials were performed on larvae to observe secondary defences (see Chapter Two*, 2.2.3 Simulated predator attack trials* for methods). Notes on the presence or absence of defensive behaviours were recorded for each trial in late instar larvae (Table A1).

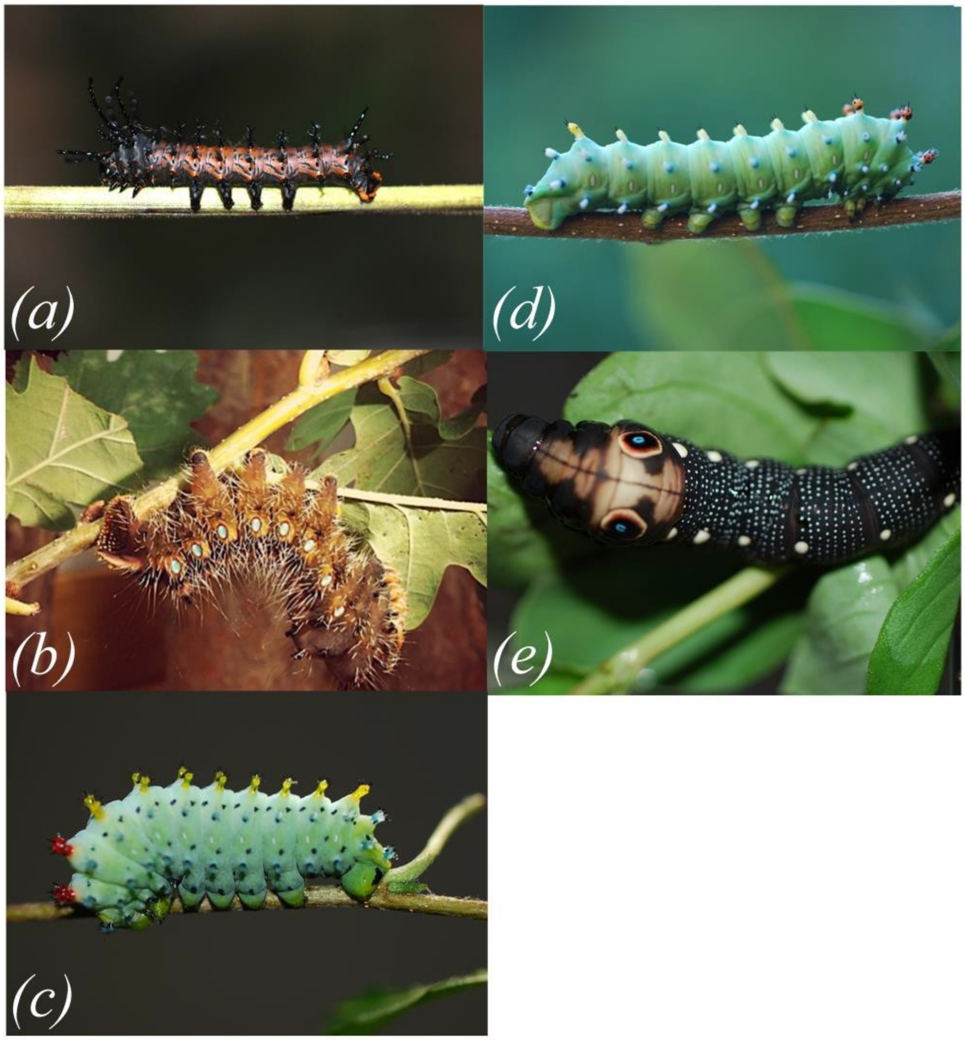


Figure A1. Appearance of late instar from each non-sound producing species tested: (a) *Citheronia splendens*; (b) *Eacles oslari;* (c) *Hyalophora cecropia;* (d) *Hyalophora columbia;* and (e) *Xylophanes falco.*

Table A1. List of defensive behaviours observed in non-sound producing caterpillars following attack.

Defence	Primary or Secondary?	Description
Regurgitation	Secondary	Regurgitation is the process by which the larvae expels fluid through the mouth in response to an attack or disturbance[1]
Major Thrashing	Secondary	Movement of the thorax from left to right vigorously >2 times[2]
Minor Thrashing	Secondary	Movement of the thorax from left to right <2 times[2]
Escaping	Secondary	Walking away from position after attack to escape predation[3]
Dropping	Secondary	Dropping off the host plant[3]
Curling	Secondary	Movement of the anterior and posterior portions of the larvae's body inward resembling the shape of the letter "C" [4]
Inflated thorax	Secondary	Enlargement of the thorax[5]
Urticating spines	Primary/Secondary	Spines that secrete a chemical defence[6]
Odour	Secondary	An unpleasant odour[7]
Defence sounds	Secondary	Air-borne signals produced by air expulsion through the mouthpart, or rubbing of the Mandibles back and forward in response to attack[9].
Eyespots	Primary	Mono or polychromatic patterns that are round or oval with round or slit pupils resembling eyes[10]

References: (1) Grant, 2007; (2) Walters et al., 2001; (3) Bateman & Fleming, 2015; (4) Deml & Dettner, 2002; (5) Hossie et al., 2013; (6) Stamp & Casey, 1993; (7) Carter, 1979; (9) Masters, 1979; (10) Janzen et al., 2010.

Results

Since larvae from *Darapsa myron* (Sphingidae: Macroglossinae) and *Eupackardia calleta* (Saturniidae: Saturniiae) did not survive until the late instars, reports on their defences were excluded. Reports on the defences of non-sound producing Bombycoidea species during simulated predator attack trials are included for late instar larvae only (instars IV and

V). All of the larvae possessed a visible primary defence: (1) *C. splendens* and *E. oslari* possessed long hairs (Fig. A1a-b); (2) *H. columbia* and *H. cecropia* possessed colourful urticating spines (Fig. A1c-d); and (3) *X. falco* possessed eyespots (Fig A1e). Overall, a total of seven secondary defences were observed amongst the species (Table A2). All of the species exhibited at least one secondary defence in response to attack. Minor thrashing was the most common behaviour, employed by three species: *C. splendens*, *H. cecropia* and *H. columbia*. Inflated thorax was the least common behaviour employed only by *X. falco*.

Table A2. Summary of secondary defences observed in non-sound producing species in late instar larvae during simulated predator attack trials.

	# tested	**Regurgitation**	**Major Thrash**	**Minor Thrash**	**Escape**	**Drop**	**Curl**	**Inflated Thorax**
Citheronia splendens	10	x	x	x				
Eacles oslari	2		x					
Hyalophora cecropia	10			x	x			
Hyalophora columbia	8			x	x			
Xylophanes falco	2							x

Discussion

In Chapter Two I tested hypotheses to explain why some Bombycoidea caterpillars produce defence sounds while others do not. Here, I show that some non-sound producing caterpillars have alternative antipredator strategies. Primary defences include eyespots, hairs, and defensive spines. Secondary defences include regurgitation, thrashing, and escape. It has been suggested that species will not invest in additional defences if the ones they already have are sufficient (Edmunds, 1974). Perhaps these species do not produce defence sounds because the alternative defences that they have are already sufficient to provide protection from predators.

Alternatively, these species may be less pursued by predators with a well-development auditory system, or in general they may face less selection pressure from predators. Future studies that test the effect of the antipredator defences on live predators would provide more insight to the survival value of these displays.

APPENDIX B: Supplemental Material for Chapter Four

Introduction

In Chapter Four of this thesis I tested the startle and habituation response of avian predators to the defence sounds of *Amorpha juglandis*. To test the startle response, I recorded the frequency of startle behaviours that occurred over the course of 6 exposures to the sounds (Fig. B1). The total number of startle behaviours was used to calculate a total startle score for each experimental group (Table B1). To test habituation, I measured the time it took the birds to return to feed on mealworms between exposures to sounds (Fig. B2), and the total time between the first and sixth approach to feed (Table B2). This appendix contains supplementary material on the results of these tests to measure startle and habituation (see Chapter Four for details on sampling and statistical analysis).

