Efficacy of the European Earwig (*Forficula auricularia*) as a Generalist Biocontrol Agent

by

Dennis Quach

B.Sc., Simon Fraser University, 2014

Thesis in Partial Fulfillment of the Requirements for the Degree of Master of Pest Management

in the Department of Biological Sciences Faculty of Science

© Dennis Quach 2019

SIMON FRASER UNIVERSITY Fall 2019

Copyright in this work rests with the author. Please ensure that any reproduction or re-use is done in accordance with the relevant national copyright legislation.
Approval

Name: Dennis Quach
Degree: Master of Pest Management
Title: Efficacy of the European Earwig (Forficula auricularia) as a Generalist Bicontrol Agent

Examining Committee: Chair: Leithen M’Gonigle
Assistant Professor

Jenny S. Cory
Senior Supervisor
Professor

Margo M. Moore
Supervisor
Professor Emerita

Tamara Richardson
Supervisor
Principal Investigator
Cornucopia Crop Consulting

Paul Abram
External Examiner
Research Scientist
Biological Control of Insect Pests
Agriculture and Agri-Food Canada

Date Defended/Approved: December 13, 2019
Abstract

The European Earwig (*Forficula auricularia*) has been the subject of scientific curiosity and public disdain since its introduction to North America due to its controversial status as both a natural enemy of agricultural pests, and as a nuisance cohabitant of human dwellings. I aim to investigate the feasibility of utilizing the earwig as a biocontrol agent against target pests of organic apple orchards, as well as its efficacy as a generalist predator in the context of agricultural ecosystems. Through DNA gut-content analysis, and cross-seasonal field observations, I was able to confirm that earwigs are consuming apple orchard pests under natural conditions. These findings are corroborated upon further analysis of field data which show a negative association between earwig abundance and multiple species of pest prevalence at tree-level occupancy across the field season. I examine predation efficacy and consumptive thresholds of the earwig in the context of generalist predator traits through temperature controlled functional response laboratory experiments for two recognized apple pest species, the rosy apple aphid (*Dysaphis plantaginea*), and the oblique-banded leafroller (*Choristoneura rosaceana*). Earwig predation was independently affected by density and temperature, but no interaction effect was observed. Analysis of the data did not accurately describe a type II functional response relationship, showing the limitations of traditional predator-models for describing predation behaviour of generalists in biocontrol practice. The preponderance of evidence outlined in this thesis provides promising evidence for utilizing European earwigs in conservation biocontrol, elucidates their role as key predators in agroecosystems, as well as reconsiders how to approach the study of generalist predators in biocontrol research and traditional predator-prey models.

**Keywords:** *Forficula auricularia*; biological control; generalist; predator-prey models; functional response; PCR; gut content analysis; *Choristoneura rosaceana*; *Dysaphis plantaginea*; temperature; logistic regression
I dedicate this to all the teachers and mentors of my past and present. This is to all the professors, scientists, educators, and philosophers - who opened my heart and mind, and shaped me into the person I am; the kind of person who would be writing this today. Thank you.
Acknowledgements

It would be to state the obvious to say that I would not have been able to pursue and complete this thesis without the hard work, dedication, generosity, and patience of all the people involved in this project.

I would like to first thank my senior supervisor, Dr. Jenny S. Cory, for her invaluable guidance and support throughout my project. Not only has she been a dedicated scientific advisor and mentor, she has also been an invaluable source of moral and emotional support. As much as I admire Jenny as an intellectual, academic and scientist, she has also been a thoughtful, caring, and charming person to work with. I am forever grateful for her mentorship and will truly miss being her student.

I would also like to thank my committee members, Tamara Richardson, and Margo Moore for their feedback, trust, and support throughout the various stages of my project. Thank you, Tamara, for the invaluable work and dedication with regards to the field data and collections, and to your team at Cornucopia Crop Consulting, including Nicole Tunbridge for her hard work and expertise, and the farmers in Cawston for use of their orchards. This thesis would not have been possible without you all. Thank you to Margo Moore and her lab, including Cassandra Carroll, and Alison Hadwin, for their help and guidance with the PCR protocol, and training in molecular techniques.

Thank you to all the Cory lab members for their support and friendship: Paul MacDonald, Joyce Leung, Pauline Deschodt, Kari Zurowski, Kevin Colmenares-Di Maria, Leon Li, Heather Coatsworth, and Sean McCann.

Thank you to Leslie Sanders, Regine Gries, Adam Blake, and Warren Wong for their support with rearing facilities, lending of equipment, and photography services.

Special thanks to all my undergraduate student volunteers who made it possible to rear the nigh-innumerable amount of the insects required for all my laboratory experiments: Elizabeth Guinto, Alex Nott, Danny Yu, Nathan Schenkeveld, Matthew Takeuchi, Mia Misic, Nathaniel Tok, Rachel Wilson, and Amy Ly.
This research was made possible by funding and sponsors for the IPS NSERC Grant, the Sharon Clements Biological Sciences Award, and the Dr. John Yorston Memorial Graduate Scholarship.
Table of Contents

Approval ............................................................................................................................. ii
Abstract ................................................................................................................................ iii
Dedication .......................................................................................................................... iv
Acknowledgements ............................................................................................................. v
Table of Contents .............................................................................................................. vii
List of Tables ..................................................................................................................... ix
List of Figures ..................................................................................................................... xi
List of Acronyms ............................................................................................................... xiii
Preface: An Earwig Poem ................................................................................................ xiv

Chapter 1. Background Introduction ............................................................................. 1
1.1. Biological Control ..................................................................................................... 1
1.2. Generalist Predators ............................................................................................... 2
1.3. The European Earwig (Forficula auricularia) .......................................................... 3
   1.3.1. Taxonomy and Physical Characteristics ............................................................. 4
   1.3.2. Social Behaviour and Parental Care .................................................................. 5
   1.3.3. Diet and Feeding Behaviour .............................................................................. 7
1.4. Thesis Objectives ..................................................................................................... 7
1.5. Figures and Tables ................................................................................................... 9

Chapter 2. Are European earwigs effective predators of apple orchard pests? .... 11
2.1. Introduction ............................................................................................................. 11
2.1.1. Pest Management in Organic Apple Orchards .................................................... 11
2.1.2. Apple Orchard Pests .......................................................................................... 12
2.1.3. Biological control ............................................................................................... 14
2.1.4. Generalist Predators .......................................................................................... 16
2.1.5. Earwigs ............................................................................................................. 17
2.1.6. Objectives of this study ..................................................................................... 18
2.2. Methods .................................................................................................................. 19
2.2.1. Field Surveys ..................................................................................................... 19
2.2.2. Gut Content Analysis ....................................................................................... 21
2.2.3. Rearing protocol ............................................................................................... 22
2.2.4. Statistical Analyses ........................................................................................... 23
2.3. Results ..................................................................................................................... 24
2.3.1. Field Surveys ................................................................................................... 24
2.3.2. Earwig Abundance and Prevalence .................................................................. 25
2.3.3. Half-life of detection ......................................................................................... 26
2.3.4. PCR screening of field-collected earwigs ......................................................... 26
2.3.5. Effect of earwigs on pest prevalence ............................................................... 27
2.3.6. Logistic regression ................................................................. 27
2.4. Discussion ......................................................................................... 28
  2.4.1. Seasonal prevalence of earwigs and pests ................................. 28
  2.4.2. DNA gut content analyses .............................................................. 28
  2.4.3. Half-life of detection and reliability of detection ......................... 31
  2.4.4. Are earwigs having a measurable effect on pest prevalence in the field? 32
  2.4.5. Earwigs as an ecological indicator for pest prevalence? ............ 35
2.5. Figures and Tables ........................................................................... 37

Chapter 3. How does temperature affect the predation capacity of a generalist predator? .......................... 51
  3.1. Introduction ......................................................................................... 51
    3.1.1. Earwigs as generalist predators .................................................... 51
    3.1.2. Generalists in Biocontrol ................................................................. 51
    3.1.3. Functional Response and Temperature ........................................... 53
    3.1.4. Objectives of this study ................................................................. 56
  3.2. Methods ........................................................................................... 56
    3.2.1. Rearing protocol ........................................................................... 56
    3.2.2. Functional Response Experiments ............................................... 58
    3.2.3. Statistical analysis ....................................................................... 59
  3.3. Results ............................................................................................. 59
    3.3.1. Rosy Apple Aphid ....................................................................... 59
    3.3.2. Oblique-banded leafroller ............................................................. 60
  3.4. Discussion ......................................................................................... 60
    3.4.1. Temperature and Density on Predation ........................................ 60
    3.4.2. Generalist predator traits ............................................................... 63
    3.4.3. Implications for biocontrol ......................................................... 64
  3.5. Figures and Tables ........................................................................... 66

Chapter 4. Concluding Remarks ................................................................. 70

References ............................................................................................ 72
List of Tables

Table 1-1. Summary descriptions of the three major categories where biological control as been successfully implemented in pest management; importation biocontrol, augmentation biocontrol (which can be sub-categorized into inoculative and inundative biocontrol), and conservation biocontrol. ................................................................. 10

Table 2-1. Aphid-specific and Lepidoptera-specific PCR primers and target DNA species tested for specificity against a background of earwig DNA. Aphid primer sequences were obtained from Romeu-Dalmau et al. (2012), and Lepidoptera primer sequences were obtained from Herbert et al. (2004). 37

Table 2-2. Summary of total number of individual earwigs testing positive for either aphid or lepidopteran DNA via PCR screening in 2015 and 2016............ 45

Table 2-3. Pairwise correlations, using unstructured REML multivariate factor analysis of mean number of earwigs on proportion of trees occupied by target pest groups (pooled data for both field seasons)....................... 45

Table 2-4. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (A = At least 1 or more Aphid species, B = Rosy Apple Aphid) as a function of number of earwigs present. The logistic curves are fitted using maximum likelihood ratio tests. Y axis shows the probability of the outcomes (1= positive for occupancy, 0 = negative for occupancy). The total population of points are representative of the distribution of data points that determined the fitted curve. NS = non-significance, * = significant effect of # earwigs on probability of specified pest occupancy ................................................................................................................... 46

Table 2-5. Mosaic plots for logistic regression analyses on the predicted probabilistic outcomes of a tree being occupied by a pest category (C = Green Apple Aphid, D = Apple Grain Aphid, E = Woolly Apple Aphid) ............... 47

Table 2-6. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (F = At least 1 or more Lepidoptera Species, G = Apple Clearwing Moth) as a function of number of earwigs present. The logistic curves are fitted using maximum likelihood ratio tests. Y axis shows the probability of the outcomes (1= positive for occupancy, 0 = negative for occupancy). The total population of points are representative of the distribution of data points that determined the fitted curve. Parameter estimates of effects tests are found in Table 4. NS = non-significance, * = significant effect of # earwigs on probability of specified pest occupancy ................................................................................................................... 48

Table 2-7. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (H = Eye-spotted Budmoth, I = Leafroller spp.) .................. 49
Table 2-8. Logistic regression analysis parameters, using a maximum likelihood ratio test on predicted probability of pest occupancy as a function of increasing number of earwigs per tree. Where β is defined as the parameter estimate of the effect of number of earwigs on pest occupancy. Number of observations analyzed for each pest group were determined from date of first detection until 1 collection date after seasonal disappearance ........ 50

Table 3-1. Parameter estimates obtained from fitted type II Holling equations for rosy apple aphid *Dysaphis plantaginea* and oblique-banded leafroller (*Choristoneura rosaceana*) (N = 48 per group for RAA, N = 120 for OBLR) T= time = 1 day. Parameter estimates that crossed above 1 for ‘a’ or below 0 for ‘h’ within the SE were omitted. Upper asymptote = maximum % prey consumed in 1 day. Search rate = proportion of 1 day to encounter 1 prey item. ................................................................. 66

Table 3-2. Results of the effects of density broken down by temperature on proportion of oblique-banded leafroller (2nd instar) (OBLR), and rosy apple aphid (RAA) consumed over time (24 h) by earwigs using a generalized linear model (binomial distribution) using a logit function. (N = 120 observations for OBLR and N= 48 per group for RAA)............... 66

Table 3-3. Factor effect of temperature, density, and interaction effect on proportion of oblique-banded leafroller (2nd instar) (OBLR), and rosy apple aphid (RAA) consumed over time (24 h) by earwigs using a generalized linear model (binomial distribution) using a logit function. (N = 600 observations for OBLR and N= 240 for RAA)........................................... 67
List of Figures

Figure 1-1. Egg mass of a female European earwig (*Forficula auricularia*) under laboratory rearing conditions. Eggs are approximately 1mm in length, with females being able to lay as many as 30-60 eggs per cluster. .......... 9

Figure 1-2. An adult female European earwig, foraging underneath a leaf occupied by Rosy Apple Aphid (*Dysaphis plantaginea*). .................................................. 9

Figure 2-1. Mean proportion of trees occupied by different aphid species in 2015 (left) and 2016 (right). (N = 30 trees per time point in 2015; N = 30-50 trees per time point 2016) ........................................................................................ 38

Figure 2-2. Mean proportion of trees occupied by different Lepidoptera species in 2015 (left) and 2016 (right). (N = 30 trees per time point in 2015; N = 30-50 trees per time point in 2016). ............................................................... 39

Figure 2-3. Earwig density (mean number of earwigs per tree) across the field season for 2015 (left) and 2016 (right). Error bars correspond to standard error of mean number of earwigs sampled per date across 3 field sites. Total N = 626 trees. Red dashed line represents theoretical maximum limit on mean number of earwigs collected per date, as maximum number of earwigs collected for gut content analysis was capped off at 50 earwigs per site, per date. ..................................................................................................... 40

Figure 2-4. Mean proportion of trees occupied by earwigs (green), aphids (red), or both earwigs + aphids on the same tree (purple dotted-line) across the two field seasons. (N = 30 trees per time point for 2015; N=30-50 trees per time point for 2016) .................................................................................. 41

Figure 2-5. Mean proportion of trees occupied by earwigs (blue), Lepidoptera (red), or both earwigs + Lepidoptera on the same tree (purple dotted-line) across the two field seasons. (N = 30 trees per time point for 2015; N=30-50 trees per time point for 2016) ............................................................................ 42

Figure 2-6. Half-life of detection results, shows mean proportion of earwigs testing positive for the Aphid DNA (A), and Lepidoptera DNA (B) at 0, 8, 24, 42, and 72 hours post consumption of Rosy Apple Aphid (Aphid), or Oblique-banded-leafroller (Lepidoptera), using COI mitochondrial aphid and lepidoptera-specific DNA primers. (n = 30, per treatment group, per time point) ......................................................................................................... 43

Figure 2-7. Total number of individual earwigs testing positive for Aphids (red) or Lepidoptera (green) across the two field seasons. The stack bar graphs show the proportion of total tested relative to the number of individuals that test negative (grey). ..................................................................................................... 44

Figure 3-1. Functional responses of *Forficula auricularia* adults at five densities of *Dysaphis plantaginea*. The type II Holling disc equation was fitted separately for each temperature (n = 48) for all temperature treatments. RMSE values are represented as units of dependent variable (proportion of total prey items consumed over time period) ............................................ 68
Figure 3-2. Functional responses of *Forficula auricularia* adults at five densities of *Choristoneura rosaceana*. The type II Holling disc equation was fitted separately for each temperature (n = 48) for all temperature treatments. RMSE values are represented as units of dependent variable (proportion of total prey items consumed over time period).
List of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEW</td>
<td>European Earwig (<em>Forficula auricularia</em>)</td>
</tr>
<tr>
<td>RAA</td>
<td>Rosy Apple Aphid (<em>Dysaphis plantaginea</em>)</td>
</tr>
<tr>
<td>OBLR</td>
<td>Oblique-banded leafroller (<em>Choristoneura rosaceana</em>)</td>
</tr>
<tr>
<td>COI</td>
<td>Cytochrome Oxidase I</td>
</tr>
<tr>
<td>AAFC</td>
<td>Agriculture Agri-Food Canada</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
</tbody>
</table>
Preface: An Earwig Poem

Crawling, chewing, climbing, the earwig goes
under the canopy, beneath the hedgerows.