Data B1

To reduce the number of research animals, birds used in our experiment had served as controls in a previous unpublished study by Dr. Frances Bonier et al. (IACUC 13-030-BIOL, Queen's University). In that study some of the birds had previously been administered an antimalarial treatment: Primaquine (10mg/kg) & Chloroquine (25mg/kg) (Birds 2, 5, 30), or Atovaquone (5.3 mg/kg) & Proguanil hydrochloride (13.2 mg/kg) (Birds 11, 18, 20) or no treatment (3, 4, 8, 9, 21, 27). While the pharmokenetics of antimalarial treatments are currently unknown for birds, they have a short half-life in humans (<2 days) and may be eliminated even faster in birds as blood flow from the external iliac vein (non-existent in mammals) allows for rapid metabolism (Baird et al., 2004; Beerahee, 1999; Knowles et al., 2010; Vermeulen et al., 2002). As a precaution, we waited 36-38 days before conducting our experiment to avoid any potential effects of previous treatments. We have no reason to believe that previous treatments would affect the behaviour of the birds.

Figure B1. Mean startle scores and proportions of startle behaviour types for each contact event with the dish: (a) Experimental phase one; (b) Experimental phase two; (c) Control phase.

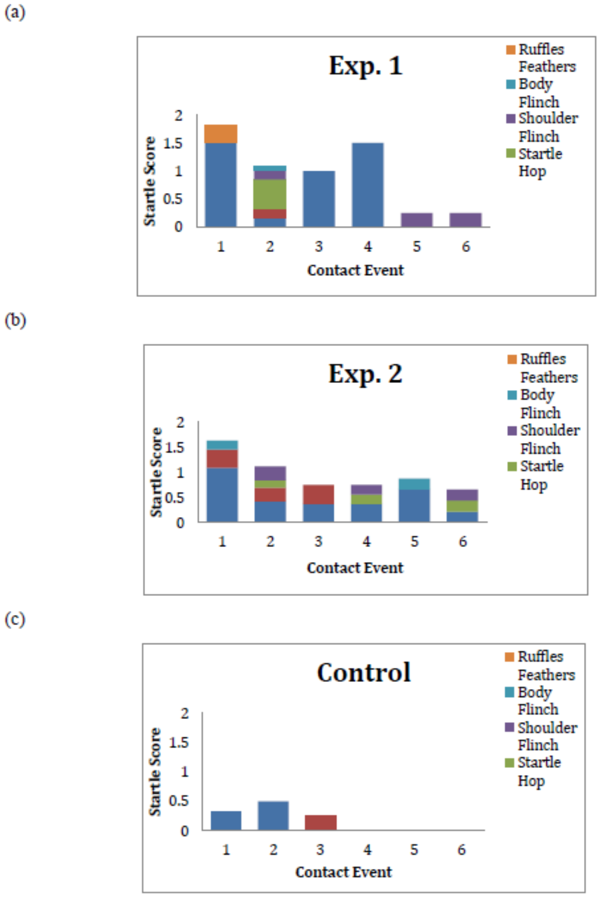
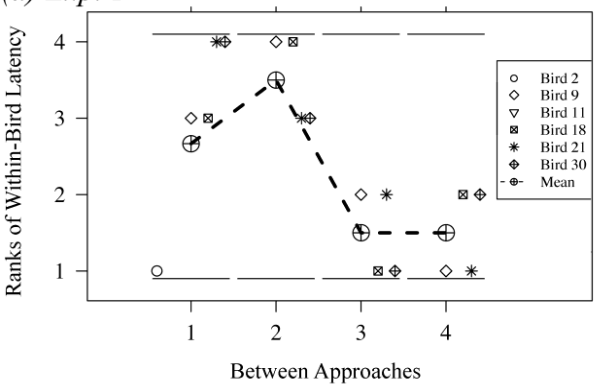


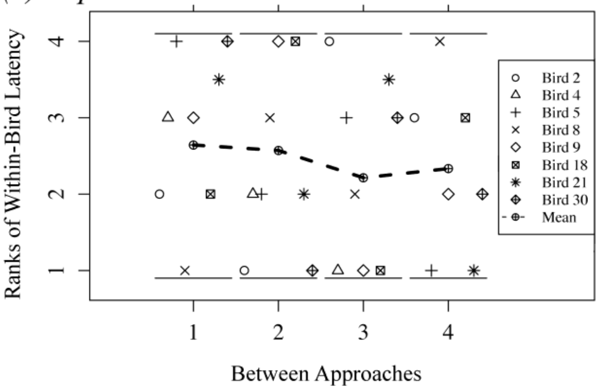
Table B1. Mean startle scores, behaviour types and sample sizes for each dish contact event.

Exp 1. (70 dB SPL)	**Contact Event**					
Behaviour	**1**	**2**	**3**	**4**	**5**	**6**
Fly	1.52	0.17	1	1.5	0	0
Wing Flap	0	0.17	0	0	0	0
Startle Hop	0	0.51	0	0	0	0
Shoulder Flinch	0	0.17	0	0	0.25	0.25
Body Flinch	0	0.17	0	0	0	0
Ruffles Feathers	0.31	0	0	0	0	0
Mean Startle Score	**1.83**	**1.2**	**1**	**1.5**	**0.25**	**0.25**
SD	**0.41**	**0.45**	**0.86**	**1**	**0.5**	**0.5**
n	**6**	**5**	**4**	**4**	**4**	**4**
Exp 2. (60 dB SPL)	**Contact Event**					
Behaviour	**1**	**2**	**3**	**4**	**5**	**6**
Fly	1.09	0.42	0.38	0.38	0.66	0.22
Wing Flap	0.36	0.28	0.38	0	0	0
Startle Hop	0	0.14	0	0.19	0	0.22
Shoulder Flinch	0	0.28	0	0.19	0	0.22
Body Flinch	0.18	0	0	0	0.22	0
Ruffles Feathers	0	0	0	0	0	0
Mean Startle Score	**1.63**	**1.13**	**0.75**	**0.75**	**0.88**	**0.66**
SD	**0.74**	**0.99**	**0.89**	**0.89**	**0.99**	**0.67**
n	**8**	**6**	**6**	**5**	**5**	**4**
Control	**Contact Event**					
Behaviour	**1**	**2**	**3**	**4**	**5**	**6**
Fly	0.33	0.5	0	0	0	0
Wing Flap	0	0	0.25	0	0	0
Startle Hop	0	0	0	0	0	0
Shoulder Flinch	0	0	0	0	0	0
Body Flinch	0	0	0	0	0	0
Ruffles Feathers	0	0	0	0	0	0
Mean Startle Score	**0.33**	**0.5**	**0.25**	**0**	**0**	**0**
SD	**0.88**	**0.93**	**0.71**	**0**	**0**	**0**
n	**9**	**8**	**8**	**7**	**7**	**6**

(a) Exp. 1



(b) Exp. 2



(c) Control

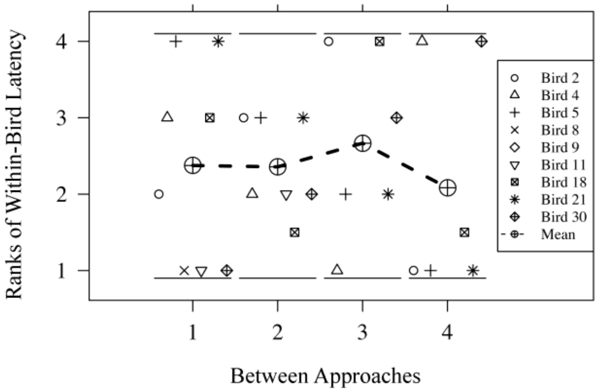


Figure B2. Changes in latency to return to feeding dish over the course for four contacts with the feeding dish following the first attempt: (a) Experimental phase one; (b) Experimental phase two; and (c) Control phase. The dotted line represents the overall mean within each phase.

Table B2. Time differences between the first and sixth contacts with the feeding dish for individual birds, standard deviations and sample sizes for each phase.