He hides in the dampness and darkness nearby,
appearing quite fierce, but perhaps just shy?

With pincers, tough armor, and scaly brown feet,
he comes out at night, to hunt and to eat.

But what does he eat?
Is his appetite small or big?

What is the preferred food,
of the enigmatic earwig?
Can he eat human brains?

Does he climb into ears?
Is this legend, or fact?

Or irrational fear?

Is he friend? Is he foe?

Is he bad? Is he good?

Is he really a pest?

Or just misunderstood?

To answer these questions, there is one thing you can do.

You must ask the earwig, yourself,
to find out what is true.

The earwig has a story
for all who would hear.

Are you willing to listen?
Will you lend him your ear?
Chapter 1.

Background Introduction

1.1. Biological Control

Biological control (or biocontrol) can be defined as the importation, augmentation, or conservation of natural enemies in order to manage a pest population (Clausen 1978; Rusch et al., 2010). Pest suppression and regulation via natural enemies, whether natural or human-mediated, has been recognized as one of the most valuable ecosystem services to humans, with estimations citing a value of upwards of 400 billion dollars (US) annually worldwide (Costanza et al., 1997; Rusch et al., 2010). However, the traditional way of measuring the efficacy of long-term biocontrol depends on the ability to reduce pest population densities down to a lower equilibrium such that the damage they can cause will be below a specified economic threshold (Clausen, 1978; Luck, 1990; Symondson et al., 2002).

Biological control can be broadly described into three categories; importation, augmentation, and conservation (for summary, see Table 1-1). In practice, the type of biocontrol strategy chosen and tactics utilized will depend on ecological context of the pest system as well as the goals and targets for pest management. Importation (or classical) biocontrol refers to the importation of natural enemies of non-native pests from their native range in order to suppress pest populations (Clausen, 1978; Luck, 1990; Rusch et al., 2010). The first modern historical example of successful importation biocontrol occurred in 1889, where the United States Department of Agriculture (USDA) utilized the predaceous vedalia lady beetle, Rodolia cardinalis to control a pervasive citrus orchard pest, the cottony-cushion scale, Icerya purchasi. (Hajek, 2004; Rusche et al., 2010). Since then, the field of biological has been adopted into many integrated pest management programs and has been employed in a wide array of ecological systems such as greenhouse systems, forestry, agriculture, urban, and semi-natural habitats (Barbosa, 1993; Crowder & Harwood, 2014; Hajek, 2004; Hoy, 1994; Gurr et al., 2000). The
strategies, techniques, and methodology employed in biocontrol research, development, and implementation vary greatly across applications and fields.

The criterion by which we can reliably predict what makes an effective biocontrol agent is heavily context dependent and remains an open question and will be discussed in further detail in Chapters 2 and 3.

1.2. Generalist Predators

The terms “generalist” and “specialist” have been typically used to describe a species’ ecological niche breadth across one or more dimensions (Loxdale et al. 2011; Symondson et al., 2002). However, in the literature, specialism/generalism can be generalized (as opposed to specialized) to describe a gradient in resource utilization, whereby specialists use more limited, closely related components, while generalists occupy broader, distantly related components (Dib et al., 2010; Loxdale et al. 2011). It is therefore easy to confound what we mean when we say “resource” whilst comparing the niche-breadth across multiple species. Ultimately, “resources” describe some configuration of space, time and/or energy whereby species can employ differing strategies in order to maximize their reproductive fitness. For this reason, there are a multitude of ways to measure “specialization/generalization” which can be measured across multiple dimensions and contexts. For example, a species can be a habitat generalist but be a dietary specialist at the same time (Peers et al., 2012; Symondson et al., 2002). To complicate things further, specialism/generalism terminology can be applied at different scales and may be applied differently depending on the context (eg. the level of dietary specialization can vary across, individuals, populations, or species). It should come as no surprise that discussing specialism/generalism can easily lead to misunderstanding, particularly in debate (Dennis et al., 2011; Loxdale et al., 2011), since specialism need not be sufficiently defined based on a single trait dimension. Conversely, requiring a species to be a generalist across all measurable trait dimensions in order to be categorized as one would be equally unreasonable. This notion is consistent with some evidence in the literature that show that trends across species which exhibit generalism in some traits, often have other traits associated with specialism
(Kassen, 2002; Loxdale et al. 2011). The debate between the merits and trade-offs of traits associated with generalists vs specialists has been an ongoing dispute throughout the historical use of biological control (Buxton & Madge, 1976; Dennis et al., 2011; Symondson et al., 2002) and will, to some degree, be addressed in Chapter 3.

1.3. The European Earwig (*Forficula auricularia*)

The European Earwig (*Forficula auricularia* Linnaeus) (EEW) is a species of insect from the Order Dermaptera, native to Europe, and western Asia (Clausen, 1978). Early reports of their presence in North America date back as early as 1909 (Fulton, 1924), and 1916 in British Columbia, Canada (Beall, 1932) although they are suspected to have arrived much earlier. Since then, they have been globally widespread and are currently considered among entomologists to be a cosmopolitan species and naturalized members of the ecosystems wherever they are found across North America, Europe, and Asia.

The common name “earwig” has been suggested to have originated from the Anglo-Saxon term “earwicga” which translates to “ear wiggler”, or “ear crawler” (Fulton, 1924). However, there is lack of consensus among entomological etymologists whom argue that the derivation may be a mistranslation of “ear-wing”, referring to their lobe-shaped pronotum where their wings reside.

Earwigs are mysterious creatures and have captured both the attention and acrimony of humans for as long as people have written about them. However, very little is known about the origin of the apocryphal superstition that they crawl into the ears of sleeping humans (Crumb et al., 1941). The earliest recorded account was written by James Latta in 1795:

> The creature called forficula or earwig is said to make its way into the ear, and to occasion no only deafness, but violent pain by its biting; and there is an instance on record of a woman, in whose ear a nest of these insects were lodged, and reduced her to the greatest distress.

However, there has been no scientific or medically documented account of EEWs crawling into human ears, or harming humans. Their pincer-like cerci, which humans
fear, are not capable of inflicting physical damage to humans. However, to this day, there is still ambiguity among entomologists as to what the cerci are used for (Fulton, 1924; Jacobs, 2009). There is some evidence that they use their pincers for defense against predators, although some have speculated that the prominent sexual dimorphism may suggest they can also use them for mating, or mate competition among males. (Fulton, 1924; Jacobs, 2009; Walker & Fell, 2001).

Although anecdotal stories and urban myths are still occasionally shared today, incidents where earwigs may accidentally crawl into the ears of humans are suspected to be rare – no more likely than house spiders, or other insects which may frequently co-habit with humans. The overwhelming negative perception due to their unnerving appearance, and their proclivity to hide in unexpected places has caused EEWs to be unfairly maligned and often categorized as nuisance urban pests for as long as they have cohabited with humans. To this day, the relationship between humans and earwigs has not changed much, causing them to be misunderstood among scientists and gardeners alike (Buxton & Madge, 1976; Crumb et al., 1914; Orpet et al., 2019).

1.3.1. Taxonomy and Physical Characteristics

European earwigs are taxonomically categorized into the Order Dermaptera, which translates to ‘skin wings’, referring to the leathery texture of the forewings of insects in this group. Although they had been previously categorized into Orthoptera, along with crickets and grasshoppers, due to their hemimetabolous life stages. EEWs belong to the largest family in Dermaptera, Forficulidae with over 460 species and 60 genera found worldwide, arranged into 8 subfamilies. (Hopkins, 2008)

The adults are brown to red-brown in color, although paler ventrally. They have distinct physical characteristics such as a shield-shaped pronotum, chewing mandibulate mouthparts, and a set of heavily sclerotized forcep-shaped cerci. Although adult earwigs appear to be wingless, they have two membranous hind wings beneath their abbreviated forewings. These wings are seldomly seen as they open and close very quickly on the rare occasion that they use them to take flight across short distances, although very little is known about earwig flight pattern behaviour (Lamb, 1974). Adult EEWs display
conspicuous sexual dimorphism, with males having larger and prominently curved cerci ranging from 4-9mm in length, and females with straighter shaped cerci which are approximately 3mm in length (Figure 1-2). The second tarsal segment is lobed, extending distally below the third tarsal segment. The bead-like antenna segments of adult earwigs can vary between 11-15 segments.

EEWs undergo 4 nymphal stages. Nymphs are typically pale in color, with the body color darkening from gray-brown to dark brown as they mature. The characteristic cerci are present in all instars and grow in size with each molt. Distinguishable sexually dimorphic characteristics among nymphs are not present, as the cerci remain wire-like and flexible until the final instar.

The eggs of EEWs are often found in well-drained soil nests and are typically cream to pearly white in color and oval shaped. The size of the eggs are approximately 1mm in length and 0.85 mm in width when first deposited, but absorb water and nearly double in volume before hatching. The mean number of eggs per cluster has been reported to range from 30-60 eggs for the first cluster and for populations of earwigs with second seasonal broods, typically producing half as many eggs (Figure 1-1).

1.3.2. Social Behaviour and Parental Care

The typical shy and elusive behaviour of European Earwigs has ironically contributed to their negative reputation. Earwigs are largely nocturnal, spending most of the time during day hidden under plant debris, cracks, crevices and dark, moist locations where they can sometimes aggregate in large numbers. For this reason, humans are often displeased to find them unexpectedly near their homes and dwellings. There is a moderate level of scientific evidence that suggest this aggregation behaviour is pheromone-mediated, as hiding spots previously occupied by adults and nymphs alike tend to elicit aggregation behaviour in other earwigs (Hehar, 2007; Sauphanor, 1992).

Although they remain relatively inactive while hiding during the day, nighttime activity can be influenced by weather. There is some evidence that suggests earwigs are most active under conditions where temperature is most stable, with activity being maximized
at higher minimum temperatures, and less activity during higher maximum temperatures (Chant & McLeod, 1952). Higher relative humidity has also been shown to suppress earwig movement, whereas increased wind velocity and greater cloud cover has been shown to be positively correlated with increased earwig activity (Chant & McLeod, 1952).

Social and maternal behaviour in European earwigs is surprisingly complex and sophisticated. The timing of seasonal mating behaviour appears to be largely dependent on geographic region where the populations reside. Males and females tend to mate in late summer, and/or early autumn, where they construct subterranean nests in well-drained soil areas in which they overwinter as mated pairs. Mating behaviour can occur once or twice per season, and will depend on the length of the summer season and the geographic region. In British Columbia, EEWs are able to mate and produce eggs twice per year (Beall, 1932; Fulton, 1924; Thesing et al., 2015). At the time of oviposition, the female will become territorial and drive the male out of the nest. Maternal care and investment are high in earwigs, which is a trait that is considered relatively rare for insects. Maternal care for the eggs is meticulous, as females are known to manipulate them frequently. Earwig females will guard them and clean them to prevent growth of fungi and other pathogens, rolling them, and even relocating them into a new nest in order to provide optimal temperature and humidity for the eggs. For this reason, it can be very difficult to rear EEWs under laboratory conditions with high fidelity (Crumb et al., 1941; Thesing et al., 2015).

As the time of hatching approaches, the female will spread out the eggs into a single layer and continue to monitor them until hatching occurs. Maternal care continues after hatching, as the females guard the nymphs and provide them with food, where she will carry it to the nest or feed them via regurgitation (Lamb, 1976). Although there is no evidence that cooperative brood care exists, there is some evidence that mated females can foster un-related orphaned broods of eggs or nymphs under certain laboratory conditions (Crumb et al., 1941; Fulton, 1924; Thesing et al., 2015).
1.3.3. **Diet and Feeding Behaviour**

Earwigs are known to be polyphagous insects, feeding on a wide variety of plant and animal matter, although they are categorized as generalist predators (Beall, 1932; Crumb et al., 1941; Fulton, 1924a; Lamb, 1974). Undoubtedly this has contributed to the controversy surrounding the earwig and brings to question whether it is a beneficial insect due to its capacity for predation on garden and agricultural pests, or if this would be offset by its facultative phytophagous behaviour. It appears that in many ways, the earwig can be viewed as a true generalist, able to adapt to multiple resources when food sources are scarce. There have even been reports of earwigs consuming algae and fungi (Buxton and Madge, 1976; Crumb et al., 1941). The EEW’s versatile dietary breadth has likely contributed to its successful proliferation, allowing it to establish itself into multiple ecosystems beyond its native range.

Reports of earwigs as phytophagous pests date as far back as 1924 (Fulton, 1924), where they have been cited as being able to do significant damage to vegetables such as beans, cabbage, celery, chard, cauliflower, cucumber, lettuce, peas, potato, and rhubarb. Because they are primarily nocturnally active, they often seek shelter during daylight hours within the crevices of food crops such as the heads of cauliflower, and ears of corn, which are likely to be eaten. Unsurprisingly, this has contributed to the human disdain for the earwigs, as their food might be contaminated by earwig frass, or aggregation pheromones, which leave behind an unpleasant odor (Crumb et al., 1941; Hehar, 2007; Sauphanor, 1992). Anecdotal reports have also attributed earwigs as a consumer of softer, tender plants of various ornamental flower species like dahlia, carnations, and pinks.

1.4. **Thesis Objectives**

The overall aim of my thesis is to investigate the feasibility of utilizing the “native” generalist predator, the European earwig (*Forficula auricularia*) as a biocontrol agent for target pests in organic apple orchard pest management. My project addresses this by asking the following questions:
Chapter 2 (Field data and DNA gut content screening):

1. Are earwigs consuming the target apple orchard pests under natural agricultural conditions?

2. Is there a detectable correlation between pest prevalence and earwig abundance across the season?

Chapter 3 (Laboratory Functional Response Experiments):

1. What is the limit of their predation rate across pest species?

2. How do additional environmental factors such as temperature affect predation rate?

Throughout this inquiry, I will also visit broader topics that may be beyond the empirical scope of this thesis but are relevantly connected to this research such as the role generalist predators play in agroecosystems, the methodological limitations of predator-prey models in biocontrol research, how we ought to approach assessing desirable traits in biocontrol candidates. In doing so, I hope to exonerate the reputation of earwigs; to allow the public to view them as beneficial members of the ecosystem, with which humans ought to embrace as harmonious cohabiters in both our urban and agricultural landscape and our world.
1.5. Figures and Tables

Figure 1-1. Egg mass of a female European earwig (Forficula auricularia) under laboratory rearing conditions. Eggs are approximately 1mm in length, with females being able to lay as many as 30-60 eggs per cluster. 
Photo Credit: Tamara Richardson (2016)

Figure 1-2. An adult female European earwig, foraging underneath a leaf occupied by Rosy Apple Aphid (Dysaphis plantaginea).
Photo Credit: Warren Wong (2016)
Table 1-1. Summary descriptions of the three major categories where biological control as been successfully implemented in pest management; importation biocontrol, augmentation biocontrol (which can be sub-categoized into inoculative and inundative biocontrol), and conservation biocontrol.