Bird ID	**Control**	**Exp. 1 (70 dB SPL)**	**Exp. 2 (60 dB SPL)**
Bird 2	6	162	167
Bird 4	8	NA	146
Bird 5	3	NA	6
Bird 8	17	NA	815
Bird 9	NA	156	414
Bird 11	4	NA	NA
Bird 18	6	1100	1388
Bird 21	27	50	120
Bird 30	2.9	15	8
Mean	**9.24**	**531.75**	**378.57**
SD	**8.49**	**479.41**	**524.6**
n	**8**	**5**	**8**

APPENDIX C: Backyard feeder experiments

Introduction

Defence sounds in insects are widespread, yet few studies have tested their functions on live predators (Conner, 2014). Two of the main proposed functions of defence sounds are aposematism and startle (Edmunds, 1974). Aposematic displays are proposed to function by warning predators of a secondary chemical defence. Startle displays are proposed to function by causing predators to hesitate or withdraw away from prey (Edmunds, 1974). It is argued that it is necessary to measure the escape and return response of predators to determine the true survival value of startle displays (Götz & Janik, 2013; Ratcliffe & Fullard, 2005). Field studies are necessary for measuring the escape response of predators to proposed startle displays. However, most studies that test the startle hypotheses either do not use real predators, or they test the displays in restricted environments (i.e. the laboratory, outdoor aviaries) where birds are not given the option to fully escape from prey after being startled (see Chapter Four).

To overcome the common limitations that lab studies pose on testing startle displays I conducted pilot trials to test the response of wild birds to Bombycoidea defence sounds at an outdoor feeder during the summer of 2014. I placed a sensor activated feeding dish on a pre-existing feeder platform, where a variety of birds were reported to feed from for over a year. A speaker activated by a sensor in a feeding dish broadcasted the defence sounds from different Bombycoidea species. I video recorded the response of the birds to the recordings of the sounds when they made contact with the feeding dish while feeding on mealworms. However, the experiments were not carried through, because I had no way to verify the identity of the birds, which is important for providing support for the startle hypothesis by showing the escape and return response of the birds (Sargent, 1990). The findings of these pilot trials are presented here.

Methods

The backyard feeder experiments were conducted on June 6th, 2014, June 29th 2014, June 10th, 2014 and August 26th, 2014 between the hours of approximately 10 am and 5 pm at an outdoor feeder located in Gatineau, QC. A sensor activated feeding dish was placed on the feeder platform and mealworms were placed as bait inside the dish. The feeding dish was connected to speakers located directly below the dish, affixed to the platform. The speakers were programmed to trigger playback of the defence sounds when contact is made by the bird with the feeding dish (See Chapter Four, *4.2.3 Sound playback*). The sounds were played at approximately 70 dB SPL measured at 22 cm from the speaker. Defence sounds from 4 different species of caterpillars that produced 4 different sound types were played: (1) vocalizing - *Amphion floridensis* (Sphingidae: Macroglossinae); (2) clicking - *Antheraea polyphemus* (Saturniidae: Saturniinae)*;* (3) chirping -

Nyceryx Magna (Sphingidae: Macroglossinae); and (4) whistling - *Amorpha juglandis* (Sphingidae: Sphinginae). The sound recordings were obtained from Dr. Jayne Yack (Carleton University, Ottawa, Canada). A Sony camcorder (DCRTRV19) on a tripod (60 cm high) was placed 1 m away from the platform to record the reactions of the birds to the sounds (Fig. C1). I remained hidden from the feeder behind trees throughout the experiments.

Figure C1. Experimental set up of outdoor feeder experiments. The sensor-activated feeding dish was placed on top of a platform containing the speaker located below. The speaker was connected to a laptop with pre-loaded defence sounds for playback when the sensor was triggered by a bird pecking at the disk. Before trials started a tarp was placed over the laptop. A video camera was placed ~1 m away from the platform to record the reactions of the birds.

Results

Experiments were conducted on three separate testing days (June 6, 2014, June 10, 2014, and August 26, 2014). The feeder was visited by house sparrows *Passer domesticus* (Passeridae). and black-capped chickadees *Poecile atricapillus* (Paridae). Overall, a total of 37 visits to the feeder were recorded. Nine of the visits were from *Poecile atricapillus* and 28 of the visits were from *Passer domesticus.* Out of the 37 visits, the sensor failed to trigger 5 times. On 8 of the visits, the birds approached the feeding dish but did not trigger the speaker by pecking on the dish (Table C1).

Two types of avian startle behaviours were observed after the defence sounds were broadcast. Flying away was observed on 3 visits after the sounds of *A. juglandis* and *N. magna* were broadcast on June 10, 2016 (Table C2). Wing flinching was observed on 3 visits after the sounds of *A. juglandis, A. floridensis* and *N. magna* were broadcast on June, 10, 2014 (Table C2). Additionally, tail fanning was observed in a black-capped chickadee on one visit to the feeder after the defensive whistles of *A. juglandis* was broadcasted on June 10, 2014 (Fig C2a). On two of the visits, it was possible to confirm that the same bird returned to the feeding dish due to the camera angle and flight path of the bird. The birds responded by flying away upon hearing the sounds on the first visit, and exhibited no response upon hearing the sounds on the second visit (Fig C2b).

Table C1. Summary of behaviours observed in avian predators (*Passer domesticus* and *Poecile atricapillus)* to Bombycoidea defence sounds.

Behaviour	**Total**
No response	13
Fly away	3
Wing flinch	3
Tail flare	1
NA, sensor not triggered	8
Total visits by *Passer domesticus*	**28**
Total visits by *Poecile atricapillus*	**9**
Total visits to feeder	**37**

Table C2. Responses of birds to the defence sounds of Bombycoidea caterpillars showing date and time of visit to the feeder, bird species, defence sound played and response.

Date	**Time**	**Avian predator species**	**Defence sound**	**Response**
June 6, 2014	12:50:40	*Poecile atricapillus*	*NA, sensor not triggered.*	NA
June 6, 2014	12:53:15	*Poecile atricapillus*	*NA, sensor not triggered.*	NA
June 6, 2014	12:56:16	*Poecile atricapillus*	*NA, sensor not triggered.*	NA
June 6, 2014	12:58:15	*Poecile atricapillus*	*N. magna, A.juglandis*	No response
June 6, 2014	13:03:14	*Passer domesticus*	*A. polyphemus, N. magna, A. juglandis, A.*	No response
June 10, 2014	14:37:26	*Passer domesticus*	*N. magna*	Fly away
June 10, 2014	14:37:44	*Passer domesticus*	*A. juglandis*	Fly away
June 10, 2014	14:44:56	*Passer domesticus*	*A. polyphemus, N. magna*	No response
June 10, 2014	14:45::17	*Poecile atricapillus*	*NA, sensor not Triggered.*	NA
June 10, 2014	14:48:13	*Poecile atricapillus*	*NA, sensor not Triggered.*	NA
June 10, 2014	14:48:45	*Passer domesticus*	*A. juglandis*	Fly away
June 10, 2014	14:49:04	*Passer domesticus*	*A. floridensis, A. polyphemus*	No response
June 10, 2014	14:52:32	*Poecile atricapillus*	*A. juglandis*	No response
June 10, 2014	14:52:39	*Poecile atricapillus (same bird as*	*A. floridensis*	Tail flaring
June 10, 2014	14:52:55	*Poecile atricapillus (same bird as at*	*A. polyphemus*	No response
June 10, 2014	15:09:17	*Passer domesticus*	*A. floridensis*	Wing flinch
June 10, 2014	15:09:30	*Passer domesticus (same bird as at*	*N. magna*	No response
June 10, 2014	15:14:25	*Passer domesticus*	*A. juglandis*	Wing flinch
June 10, 2014	15:14:30	*Passer domesticus (same bird as at*	*A. floridensis*	No response
June 10, 2014	15:18:14	*Passer domesticus*	*A. polyphemus, N. magna, A. juglandis, A.*	No response
June 10, 2014	15:32:57	*Passer domesticus*	*A. polyphemus*	No response
June 10, 2014		*Passer domesticus (same bird as at*	*N. magna*	Wing flinch
June 10, 2014	15:37:11	*Passer domesticus*	*A. polyphemus, N. magna, A. juglandis*	No response
June 10, 2014	15:53:15	*Passer domesticus*	*A. polyphemus, N. magna, A. juglandis*	No response
June 10, 2014	15:57:17	*Passer domesticus*	*Sensor not triggered*	NA
June 10, 2014	15:59:14	*Passer domesticus*	*Sensor not triggered*	NA
June 10, 2014	16:06:21	*Passer domesticus*	*Sensor not triggered*	NA

June 10, 2014	16:14:13	*Passer domesticus*	*A. polyphemus, N. magna*	No response
June 10, 2014	16:10:19	*Passer domesticus*	*A. polyphemus*	No response

Figure C2. (a) Tail flaring observed in the black-capped chickadee when the defensive whistle of *A. juglandis* was played. (b) No startle response observed to the defence sounds in 17 out of 28 visits, like in the black-capped chickadee shown here.

Discussion

The survival value of prey startle displays has been difficult to determine because few studies have captured the reaction of predators to prey startle displays in the wild. In these experiments I attempted to record the startle response of avian predators in the field to the recordings of defence sounds from four species of Bombycoidea caterpillars. On the majority of visits the birds did not exhibit a noticeable behavioural response to the defence sounds. On a handful of visits, birds responded by flying away, flinching their wings, and in one case flaring their tail to the vocalizing sounds of *A. floridensis,* rasping sounds of *N. magna,* and whistling sounds of *A. juglandis*. Tail fanning in birds sometimes represents a form of defensive posturing use to scare prey (Schowalter, 2000), and flying away and wing flinching are considered to be avian startle reactions (Olofsson et al., 2012b; Schlenoff, 1985). Thus is appears that some of the birds exhibited a startle response to the defence sounds. It may be possible that many visits did not result in any behavioural response because the same birds that were initially startled by the sounds eventually habituated to them on subsequent revisits. While I had no way to verify the identity of the birds who visited the feeder, the possibility that some of the same birds repeatedly came back to the feeder should not be ruled out because the experiments were all conducted within a short period of time on each day (i.e. ~4 hours) and there were sometimes very short intervals between visits (a few minutes) (see Table C1). However, without a bird band tracker to confirm the identity of the birds it is impossible to know whether the same birds were returning to the feeder. Future studies that can experimentally test the predator responses to defence sounds in wild birds while tracking their identification will be invaluable to providing insights about the survival value of startle displays.

References

Abrahams, M.V., Mangel, M., & Hedges, K. (2007). Predator-prey interactions and changing environments: who benefits? *Philosophical Transactions: Biological Sciences, 362*(1487), 2095-2014.

Adams, R.A., & Pedersen, S.C. (2000). *Ontogeny, Functional Ecology, and Evolution of Bats.* Cambridge, UK: Cambridge University Press.

Alexander, R.D. (1957). Sound production and associated behaviour in insects. *The Ohio Journal of Science, 57*(2), 101-1132

Allen, B.J.M., Domenici, P., Munday, P.L., & McCormick, M.I. (2015). Feeling the heat: the effect of acute temperature changes on predator-prey interactions in coral reef fish. *Conservation Physiology, 3*(1), cov011.

Ananthakrishnan, T.N. (2005). Perspectives and dimensions of phenotypic plasticity in insects. In T.N. Ananthakrishnan & D. Whitman (Eds.), *Insect Phenotypic Plasticity* (pp. 1-49). Enfield, NH: Science Publishers, Inc.

Arnqvist, G., & Johansson, F. (1998). Ontogenetic reaction norms of predator-induced defensive morphology in dragonfly larvae. *Ecology, 79*(6), 1847-1858.

Baird, K.J., & Hoffmann, S.L. (2004). Primaquine therapy for malaria. *Clinical Infectious Diseases, 39*(9), 1336-1345.

Balderrama, N., & Maldonado, H. (1973). Ontogeny of the behaviour in the praying mantis. *Journal of Insect Physiology, 19*(2), 319-336.

Barr, B.A. (1969). Sound production in Scolytidae with emphasis on the genus *Ips. Canadian Entomology, 101*, 636-672.

Barton, B.T. (2014). Reduced wind strengthens top-down control of insect herbivore. *Ecology, 95*(9), 2375-2381.

Barton Browne, L., & Raubenheimer, D. (2003). Ontogenetic changes in the rate of ingestion and estimates of food consumption in fourth and fifth instar *Helicoverpa armigera* caterpillars. *Journal of Insect Physiology, 49*(1), 63-71.

Barton Browne, L. (1995). Ontogenetic changes in feeding behaviour. In R.F. Chapman & G de Boer (Eds.) *Regulatory Mechanisms in Insect Feeding* (pp. 307-342). New York, NY: Chapman & Hall.

Bateman, P.W., & Fleming, P.A. (2015). Invertebrates. In W.E. Cooper & D.T. Blumstein (Eds.), *Escaping from predators: An integrative view of escape decisions* (pp. 177-198). Cambridge, UK: Cambridge University Press.

Bates, D.L. & Fenton, M.B. (1988). Aposematism or startle? Predators learn their responses to the defences of prey. *Canadian Journal of Zoology, 68*(1), 49-52.

Bauer, T. (1976). Experimente zur frage der biologischen Bedeutung des stridulationsverhaltens von Käfern. *Zeitschrift für Tierpsychologie, 42*(1), 57-65.

Beason, R.C. (2004). What can birds hear? *USDA National Wildlife Research Center – Staff Publications*, *78*. Retrieved from http://digitalcommons.unl.edu/icwdm_usdanwrc/78

Beerahee, M. (1999). Clinical pharmacology of atovaquone and proguanil hydrochloride. *Journal of Travel Medicine, 6,* S13-S17.

Bennet-Clark, H.C. (1999). Resonators in insect sound production: How insects produce loud pure-tone songs. *The Journal of Experimental Biology, 202*, 3347-3357.

Berger, D., Walters, R., & Gotthard, K. (2006). What keeps insects small? Size dependent predation on two species of butterfly larvae. *Evolutionary Ecology, 20*, 575-589.

Bernays, E.A. (1997). Feeding by lepidopteran larvae is dangerous. *Ecological Entomology, 22*(1), 121-123.

Blanckenhorn, W.U. (2000). The evolution of body size: what keeps organisms small? *The Quarterly Review of Biology, 75*(4), 385-407.

Blaszczyk, J.W. (2003). Startle response to short acoustic stimuli in rats. *Acta Neurobiologiae Experimentalis, 63*(1), 25-30.

Blondeheim, S.A., & Frankenberg, E. (1983). Protest sounds of a grasshopper: Predator-deterrent signal?*. *Psyche, 90*(4), 387-394.

Brakefield, P.M. (2009). Crypsis. In V.H Resh & R.T. Carde (Eds.), *Encyclopedia of Insects, Second edition* (pp. 236-238). Burlington, MA: Academic Press.

Broughton, W.B. (1963). Method in bio-acoustic terminology. In R.G. Busnel (Ed.), *Acoustic Behaviour of Animals* (pp. 3-25). New York, NY: Elsevier.

Brown, S.G., Boettner, G.H., & Yack, J.E. (2007). Clicking caterpillars: Acoustic aposematism in *Antheraea polyphemus* and other Bombycoidea. *Journal of Experimental Biology, 201*, 993-1005.

Buchler, E.R., Wright, T.B., & Brown, E.D. (1981). On the functions of stridulation by the passalid beetle *Odontotaenius disjunctus* (Coleoptera: Passalidae). *Animal Behaviour, 29*(2), 483-486.

Bura, V.L. (2010). Sound production in Bombycoidea caterpillars: A comparative study of taxonomic diversity mechanisms and function (Unpublished Master's thesis). Carleton University, Ottawa.

Bura, V.L., Fleming, A.J., & Yack, J.E. (2009). What the buzz? Ultrasonic and sonic warning signals in caterpillars of the great peacock moth (*Saturnia pyri). Naturwissenschaften, 96*(6), 713-718.

Bura, V.L, Hnain, A.K., Hick, J.N. & Yack, J. (2012). Defensive sound production in the tobacco hornworm, *Manduca sexta* (Bombycoidea: Sphingidae). *Journal of Insect Behaviour, 25*(2), 114-126.

Bura, V.L., Kawahara, A.Y., Yack, J.E. (2016). A comparative analysis of sonic defences in Bombycoidea caterpillars. *Scientific Reports, 6*(31469).

Bura, V.L., Rower, V.G., Martin, P.R., & Yack, J.E. (2011). Whistling in caterpillars (*Amorpha juglandis*, Bombycoidea): Sound-producing mechanism and function. *Journal of Experimental Biology, 214,* 30-37.