<table>
<thead>
<tr>
<th>Biocontrol Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Importation (Classical) Biocontrol</strong></td>
<td>The practice of suppressing pest populations by importing natural enemies of the target pests; often from their native range. Classical (Importation) biocontrol is often used on introduced or invasive species that are causing economic or environmental damage.</td>
</tr>
<tr>
<td><strong>Augmentation Biocontrol</strong></td>
<td>The supplementation of natural enemies; often through periodic releases without the assumption or goal of long-term establishment.</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td>Introducing natural enemies (often in smaller numbers) where they can reproduce and control for the target pest</td>
</tr>
<tr>
<td><strong>Inundation</strong></td>
<td>Introducing sufficiently large numbers of natural enemies where pest control is presumed to be done by the released individuals themselves</td>
</tr>
<tr>
<td><strong>Conservation Biocontrol</strong></td>
<td>The use of endemic natural enemies for pest suppression by protecting or enhancing environmental conditions, or minimizing environmental disturbance to encourage further establishment and occupancy of the biocontrol agent</td>
</tr>
</tbody>
</table>
Chapter 2.

Are European earwigs effective predators of apple orchard pests?

2.1. Introduction

2.1.1. Pest Management in Organic Apple Orchards

In 2017, the total Canadian farm-gate value of apples produced was estimated to be over $224 million (StatsCanada, 2016). When considering value by volume, apples are a contender for the most economically important fruit crop produced in Canada. British Columbia produces approximately 30% of the total volume of apples grown in Canada, where 98% of BC apples are being produced in the Okanagan and Similkameen valleys. However, starting in 2014, the Northwest Farm Credit Service reported a record level downturn in the demand for apples in the domestic market (AAFC, 2016). Apple growers in Canada and United States have since experienced significant profit margin compression. This has been exacerbated by more stringent market requirements as consumer preferences demand for higher quality fresh produce, with higher standards for food safety, traceability, and sustainability.

For this reason, pest management in the apple orchard industry is becoming increasingly cost-restrictive, while market demand for organic crops continues to rise. It is therefore imperative that future innovations in pest management practices and strategies will need to reconcile economic growth with the necessity of sustainability, reducing reliance on conventional chemical pesticides. Furthermore, apple growers are facing a myriad of challenges in the near future. A growing preponderance of evidence has shown the rise of insecticide-resistant populations of insect pests- not only in other agricultural systems, but apples as well (Beers et al., 2016; Malagnoux et. al, 2015; Unruh et al., 2016). As growers transition to higher density orchards, pest problems can be exacerbated through interaction effects of weather, invasive species, and climate-mediated pest outbreaks (AAFC, 2016; Hill et al., 2019). With finite resources to be allocated to deal with the
growing number of future challenges, this will likely narrow the gap between economic and action thresholds, putting a more restrictive limit on the frequency and desire for chemical pesticide use (AAFC, 2016; Beers et al., 2016; Romeu-Dalmau et al., 2012). Future growers and industry professionals will be required to adapt through innovation that is centered around longer-term planning, cost-effective investments, and more pragmatic, sustainable, pest management practices.

2.1.2. **Apple Orchard Pests**

Apple trees are relatively long-lived perennial agricultural crops. As the industry continues to shift towards more lucrative plantings of high density orchards and reduced chemical pesticide use, apple orchards can become a more attractive home to a large diversity of pests and natural enemies. Some of the arthropod pests of apples in Canada include: mites, tree-boring and fruit boring insects, defoliators, as well as phloem-feeding insects such as aphids that can act as disease vectors (Dixon, 1973; Piñol et al., 2009). The apple industry will also need to face the combined effects of climate change extending the ecological range of pre-existing pests, as well as increased rate of international trade which can also bring in new exotic pests (Bebber et al., 2013; Hulme, 2009). The recent establishment of the Brown Marmorated Stinkbug (*Halyomorpha halys*) in British Columbia is an example of this (Fogain & Graff, 2011). As the projected number of emergent pests continues to rise, so too will the demand for knowledge and technologies that can aid in their management at population levels.

However, species-specific pest population cycles are often asynchronous, and can be heavily influenced by numerous environmental and ecological factors, making it difficult to forecast long-term pest population dynamics and predict outbreaks (Beddington et al., 1978; Dib et al., 2010; Hassell, 1978; Tilman, 1996). On the other hand, by forgoing the use of conventional synthetic insecticides, organic apple orchards might have the advantage of increased natural enemy diversity. There is some evidence to suggest that this increase is associated with the perturbation of pest prevalence and density (Macfadyen et al. 2009; Rusch et al., 2010; Symondson et al., 2002).
Currently, there are two pest groups in particular, aphids (Hemiptera) and Lepidoptera, which cause a significant amount of economic damage in the apple orchard industry (Cross et al., 2016; Jones et al., 2016; Orpet et al., 2019). Most of the species in these groups are currently classified as moderate to high threats to apple crops by federal pest management reports in Canada (AAFC, 2016).

**Aphids (Hemiptera: Aphididae)**

Aphids are phloem feeding insects with piercing mouthparts. Although they do not typically cause direct damage to fruits, aphids are extremely prolific and will reproduce parthenogenically throughout the growing season, causing outbreaks to appear suddenly and unpredictably when environmental conditions are ideal (Dib et al., 2010; Dixon, 1973). Although aphid outbreaks seldomly result in apple tree mortality, feeding by aphids early in the season can cause severe deformation of leaves and shoots, resulting in economic damage due to stunted growth and deformation of fruit. Furthermore, aphids produce honeydew when feeding, which can facilitate growth of plant pathogens like the sooty mold, *Fumago vagaus* (Piñol et al., 2009; Romeu-Dalmau et al., 2012).

Management of aphids via biocontrol methods can be further exacerbated by their mutualistic relationship with certain species of ants, which deter natural enemies of aphids, as they utilize the sugar-rich content of the honey dew as a food source (Kaneko, 2003; Karami-jamour et al., 2018).

**Lepidoptera**

Lepidopterans are a diverse group of insects, with a wide range of feeding habits, life history traits, and reproductive cycles. For this reason, this pest group can cause significant damage to apple trees in a variety of ways. Pestiferous levels of outbreaks of various lepidopteran pests can occur at lower population densities, making it potentially difficult for pest management monitoring. For example, low numbers of tree-boring species like the Apple Clearwing Moth (*Synanthedon myopaeformis* (Borkhausen)), can cause significant mortality on younger trees, particularly after they have been newly grafted (Judd et al., 2016). Many pest species of Lepidoptera also exhibit defensive behaviour like rolling themselves in foliage, or building hibernacula, making them less
exposed to natural predators (Cossentine et al., 2010). Tortricids like the Oblique-banded leafroller (Choristoneura rosaceana (Harris)) can feed on the leaves of apple trees, which can cause moderate levels of defoliation early in the season, and direct feeding damage to the fruit later in the season (Brunner, 1999; Sanderson, 1909).