Carisio, L., Claudia, P., & Rolando, A. (2004). Stridulation variability and morphology: An examination in dung beetles of the genus *Trypocopris* (Coleoptera, Geotrupidae). *Population Ecology, 46*(1), 27-37.

Caro, T. (2005). *Antipredator defenses in birds and mammals.* Chicago, IL: The University of Chicago Press, Ltd.

Carter, D.J. (1979). *The observer's book of caterpillars*. London, UK: Frederick Warne.

Castellanos, I., Pedro, I.Z, & Caldas, B.A. (2015). Morphology and antipredator behavior in larval *Orgyia leucostigma* (Lepidoptera: Lymantriidae). *Journal of the Lepidopterists' Society, 69*(4), 327-331.

Chapman, R.F. (2013). Mechanical communication: producing sound and substrate vibrations. In S.J. Simpson & A.E. Douglas (Eds.), *The Insects Structure and Function* (pp. 680-701). Cambridge, UK: Cambridge University Press.

Chattopadhyay, J. (2015). The structure and defensive efficacy of glandular secretion of the larval osemeterium in *Graphium agamemnon* Linnaeus, 1758 (Lepidoptera: Papilionidae). *Turkish Journal of Zoology, 35*(2), 245-254.

Claridge, M. (2006). Insect Sounds and Communication - An Introduction. In S. Drosopoulos & M.E. Claridge (Eds.), *Insect sounds and communication: Physiology, Behaviour, Ecology and Evolution* (pp. 3-10). Boca Raton, FL: Taylor & Francis Group.

Cocroft, R.B. (1999). Offspring-parent communication in a subsocial treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*). *Behaviour, 136*(1)*,* 1-21.

Cocroft, R.B., & De Luca, P. (2006). Size-frequency relationships in insect vibratory signals. In S. Drosopoulos & M.F Claridge (Eds.), *Insect sounds and communication: Physiology, behaviour, ecology and evolution* (pp. 99-110). Boca Raton, FL: Taylor & Francis Group.

Coley, P.D., Bateman, M.L., & Kursar, T.A. (2006). The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos, 115*(2), 219-228.

Conner, W.E. (2014). Adaptive sounds and silences: Acoustic anti-predator strategies in insects. In B. Hedwig (Ed.), *Insect Hearing and Acoustic Communication*, *Animal Signals and Communication 1* (pp. 64-80). Berlin: Springer-Verlag.

Conner, W.E., & Corcoran, A.J. (2012). Sound strategies: The 65-million-year-old battle between bats and insects. *Annual Review of Entomology, 57*(1)**,** 21-39.

Corcoran, A.J., Barber, J.R., Hristov, N.I., & Conner, W.E. (2011). How do tiger moths jam back sonar? *Journal of Experimental Biology, 214*, 2416-2425.

Corcoran, A.J., Conner, W.E., & Barber, J.R. (2010). Anti-bat tiger moth sounds: Form and function. *Current Zoology, 56*(3), 358-369.

Cornell, J.C., Stamp, N.E., & Bowers, M.D. (1987). Developmental change in aggregation, defence, and escape behavior of buck moth caterpillars, *Hemileuca lucina* (Saturniidae). *Behavioral Ecology and Sociobiology, 20*(6), 383-388.

Cramp, S. (1980). *Handbook of the Birds of Europe, the Middle East and North Africa: Ostrich to ducks.* London, UK: Oxford University Press.

Curio, E. (1965). Die schutzanpassungen dreier raupen eines Schwarmers (Lepidoptera, Sphingidae) auf Galapagos. *Zoologische Jahrbuecher Systematik, 92*, 487-522.

Darwin, C. (1887). *The Life and Letters of Charles Darwin.* London, UK: John Murray.

Deml, R., & Dettner, K. (2002). Morphology and classification of larval scoli of Saturniinae and Hemileucinae (Lepidoptera: Saturniidae). *Journal of Zoological Systematics and Evolutionary Research, 40*(2), 82-91.

Dethier, V.G. (1954). Evolution of feeding preferences in phytophagous insects. *Evolution, 8*, 33-54.

Dooling, R.J. & Searcy, M.H. (1985). Temporal integration of acoustic signals by the budgerigar (*Melopsittacus undulatus).Journal of the Acoustical Society of America, 77* (5), 1917-1920.

Dukas, R., Kamil, A.C. (2001). Limited attention: the constraint underlying search image. *Behavioural Ecology, 12*(2), 192-199.

Dunning, D.C. (1968). Warning sounds of moths. *Zeitschrift fur tierpsychologie, 25*(2), 129-138.

Dussourd, D.E. (1993). Foraging with finesse: caterpillar adaptations for circumventing plant defenses. In N.E. Stamp & T. Casey (Eds.), *Ecological and evolutionary constraints of caterpillars* (pp. 92-31). New York, NY: Chapman and Hall.

Dyar, H.G. (1890). The number of molts of Lepidopterous larvae. *Psyche, 5* 420-422.

Edmunds, M. (1974). *Defence in animals: a survey of anti-predator defences.* New York, NY: Logman Group Ltd.

Einsporn, R.L., & Habtzghi, D. (2013). Combining paired and two-sample data using a permutation test. *Journal of Data Science, 11,* 767-779.

Elsner, N., & Popov, A.V. (1978). Neuroethology of acoustic communication. In J.E. Threherne, M.J. Berridge and V.B. Wigglesworth (Eds.) *Advances in Insect Physiology,* pp. 229-356. New York, NY: Academic Press Inc.

Endler, J.A. (1984). Progressive background matching in moths, and a quantitative measure of crypsis. *Biological Journal of the Linnean Society, 22*, 187-231.

Etile, E., & Despland, E. (2007). Developmental variation in the forest tent caterpillar: life history consequences of a threshold size for pupation. *Oikos, 117*(1), 135-143.

Ewing, A.M. (1989). *Arthropod bioacoustics: Neurobiology and behavior*. New York, NY: Cornell University Press.

Exnerová, A., Štys, P., Kristin, A., Volf, O., & Pudil, M. (2003). Birds as predators of true bugs (Heteroptera) in different habitats. *Biologica, 58*(2), 253-264.

Fabian, D., & Flatt, T. (2012). Life history evolution. *Nature Education Knowledge, 3*(10), 24.

Fallow, P.M., Gardner, J.L., & Magrath, R.D. (2011). Sound familiar? Acoustic similarity provokes responses to unfamiliar heterospecific alarm calls. *Behavioral Ecology, 22,* 401-410.

Fallow, P.M., Pitcher, B.J., & Magrath, R.D. (2013). Alarming features: birds use specific acoustic properties to identify heterospecific alarm calls. *Proceedings of the Royal Society B, 280,* 20122539.

Federley, H. (1905). Sound produced by Lepidopterous larvae. *Journal of the New York Entomological Society, 13*, 109-110.

Field, L.H. (1980). The tick sound of a giant weta, *Deinacrida rugosa* (Orthoptera: Stenopelmatidae: Deinacridinae). *New Zealand Entomologist, 7*(2)*,* 176-183.

Field, L.H., & Glasgow, S. (2001). Defence behaviour. In L.H. Field (Ed.), *The Biology of Wetas, King Crickets, and their Allies,* pp. 297-316. New York, NY: Oxford University Press.

Forrest, T.G. (1987). Insect size tactics and developmental strategies. *Oecologia, 73*(2), 178-184.

Forrest, T.G. (1994). From sender to receiver: propagation and environmental effects on acoustic signals. *American Zoologist, 34*(6), 644-654.

Frank, K.D. (2015). *Polyphemus moth*. Retrieved from http://fitlersquarepress.com/wp-content/uploads/2015/08/Book-1_Ecology_web_chapter8.pdf

Fullard, J.H., & Fenton, M.B. (1977). Acoustic and behavioural analyses of the sounds produced by some species of Nearctic Arctiidae (Lepidoptera). *Canadian Journal of Zoology, 55*(8), 1213-1224.

Futahashi, R., & Fujiwara, H. (2008). Juvenile hormone regulates butterfly larval pattern switches. *Science, 22*(319), 1061.

Gaston, K.J., Reavey, D., & Valladares, G.R. (1991). Changes in feeding habit as caterpillars grow. *Ecological Entomology, 16*(3), 339-344.

Gerhardt, H.C., & Huber, F. (2002). *Communication in Insects and Anurans.* Chicago, IL: The University of Chicago Press.

Gerling, D., Roitberg, B.D., & Mackauer, M. (1990). Instar-specific defense of the pea aphid, *Acrythosiphon pisum*: Influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelmidae). *Journal of Insect Behaviour, 3*(4), 501-514.

Gillooly, J.F., & Ophir, A.G. (2010). The energetic basis of acoustic communication. *Proceedings of the Royal Society B, 277*(1686), 1325-1331.

Gomez-Nieto, R., Horta-Junior, J.A.C., Castellano, O., Millian-Morell, L., Rubio, M.E., & Lopez, D.E. (2014). Origin and function of short-latency inputs to the neural substrates underlying the acoustic startle reflex. *Frontiers in Neuroscience, 9*(216), 1-18.

Gotthard, K. (2000). Increased risk of predation as a cost of high growth rate: an experimental test in a butterfly. *Journal of Animal Ecology, 69*(5), 896-902.

Götz, T., & Janik, V.M. (2011). Repeated elicitation of the acoustic startle reflex leads to sensitization in subsequent avoidance behaviour and induces fear conditioning. *BMC Neuroscience, 12*(30), 1471-2202-12-30.

Grant, J.B. (2007). Ontogenetic colour change and the evolution of aposematism: a case study in panic moth caterpillars. *Journal of Animal Ecology, 76*(3), 439-447.

Griesser, M. (2013). Do warning calls boost survival of signal recipients? Evidence from a field experiment in a group-living bird species. *Frontiers in Zoology, 10*(49)*,* 1-5.

Griethuijsen, L.I., Banks, K.M., & Trimmer, B.A. (2013). Spatial accuracy of a rapid defense behavior in caterpillars. *Journal of Experimental Biology, 216*, 379-387.

Guilford, T., & Dawkins, M.S. (1991). Receiver psychology and the evolution of animal signals. *Animal Behaviour, 42*(1), 1-14.

Gullan, P.J., & Cranston, P.S. (2010). *Insects: An outline of entomology 4th ed*. Sussex, UK: Wiley-Blackwell.

Grant, J.B. (2006). Diversification of gut morphology in caterpillars is associated with defensive behavior. *Journal of Experimental Biology, 209*(Pt 15), 3018-3024.

Greene, E. (1989). A diet-induced developmental polymorphism in a caterpillar. *Science*, *243*(4891), 643-646.

Green, A.J. (2001). Mass/Length residuals: Measures of body condition or generators of spurious results? *Ecology, 82*(5), 1473-1483.

Greeney, H.F., Dyer, L.A., & Smilanich, A.M. (2012). Feeding by lepidopteran larvae is dangerous: A review of caterpillars' chemical, physiological, morphological, and behavioural defences against natural enemies. *Invertebrate Survival Journal, 9*(1)*, 7-34.*

Greenlee, K.J., & Harrison, J. (2005). Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta. Journal of Experimental Biology, 208*, 1385-1392.

Hager, F.A., & Kirchner, W.H. (2013). Vibrational long-distance communication in the termites *Macrotermes natalensis* and *Odontotermes sp*. *Journal of Experimental Biology*, *216,* 3249- 3256.

Hagg, J.F., Zarobelny, M., Jorgensen, K., Vogel H., Moller, B.L., & Bak, S. (2014). Chemical defense balanced by sequestration and *de novo* biosynthesis in a Lepidopteran specialist. *PLoS ONE, 9*(10), e108745.

Hall, D.W. (2012). Luna moth. Retrieved from http://entnemdept.ufl.edu/creatures/misc/moths/luna_moth.htm

Halpin, C.G., Skelhorn, J., & Rowe, C. (2013). Predators' decisions to eat defended prey depend on size of undefended prey. *Animal Behaviour, 85*(6), 1315-1321.

Hanlon, R.T. (1999). Crypsis, conspicuousness, mimicry and polyphenism as antipredator defences of foraging octopuses on Indo-Pacific coral reefs, with a method of quantifying crypsis from video tapes. *Biological Journal of the Linnean Society, 66*(1)*,* 1-22.

Hebb, D.O. (1946). On the nature of fear. *Psychological Review, 53*(5)*,* 259-276.

Heller, K. (1996). Unusual abdomino-alary, defensive stridulatory mechanism in the bushcricket *Pantecphylus cerambycinus* (Orthoptera, Tettigonioidea, Pseudophyllidae). *Journal of Morphology, 227*(1), 81-89.

Heppner, J.B. (2008). Butterflies and moths (Lepidoptera). In J.L. Capinera, *Encyclopedia of Entomology, 2nd Edition* (pp. 630-700). New York, NY: Springer Publishing Company.

Hill, S.A. (2007). Sound generation in *Mantis religiosa* (Montodae: Mantidae): stridulatory structures and acoustic signal. *Journal of Orthoptera Research, 16*(1), 35-49.

Hodkinson, I.D., & Hughes, M.K. (1982). *Insect Herbivory.* New York, NY: Chapman and Hall.

Hollen, L.I., & Radford, A.N. (2009). The development of alarm call behaviour in mammals and birds. *Animal Behaviour, 78*(4)*,* 791-800.

Holloway, J.D. (1987). *The Moths of Borneo Part 3*. Kuala Lumpur, MY: Southen Sdn Bhd.

Hossie, T.J., Skelhorn, J., Breinholt, J.W., Kawahara, A.Y., & Sherratt, T.N. (2015). Body size affects the evolution of eyespots in caterpillars. *Proceedings of the National Academy of Sciences, 112*(21), 6664-6669.

Hossie, T., & Sherratt, T.N. (2013). Defensive posture and eyespots deter avian predators from attacking caterpillar models. *Animal Behaviour*, *86*(2), 383-389.

Hossie, T., & Sherratt, T.N. (2013). An eyespot that "blinks": an open and shut case of eye mimicry in *Eumorpha* caterpillars (Lepidoptera: Sphingidae). *Journal of Natural History, 47*(45-46), 2915-2926.

Hoy, R., Nolen, T., & Brodfuehrer, P. (1989). The neuroethology of acoustic startle and escape in flying insects. *Journal of Experimental Biology, 146,* 287-306.

Hozumi, N., & Miyatake, T. (2005). Body-size dependent difference in death-feigning behavior of adult *Callosobruchus chinensis. Journal of Insect Behavior, 18*(4), 557-566.

Hristov, N. I., & Conner, W. E. (2005). Sound strategy: acoustic aposematism in the bat-tiger moth arms race. *Naturwissenschaften, 92*(4), 164-169.

Ingalls, V. (1993). Startle and habituation responses of blue jays (*Cyanocitta cristata)* in a laboratory simulation of anti-predator defenses of *Catocala* moths (Lepidoptera: Noctuidae). *Behaviour, 126*(1/2)*,* 77-96. International Union for the Conservation of Nature and Natural Resources (IUCN). (2007). *2007 Red List*. Retrieved from: http://www.factmonster.com/ipka/A0934288.html

Janzen, D.H., Hallwachs, W., & Burns, J.M. (2010). A tropical horde of counterfeit predator eyes. *Proceedings of the National Academy of Science, 107*(26)*,* 11659-11665.

Johnson, M.L., & Zalucki, M.P. (2007). Feeding and foraging behaviour of a generalist caterpillar: are third instars just bigger versions of firsts? *Bulletin of Entomological Research, 97*(1), 81-88.

Knowles, S.C.L., Palinauskas, V., & Sheldon, B.C. (2010). Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology, 23(3),* 557-569.

Langellotto, G.A., Denno, R.F., & Ott, J.R. (2000). A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology, 81*(3), 865-875.

Lederhouse, R.C. (1990). Avoiding the Hunt: Primary Defences of Lepidopteran Caterpillars. In D.L. Evans & J.O. Schmidt (Eds.), *Insect defences: Adaptive Mechanisms and Strategies of Prey and Predators* (pp. 175-189). Albany, NY: State University of New York Press.

Lewis, E.E., & Cane, J. (1990). Stridulation as a primary anti-predator defence of a beetle. *Animal Behaviour*, *40*(5)*,* 1003-1004.