2.1.3. Biological control

As pest management technologies continue to progress, there will be an increased focus on sustainability, making biological control (biocontrol) an attractive option (Crowder & Hardwood, 2014; Macfadyen et al., 2009; Rusch et al., 2010). Classical biological control (also referred to as importation biological control) is a well known and practiced technology, involving the importation of natural enemies from the native host range of an exotic pest species. Applications of classical biological control are not limited to agricultural systems, as they have also been implemented effectively in forest, and urban pest management as well (DeBach & Rosen, 1991; Rusch et al., 2010). Over the years, numerous successful examples of effective biocontrol have been well documented in the literature, particularly in weed and arthropod biocontrol (Crowder & Harwood, 2014; DeBach, 1964; DeBach & Rosen, 1991; Macfadyen et al., 2009; Rusch et al. 2010).

However, the successful utilization of natural enemies in pest management has not merely been limited to importation strategies. Conservation, inundative, and augmentation biocontrol strategies have also been utilized with varying levels of success. (Barbosa, 1998; Gurr et al., 2000; Symondson et al., 2002). While classical biocontrol focuses on importation of non-native natural enemies to regulate or suppress economically damaging exotic pest species, conservation biocontrol aims to facilitate ecological conditions that favor pre-existing native natural enemies in the agricultural environment. This can be done through cultural practices which protect or generate natural habitats, refuge, or corridors which can facilitate or accelerate immigration of natural enemy species into the agricultural system (DeBach, 1991; Gurr et al., 2000; Rusch et al., 2010). In contrast, inundation and augmentation strategies often involve mass rearing programs of natural enemies, coupled with timed strategic releases in order
to suppress pest populations in the shorter term. Inundation and augmentation biocontrol, therefore, need not be mutually exclusive with classical biocontrol.

Further consideration of conservation biocontrol strategies into integrative pest management systems may have the pragmatic advantage of being easier to implement in the absence of the necessary restrictions associated with importation of foreign biocontrol agents (Crowder & Harwood, 2014; Louda et al., 2002). Classical biocontrol programs tend to incur longer development time, alongside higher potential risk, as importing non-native species may have unintended non-target effects in the non-native ecological range (Rusch et al., 2010; Symondson et al., 2002). Thus, more time and resources are expended on non-target testing and stringent importation and quarantine procedures are required to successfully implement classical biocontrol programs. Although this precaution is well justified, and can be worthwhile in the long-term, this can make conservation biocontrol, which aims to utilize native natural enemy species appear, not only less risky, but less costly to execute and implement.

Identifying effective conservation biocontrol candidates, however, can be challenging and difficult to demonstrate (Louda et al., 2002; McNeil et al., 2010). One reason is that these economically damaging pest outbreaks are still perceived as a problem that occurs in spite of the fact that these natural enemies already exist in their respective native range with multiple pest species present (Hokkanen & Lynch, 1995; Louda et al., 2002). However, it is difficult to interpret this in the context of the confounding factors associated with agricultural practices such as broad-spectrum insecticide use, habitat disruption, and hedge row removal; all of which are likely to negatively impact natural enemy populations which would otherwise be effective in conservation biocontrol services (Brunner 1999; Malgnoux et al., 2015; Schmidt et al., 2003). It is therefore important to properly evaluate the entirety of the ecological and environmental context in which these pest outbreaks occur when developing hypotheses on the role of particular natural enemy species in agroecosystems.

In an apple orchard system, there can be numerous factors that can play a role in insect pest population dynamics (Cross et al., 2015; Dib et al., 2010; Hill et al., 2019; Jones et
Predator-prey dynamics, in particular, can be inscrutable, as interaction effects of various abiotic and biotic factors can make it difficult to interpret field data in the context of traditional population-level models (Gurr et al., 2000; Symondson et al., 2002). It is, therefore, extremely difficult to prove the causal effects of natural enemy mediated pest suppression. However, certain conditions need to be met in the consideration of a biocontrol agent. Two of the most basic conditions can be summarized by the following:

1. The biocontrol agent must be able to consume the target pests under natural field conditions
2. The biocontrol agent must have a measurable effect on the overall pest impact in the agricultural system.

Although these two conditions are not exhaustive when it comes to predicting the potential success of a biocontrol agent, failure to meet at least one of these conditions would usually preclude a biocontrol agent from candidacy.

2.1.4. Generalist Predators

Invertebrate generalist predators are most widely used in management of greenhouse pests, where they are periodically released to suppress pest populations, but where they are not expected to survive in the long term. Labybird beetles, parasitoids and true bugs are the most commonly used groups for commercial greenhouse management and have been very successful (Chang & Kareiva, 1999; Gurr et al., 2000; Symondson et al., 2002). Other native generalist predators, such as carabid beetles and spiders, have received more attention in relation to conservation biocontrol in outdoor agriculture and forestry (Barbosa, 1998; Holland, 2001; Symondson et al., 2002). The generalist/specialist dichotomy in the biocontrol literature is often invoked when weighing the pros and cons associated with the perceived risks and benefits of using certain biocontrol agents and can be a useful way to highlight certain characteristics when considering biocontrol candidates (Beddington et al., 1978; Hokkanen & Lynch, 1995; Schmidt et al., 2003; Symondson et al. 2002). Traditionally, using specialists, like parasitoids, in insect biocontrol can be quite pragmatic, as their perceived host-specificity
implies an associated lower risk to non-target organisms (Beddington et al., 1978; DeBach, 1991). Parasitoid examples in the literature often credit their success in part due to their ability to closely track pest population cycles, as their life history and phenology are necessarily closely linked with their host (Hassell, 1978; Hoy, 1994; Kassen, 2002; Nicholson & Bailey, 1935; Tilman, 1996). However, generalists have many traits that can offer different advantages in pest management. Being polyphagous, generalists have the potential benefit to target multiple pests at once. Unlike host-specific parasitoids, generalist predator population dynamics are not entirely dependent on the availability of specific prey species, making them potentially more resilient to environmental and ecological stochasticity (Chiverton, 1986; Koss et al., 2003; Landis et al., 1997; Menalled et al., 1999). Prey population crashes, for example, may not impact them as much, as they are theoretically capable of switching to different hosts during periods of unpredictable prey availability. This might make them more likely to persist in the environment for a longer period of time after release, making them an attractive option when sustainability is a priority (Symondson et al., 2002). However, the utility of these characteristics will depend on many different factors. Obviously, using generalist predators will require more extensive non-target testing, as the risk of them doing unintended harm to non-target organisms or the environment is theoretically higher in comparison to host-specific specialists (Crowder & Harwood; 2014; Hokkanen & Lynch, 1995; Kassen, 2002; Louda et al., 2002). However, in conservation biocontrol, the focal predators are native (or naturalized) so any risk is considerably reduced (Barbosa, 1998; Hokkanen & Lynch, 1995; Symondson et al., 2002). Other factors like prey preference, and density-dependent effects like functional response and predation thresholds will likely determine whether or not a generalist predator can have a desirable effect on pest suppression.

2.1.5. Earwigs

European Earwigs (*Forficula auricularia*) (EEW) are generalist predators found commonly among natural enemy assemblages throughout agricultural ecosystems. Although native to North-Central Europe, since their accidental introduction into North America in the late 1800s, they are now considered naturalized members of
agroecosystems (Beall, 1932; Clausen, 1978; Fulton, 1942). There have also been studies conducted on EEWs in the United States, Oceania, and Europe in the context of apple, pear, and citrus orchards, providing strong evidence that they are consistently found with ubiquity in these fruit orchards (Cross et al., 2015; Dib et al., 2010; Helsen et al., 1998; Orpet et al., 2019; Romeu-Dalmau et al., 2012). This might suggest that they might play an important role as a key predator in these ecosystems, which would make European earwigs promising potential lower-risk candidates for conservation biocontrol (Barbosa 1998; Hokkanen & Lynch, 1995).

EEWs have been cited as an important natural enemy of various economically important pests such as codling moth *Cydia pomonella*, rosy apple aphid *Dysaphis plantaginea*, and oblique-banded leafroller, *Choristoneura rosaceana*. However, they have also been cited as facultative omnivores (Beall, 1932; Fulton, 1942; Orpet et al., 2019), consuming plant material in addition to prey insects. There has also been anecdotal evidence that they have the capacity to damage soft-skinned fruits, including pre-damaged fruit and even soft-skinned apple varieties. This further exacerbates their controversial status, as academics and professionals debate their net effect on humans as members of the agricultural ecosystem. Past experimental work suggests that the presence of predatory EEWs are negatively associated with abundance of aphid pests in apple orchard systems (Caroll & Hoyt, 1984; Cross et al., 2015, Dib et al., 2010; Orpet et al., 2019). However, only a sparse amount of evidence exists that can conclusively demonstrate that they are consuming the specified prey under natural conditions (Orpet et al., 2019; Unruh et al., 2016), highlighting the need to provide evidence that will allow us to further evaluate their impact beyond merely correlational data.

2.1.6. Objectives of this study

The overall aim of this study is to investigate the potential for European Earwigs to be effective biocontrol agents in the context of organic apple orchard systems. My specific research questions were:

**Q1.** Are earwigs present in the field at the same time the target pests are present during the growing season?
Q2. If they are both present at the same time, are earwigs consuming the target pests in the field?

Q3. Is there a detectable pattern of correlation between earwig abundance and pest prevalence?

2.2. Methods

2.2.1. Field Surveys

All field work and pest surveys of trees took place in three commercial organic apple orchards in Cawston, British Columbia, Canada at the following farms: Rbt1 (49°09’45”N 119°43’50”W), Jrn1 (49°09’31”N 119°44’01.95”W), Slv1 (49°11’28”N 119°43’26”W). Earwig collections took place at recurring two week intervals across two field seasons (From April 23rd – August 21st in 2015, and May 13th – August 19th in 2016). All of the farms were similar in size, and landscape features. Sampling and surveys took place in Gala apple and Ambrosia apple variety orchard blocks. No earwig collections took place from these orchards prior to the study. Climate data, including mean, minimum, and maximum temperature, were also recorded. Due to logistical reasons, it was not possible to sample at the exact time for each collection date at each farm, therefore, collections took place between early dawn and late afternoon. Earwigs were systematically collected from 10 trees per farm, per collection date, with staggered locations of sampling points where half the samples were collected from the interior of the orchard block, and the other half closer to the edge of the block. This was done in order to account for non-random distribution of the insects. In order to obtain an adequate sample size for the gut content screening, twenty trees were sampled instead of ten from the farms Slv1 and Jrn1 on July 24 – August 21 in 2015. This was repeated for all 3 farms for 2016 on May 27 and June 10 sampling dates, while 12 trees were sampled from Slv1 and 13 trees from Jrn1 farms on August 19 in 2016. No tree was sampled or surveyed more than once, and sampling blocks were staggered in order to minimize potential non-independence associated with insect movement between sampling dates.

The first collections in April and May 2015 were conducted using beating trays on the branches of the trees. This method proved to be ineffective, as it would yield low capture
rates, and increased the likelihood that developing fruit would be damaged. The collection method was therefore changed to cardboard traps which were hung approximately one meter above the ground level on each apple tree. Traps were hung one week prior to each collection date.

In 2015, earwigs were collected in situ and promptly preserved in 70% ethanol and stored on dry ice until taken to the laboratory where they were kept at -80°C until they were processed. In 2016, the earwigs were initially stored in a -10°C cooler instead of dry ice with all other aspects of the collection protocol remaining unchanged. In order to confirm that this did not affect yield or quality of the DNA, primary testing was conducted using a spectrophotometer, and agarose gel electrophoresis of PCR products obtained using universal barcoding COI mitochondrial DNA primers (Folmer et al., 1994). Up to 20 earwigs per site per collection date were screened for Lepidoptera and aphid DNA. For earlier collection dates, if less than 20 earwigs were site were collected, all of the earwigs were tested. In 2016, the number of earwigs collected was capped at 50 earwigs between June 24th – August 5th. One day prior to each earwig collection, a full tree census was conducted from the same trees to determine which pests and beneficial arthropods were co-habiting with the earwigs. Trees were surveyed visually, and presence/absence of each pest and natural enemy species was recorded. If the arthropod could not be identified taxonomically with confidence down to the species level, it was labelled as such and categorized into non-species specific groups (eg. Spider spp., Ladybird spp., etc.).

Insects surveyed included: rosy apple aphid, green apple aphid, apple grain aphid (Rhopalosiphum insertum (Walker), woolly apply aphid, apple leaf blister mite Phytophus mali (Burts), apple clearwing moth (adults), leafroller (oblique-banded and other species), eye-spotted budmoth, ant (species unspecified), apple leaf-curl midge, thrip spp., and apple mealy bug. Beneficial insects surveyed included: syrphid flies (eggs and larvae), aphid midges (Aphidoletes spp.), ladybird beetle species (adults, eggs, and larvae), and spiders.
2.2.2. Gut Content Analysis

DNA Extraction

DNA was extracted from whole earwig abdomens using QIAGEN™ DNeasy tissue kits, using the manufacturer’s protocol. Earwig abdomens were first homogenized using an autoclaved hand pestle in a centrifuge tube prior to proteinase K treatment in the lysis buffer. The whole body was used for pest insects using the same method when processing samples for primer specificity testing. Concentration and purity of the DNA were checked using a NanoDrop 2000 UV-Vis Spectrophotometer to verify by A260/A280, and A260/A230 absorption readings. Total DNA was eluted in 40µL of elution buffer AE and stored at -20°C in order to obtain sample concentrations of approximately 100-200 ng/µL.

PCR protocol

The PCR protocol and aphid-specific COI region mitochondrial DNA primers (AphF1, AphR3) followed the methods described in Romeu-Dalmau et al. (2012). Lepidoptera-specific primers (LepF1, LepR1) and protocol were obtained from Herbert et al. (2004), and Ball & Armstrong, (2006). The PCR primers used in this study were verified using Barcode of Life (BOLD) database and region-specificity of the sequences were confirmed using DNA nucleotide BLAST. Prior to the field surveys, the primers were tested to ensure specificity for their respective pest groups for both pure target DNA and in a background of earwig DNA (Table 2-1). The species chosen for the screening were species deemed to be economically significant and based on predictions on what we expected to find in the field at the chosen field sites, and thus, not necessarily exhaustive of all the possible aphid and Lepidoptera species found naturally in the field.

PCR reactions were carried out in 25µL volumes containing 1µL of resuspended DNA (approximately 150-250 ng/µL), 5 mM of dNTPs, 50 mM MgCl₂ in 10x reaction buffer, 50 ng of each primer, and 0.6U of Platinum Taq polymerase (Invitrogen Corporation, CA, USA). Aphid samples were amplified in a thermal cycler for 35 cycles at 94°C for 20s, 50°C for 30s, and 72°C for 45s. Lepidoptera samples were amplified in a thermal cycler for 35 cycles at 94°C for 20s, 55°C for 30s, and 72°C for 45s. Initial denaturation
step was carried out at 94°C for 2 minutes, and a final extension step at 72°C for 2 minutes. Pure DNA of the target pest and autoclaved DNase free distilled water was used as positive and negative controls respectively. Each extracted sample was tested independently for each pest. These samples were tested three times and considered positive if pest DNA was detected in one of them. The results were screened via DNA gel electrophoresis to determine presence/absence based on whether bands were present, using a 1,000 bp ladder.