Lichter-Marck, I.E., Wylde, M., Aaron, E., Oliver, J.C., & Singer, M.S. (2015). The struggle for safety: effectiveness of caterpillar defenses against bird predation. *Oikos*(124), 525-533.

Lind, J., & Cresswell, W. (2005). Determining the fitness consequences of antipredation behavior. *Behavioural Ecology, 16*(5), 945-956.

Maldonado, H. (1970). The deimatic reaction in the praying mantis *Stagmatoptera biocellata*. *Zeitschrift für Physiologische*, *68*(1)*,* 60-71.

Mänd, T., Tammaru, T., & Mappes, J. (2007). Size dependent predation risk in cryptic and conspicuous insects. *Evolutionary Ecology*, *21*(5), 485-498.

Marden, J.H., & Chai, P. (1991). Aerial predation and butterfly design: how palatability, mimicry, and the need for evasive flight constrain mass allocation. *American Naturalist, 138*(1), 15-36.

Marler, P. (1955). Characteristics of some animal calls. *Nature, 176*, 6-8.

Masters, W.M. (1979). Insect disturbance stridulation: Its defensive role. *Behavioural Ecology and Sociobiology, 5*(2)*,* 187-200.

Masters, W.M. (1980). Insect disturbance stridulation: Characterization of airborne and vibrational components of the sound. *Journal of Comparative Physiology A, 135*(3)*,* 259-268.

Matthews, R.W., Matthews, J.R. (2010). *Insect Behaviour, Second Edition.* New York: NY: Springer.

McClure, M., & Despland, E. (2011). Defensive responses by a social caterpillar are tailored to different predators and change with larval instar and group size. *Naturwissenschaften, 98*, 425-434.

McEwen, B.S., & Sapolsky, R.M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology, 5*(3), 205-216.

Mohl, B., & Miller, L.A. (1976). Ultrasonic clicks produced by the peacock butterfly: A possible bat-repellent mechanism. *Journal of Experimental Biology, 64*(3)*,* 639-644.

Mondor, E.B., Tremblay, M.N., Awmack, C.S., & Lindroth, R.L. (2004). Divergent pheromone- mediated insect behaviour under global atmospheric change. *Global Change Biology, 10*(10)*,* 1820-1824.

Morales, M.A., Barone, J.L. & Henry, C.S. (2008). Acoustic alarm signally facilitates predator protection of treehoppers by mutualist ant bodyguards. *Proceedings of the Royal Society B, 275*(1645), 1935-1941.

Morris, D. (1956). The feather postures of birds and the problems of the origin of social signals. *Behaviour, 9*(2/3)*,* 75-113.

Morris, G.K., DeLuca, P.A., Norton, M., & Mason, A.C. (2001). Calling-song function in male haglids (Orthoptera: Haglidae, *Cyphoderris*). *NRC Research Press*. Retrieved from http://cjz.nrc.ca

Miller, L.A. & Surlykke, A. (2001). How some insects detect and avoid being eaten by bats: Tactics and countertactics of prey and predator. *Bioscience, 51*(7), 570-581.

Motulsky, H., & Christopoulos, A. (2004). *Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting*. New York, NY: Oxford University Press, Inc.

Nashida, R. (2002). Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology, 47*(1), 57-92.

Nijhout, H.F., & Wheeler, D.E. (1982). Juvenile hormone and the physiological basis of insect polymorphisms. *The Quarterly Review of Biology, 57*(2), 109-133.

Nijhout, H.F. (2003). Development and evolution of adaptive polyphenism. *Evolution & Development, 5*(1), 9-18.

Nijhout, H.F. (2003). The control of body size in insects. *Developmental Biology, 261*(1)*,* 1-9.

Olofsson, M., Jakobsson, S., Wiklund, C. (2012a). Auditory defence in the peacock butterfly (*Inachis io*) against mice (*Apodemus flavicollis* and *A. sylvatiscus*). *Behavioral Ecology and Sociobiology, 66*, 209-215.

Olofsson, M., Eriksson, S., Jakobsson, S., & Wiklund, C. (2012b). Deimatic display in the European swallowtail butterfly as a secondary defence against attacks from great tits. *PLoS ONE, 7,* e47092.

Olson, H.F. (1972). The measurement of loudness. *Audio*, 18-22.

Packard, A.S. (1904). Sound production by a Japanese Saturnian caterpillar. *Journal of the New York Entomological Society, 12*, 92-93.

Peig, J., & Green, A.J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos, 118*(12), 1883-1891.

Pilz, P.K.D., & Schnitzler, H.U. (1996). Habituation and sensitization of the acoustic startle response in rats: Amplitude, threshold, and latency measures. *Neurobiology of Learning and Memory, 66*(1), 67-79.

Pittaway, A.R. (1993). *Hawkmoths of the Western Palaearctic.* London, UK: Harley Books.

Pomeroy, H., & Heppner, F. (1977). Laboratory determination of startle reaction time of the starling (*Sturnus vulgaris*). *Animal Behaviour, 25*(3)*,* 720-725.

Pressier, E.L., Bolnick, D. I., & Benard, M.F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology, 86*(2), 501-509.

Prestwich, K.N. (1994). The energetics of acoustic signaling in anurans and insects. *American Zoologist, 34*(1), 625-643.

Prudic, K.L., Oliver, J.C., & Sperling, F.A.H. (2007). The signal environment is more important than diet or chemical specialization in the evolution of warning colouration. *Proceedings of the National Academy of Sciences of the United States of America, 104*(49), 19381-19386.

Randall, J.A., & Stevens, C.M. (1987). Foot drumming and other anti-predator responses in the bannertail kangaroo rat (*Dipodomys spectabilis). Behavioural Ecology and Sociobiology, 20*(3), 187-194

Ratcliffe, J.M. & Fullard, J.H. (2005). The adaptive function of tiger moth clicks against echolocating bats: an experimental and synthetic approach. *Journal of Experimental Biology, 208*, 4689-4698.

Reader, T., Hochuli, D. (2003). Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology, 28*(6)*,*729-737.

Reinhold, K. (2010). Variation in acoustic signally traits exhibits footprints of sexual selection. *Evolution, 65*(3), 738-745.

Relyea, R.A. (2005). Constraints on inducible defenses: Phylogeny, ontogeny, and phenotypic trade-offs. In P. Barbosa & I. Castellanos (Eds.), *Ecology of Predator-Prey Interactions* (pp. 189-207). New York, NY: Oxford University Press.

Remmel, T., & Tammaru, T. (2009). Size-dependent predation risk in tree-feeding insects with different colouration strategies: a field experiment. *Journal of Animal Ecology, 78*(5), 973- 980.

Robertson, R.R. (1973). Optimal niche space of the red-winged blackbird. III. Growth rate and food of nestlings in marsh and upland habitat. *Wilson Bulletin, 85*(2)*,* 209-222.

Romer, H. (1993). Environmental and biological constraints for the evolution of long-range signaling and hearing in acoustic insects. *Philosophical Transactions of the Royal Society B, 340(*1292).

Rosi-Denadi, C.A., & Yack, J.E. (2015). Vocalization: A unique mechanism for sound production in insects. *15th International Meeting on Invertebrate Sound & Vibration XV, Ottawa, July 13-17 2015.* Ottawa, ON: Carleton University.

Rowe, C., & Guildford, T. (1999). The evolution of multimodal warning displays. *Evolutionary Ecology, 13*, 655-671.

Rowe, C., & Halpin, C. (2013). Why are warning displays multimodal? *Behavioural Ecology and Sociobiology, 67*(9), 1425-1439.

Ruxton, G.D., & Neuhauser, M. (2010). When should we use one-tailed hypothesis testing? *Methods in Ecology and Evolution, 1*(2), 114-117.

Ruxton, G.D., Sherratt, T.N., & Speed, M.P. (2004). *Avoiding attack: The evolutionary ecology of crypsis, warning signals and mimicry*. New York, NY: Oxford University Press.

Ryan, M.J. (1986). Factors influencing the evolution of acoustic communication: biological constraints. *Brain, behaviour, and evolution, 28*(1-3), 70-82.

Ryan, T.P. (2013). *Sample Size Determination and Power.* New Jersey, NY: John Wiley & Sons, Inc.

Sanborn, A., & Phillips, P.K. (1995). Scaling of sound pressure level and body size in cicadas (Homoptera: Cicadidae; Tibicinidae). *Annals of the Entomological Society of America, 88*(4), 479-484.

Sandow, J.D., & Bailey, W.J. (1978). An experimental study of defensive stridulation in *Mygalopsis ferruginea* Redtenbacher (Orthoptera: Tettigoniidae). *Animal Behaviour, 26,* 1004-1011.

Sandre, S., Tammaru, T., Esperk, T., Julkunen-Titto, R., & Mappes, J. (2007). Carotenoid-based colour polyphenism in a moth species: search for fitness correlates. *Entomologia Experimentalis et Applicata, 124*(3), 269-277.

Sargent, T.D. (1990). Startle as an anti-predator mechanism, with special reference to the underwing moths, (*Catocala*). In D.L. Evans & J.O. Schmidt (Eds.), *Insect defences: Adaptive mechanisms and strategies of prey and predators* (pp. 229-238). New York, NY: State University of New York Press.

Sarkar, A. (2003). *Development of animal behaviour.* Darya Ganj, IN: Discovery Publishing House.

Schlenoff, D.H. (1985). The startle responses of blue jays to *Catocala* (Lepidoptera: Noctuidae) prey models. *Animal Behaviour, 33*(4)*,* 1057-1067.

Schowalter, T.D. (2000). *Insect Ecology: An Ecosystem Approach*. Sand Diego, CA: Academic Press.

Sherratt, T.N. & Wilkinson, D.M. (2002). *Big Questions in Ecology and Evolution.* New York, NY: Oxford University Press.

Shettleworth, S.J. (1989). Animals foraging in the lab: Problems and promises. *Journal of Experimental Psychology: Animal Learning and Cognition, 15*(1), 81-87.

Singer, J.D. (2002). Fitting individual growth models using SAS PROC MIXED. In D.S. Moskowitz & S.L. Hershberger, *Modeling Intraindividual Variability with Repeated Measures Data: Methods and Applications* (pp. 134-170). New Jersey, NY: Lawrence Erlbaum Associates, Inc.

Skelhorn, J., & Ruxton, G.D. (2008). Ecological factors influencing the evolution of insects' chemical defenses. *Behavioural Ecology*, *19*(1), 146-153).

Skelhorn, J., Rowland, H.M., Speed, M.P. & Ruxton, G.D. (2010). Masquerade: Camouflage without crypsis. *Science, 327*(1), 51.

Skelhorn, J.K., Holmes, G.G., & Rowe, C. (2016). Deimatic or aposematic? *Animal Behaviour, 113,* e1-e3.

Slansky, F., & Scriber, J.M. (1985). Food consumption and utilization. In G.A. Kerkut (Ed.) *Regulation: Digestion, Nutrition, Excretion* (pp. 88-151). Willowdale, ON: Pergamon Press Canada Ltd.

Siddall, E.C., & Marples, N.M. (2011). Hear no evil: The effect of auditory warning signals on avian innate avoidance, learned avoidance and memory. *Current Zoology, 57*(2), 197-207.

Smee, D. (2010). Environmental context influences the outcomes of predator-prey interactions and degree of top-down control. *Nature Education Knowledge, 3*(10), 58.

Smith, R.L., & Langley, W.M. (1978). Cicada stress sound: An assay of its effectiveness as a predator defence mechanism. *Southwestern Naturalist 23*(2), 187-196.

Speed, M.P., Ruxton, G.D. (2005). Aposematism: what should our starting point be? *Proceedings of the Royal Society B, 272*(1561), 431-438.

Srygley, R.B., & Chai, P. (1990). Flight morphology of Neotropical butterflies: palatability and distribution of mass to the thorax and abdomen. *Oecologia, 84*(4), 491-499.

Stamp, N.E. (1986). Physical constraints of defense and response to invertebrate predators by pipe vine caterpillars (*Battus philenor*: papilionidae). *Journal of Lepidopterists' Society, 40*(3), 191-205.

Stamp, N.E., & Casey, T.M. (1993). *Caterpillars: ecological and evolutionary constraints on foraging.* London, UK: Chapman & Hall.

Stearn, S. (1992). *The evolution of life histories.* London, UK: Oxford University Press.

Stevens, M., & Merilaita, S. (2011). *Animal Camouflage: Function and mechanisms*. New York: NY: Cambridge University Press.

Sugiura, S., & Yamazaki, K. (2006). The role of silk threads as lifelines for caterpillars: pattern and significance of lifeline-climbing behaviour. *Journal of Economic Entomology, 31*(1), 52-57.

Thomas, J.A., & Jalili, M.S. (2004). Echolocation in insectivores and rodents. In J.A. Thomas, C.F. & M. Vater (Eds.), *Echolocation in Bats and Dolphins* (pp. 547-564). Chicago, IL: The University of Chicago Press.

Tinbergen, L. (1960). *The Dynamics of Insect and Bird Populations in Pine Woods.* Leiden, NL: E.J. Brill.

Tinbergen, L. (1960). The natural control of insects in pine woods I. Factors influencing the intensity of predation by songbirds. *Archives Néerlandaises de Zoologie 13*, 265-343.

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift fur tierpsychologie, 20*, 410-433.

Tseng, M., & O'Connor, M.I. (2015). Predators modify the evolutionary response of prey to temperature change. *Biology Letters, 11*(12), 20150798.

Tuskes, P.M., Tuttle, J.P., & Collings, M.M. (1996). *The Wild Silk Moths of North America: A natural history of the Saturniidae of the United States and Canada.* New York, NY: Cornell University Press.

Ullyett, H. (1865). Reports of the societies. *The Naturalist, 124*(1028), 285-287.

Umbers, K.D.L., Lehtonen, J. & Mappes, J. (2015). Deimatic displays. *Current Biology*, *25*(2), R58-59.

Umbers, K.D.L., & Mappes, J. (2016). Towards a tractable working hypothesis for deimatic displays. *Animal Behaviour, 113,* e5-e7.

Vanderhoff, E.N., & Eason, P.K. (2009). American robin seet calls: Aerial alarm or contact call? *Wilson Journal of Ornithology, 121*(2), 406-411.

van Nieukerken, E.J., Kaila, L., Kitching, I.J., Kristensen, N.P., Lees, D.C. et al., (2011). Order Lepidoptera. In Z.D. Zhang (Ed.) *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness* (pp. 212-221). Auckland, NZ: Magnolia Press.

Vermeulen, B., De Backer, P., & Remon, J.P. (2002). Drug administration policy to poultry. *Advanced Drug Delivery Reviews, 54*(6), 795-803.

Wagner, D.L., Schweitzer, D.F., Bolling Sullivan, J., & Reardon, R.C. (2011). *Owlet Caterpillars of Eastern North America.* Woodstock, UK: Princeton University Press.

Walters, E.T., Illich, P.A., Weeks, J.C., & Lewin, M.R. (2001). Defensive responses of larval *Manduca sexta* and their sensitization by noxious stimuli in the laboratory and field. *Journal of Experimental Biology, 204*(Pt 3), 457-469.

Wicksten, M.K. (1990). Adaptive coloration in invertebrates. *Proceedings of a symposium sponsored by the American Society of Zoologist.* University College Station: Texas A&M Sea Grant College Program.

Wilson, J.M., & Barclay, R.M. (2006). Consumption of caterpillars by bats during an outbreak of western spruce budworm. *The American Midland Naturalist, 155*(1), 244-249.

Wright, A.B. (1993). *Caterpillars of North America.* New York, NY: Houghton Mifflin Company.

Young, C.A., Bura, V.L., & Yack, J.E. (2015). Insect defense sounds: Do difference signals characteristics convey different messages? Poster session presented at the Invertebrate Sound & Vibration XV International Meeting, Ottawa, ON.

Zalucki, M.P, Clarke, A.R., & Malcolm, S.B. (2002). Ecology and behaviour of first instar larval Lepidoptera. *Annual Review in Entomology, 47*, 361-393.

Zelditch, M.L., Swiderski, D.L., & Sheets, H.D. (2012). *Geometric morphometrics for biologists: A primer (second edition).* Waltham, MA: Elsevier Inc.

Zera, A.J., & Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Anthony Zera Publications, 15.* Retrieved from http://digitalcommons.unl.edu/bioscizera/15