**Half-life of Detection:**

In order to determine the likelihood of detection of pest DNA through PCR gut screening of earwigs, we determined a timing window for prey DNA decay rates in the earwig guts. Both aphid and Lepidoptera DNA detection decay rates in the earwig gut were determined via half-life of detection feeding trials. Lab colony reared male earwigs naïve to aphids were starved for 14 days at 20°C with water. Earwigs were allowed to consume up to eight 3rd instar Rosy Apple Aphid (*Dysaphis plantaginea*) or until satiation. Thirty individuals were each were killed at time = 0, 4, 24, 48 and 72 hours, for a total of 150 earwigs. This exact protocol was repeated, using up to ten 2nd instar oblique-banded leafroller larvae (*Choristoneura rosaceana*). DNA extraction of the whole earwig gut and PCR protocol were performed as outlined above.

### 2.2.3. Rearing protocol

Earwigs used for the detection of half-life trials were reared as described in Meunier et al. (2012), and Sandrin et al. (2015). During this period, earwigs were kept in 16x10x7.5 cm polyethylene food storage containers with a 5x1.5cm hole cut out, covered and taped with a no-see-um net screen to allow for air circulation. Habitat substrate inside the container was created using a 4 cm layer of plaster of Paris mixed with distilled water. A depression was created using a 29.6 mL SOLO cup, with a small, 1.5 cm diameter tunnel etched into the edge of the depression to allow earwigs to move in and out of the depression. Food and water were provided using “Kibbles’n’Bits” branded dog food in 29.6 mL SOLO cups, while another 29.6 mL SOLO cup was provided with cut dental wicks soaked with distilled water.
Rosy Apple Aphids (RAA) were lab-reared on their secondary host plant, the broadleaf plantain, *Plantago major* throughout the time of the experiment. Colonies were collected from apple trees from various apple orchard field sites in Cawston, BC. Field collected individuals were transferred onto plantain plants and allowed to establish across four weeks at growth chambers set to 20°C with 16:8 day:night cycle. The aphid colonies underwent daily inspection and removal of aphid mummies to completely rid the colonies of parasitoid wasps across two weeks. All aphids fed to the earwigs for the half-life of detection studies were 3rd instar apterous individuals from the same cohort.

Oblique-banded leafroller (OBLR) egg masses were obtained from a lab colony from the Pacific Agriculture Research Station in Summerland, BC. Colonies were set up at room temperature between 21-23°C with 16:8 day:night cycle. Leaf roller egg masses were housed in 29.6 mL SOLO™ cups and inspected daily for hatching of neonates (newborn larvae). Newly hatched individuals were placed in 59.2 mL SOLO™ cups at a density of 7-10 individuals per cup and reared for the remainder of their life cycle on artificial McNeil’s pinto bean diet (Shorey and Hale, 1965). All leafrollers used to feed earwigs for half-life of detection were from a single cohort and were haphazardly chosen at the 2nd instar, determined through head capsule size.

2.2.4. **Statistical Analyses**

We examined whether proportional tree occupancy by pest groups exhibited a correlational response to earwig density through a multivariate analysis using an unstructured REML (restricted maximum likelihood estimation) method. This statistical method was chosen in order to reduce bias in variance and covariance estimates associated with missing numerical data for earwig number, and to account for temporal non-independence associated with seasonality with the insect groups surveyed.

In order to test the potential effect of earwig density on probability of presence of a particular pest group on a tree, a series of logistic regressions were conducted. Logistic regression curves were fitted using maximum likelihood ratio tests and mosaic plots were analyzed to assess the predictive effect of mean number of earwigs on occupancy of a pest or pest group. In order to minimize seasonal effects which will violate the model
assumptions of non-independence of response variables, analyses were truncated by date for each pest group based on seasonal occurrence, and then analyzed separately by year. The overall effect on lepidoptera and aphid prevalence was also examined in this way. All statistical analyses were conducted using the statistics program JMP 13.1.0. (logistic regression was verified using glm() function, and REML estimates using lme4 package in R 3.5.3)

2.3. Results

2.3.1. Field Surveys

Aphids

Of the four ecologically prevalent aphid species across the two seasons of surveys, three of them were economically important; Rosy Apple Aphid (*Dysaphis plantaginea* (Passerini)), Green Apple Aphid (*Aphis pomi* (DeGeer)), Woolly Apple Aphid (*Erisoma lanigerum* (Hausmann)), while one of them was consistently found early in the spring, Apple Grain Aphid (*Rhopalosiphum insertum* (Walker)).

At least one aphid species was prevalent at reliable levels of detection via surveys throughout both field seasons across all sampling dates, with Woolly Apple Aphid and Rosy Apple Aphid being the two most prominent species across both years (Figure 2-1). Overall, a larger proportion of trees were occupied by aphids in 2015 relative to 2016, with Rosy Apple Aphid, Green Apple Aphid, and Apple Grain aphids being more prevalent in 2015. A very low number of trees were occupied by Green Apple Aphid and Apple Grain Aphid (less than 5-10% of trees at their highest levels of detection) in 2016. All species of aphid showed a single peak in prevalence across both years, apart from Woolly Apple Aphid, which showed two peaks. Overall, Woolly Apple Aphid showed the highest level of consistency and lowest level of variance of detection. The magnitude and timing of aphid prevalence also differed between 2015 and 2016. Aphid prevalence peaked earlier for 2015 in early May, where 95-100% of trees surveyed were occupied by at least one aphid species. In contrast, aphid prevalence peaked two weeks later between the second and third week of May for 2016, at around 78%.
Lepidoptera

Three economically significant Lepidopteran pests were identified during the field surveys, across two families: Sesiidae; Apple Clearwing Moth (*Synanthedon myopaeformis* (Borkhausen)), and Tortricidae; Eyespotted budmoth (*Spilonota ocellana* (Denis and Schiffermüller) and Oblique-banded leafroller (*Choristoneura rosaceana* (Harris)). However, not all leafrollers detected during the surveys could be taxonomically verified with a high degree of certainty in the field and were therefore categorized into the same group labelled “Leafroller spp.”

At least one or more Lepidopteran species was found on a significant proportion of trees across all survey dates for both 2015 and 2016 (Figure 2-2). Apple Clearwing Moth was the most prevalent and consistently detectable species. In correspondence with the aphid surveys, overall pest prevalence was much lower in 2016 than 2015, with leafrollers and eye-spotted budmoth being most affected by year. Also mirroring the occupancy trends of the aphid observations, Lepidopteran prevalence, particularly for Apple Clearwing Moth, peaked two to three weeks later in 2016 than in 2015.

2.3.2. Earwig Abundance and Prevalence

The mean number of earwigs per tree peaked during the last week of June in 2015 and the first week of July in 2016 (Figure 2-3). In both years, the peak earwig numbers started to decline during the first week of August. Correspondingly, earwig prevalence profiles closely match the profile of earwig abundance for both years (Figure 2-3, Figure 2-4, Figure 2-5). In contrast to the trends observed in pest prevalence data, both earwig presence and numerical abundance were higher in 2016 relative to 2015; however, the true maximum peak density was not determined due to the cap of 50 earwigs per collection site per day being reached. Although no detectable differences in earwig density or prevalence between the field sites were observed, the total variance of earwig density was higher in 2015 relative to 2016 (data not shown). For both years, earwigs were consistently detectable across every collection date at every site.
Q1. Are earwigs present in the field at the same time as the target pests?

Overall, there was substantial temporal overlap in earwig prevalence and abundance with prevalence of both aphids (Figure 2-4), and Lepidoptera (Figure 2-5). However, only a small proportion of the trees surveyed were occupied by both earwigs and the pests, with the exception of points later in the season where the proportion of trees occupied by earwigs approached 100%. In 2015, the rise in earwig prevalence was associated the decline in aphid prevalence. However, in 2016 this pattern was not observed. A very similar relationship between earwig and Lepidoptera prevalence was observed across both years.

2.3.3. Half-life of detection

Overall, both aphids and Lepidoptera showed a non-linear decrease in the rate of detection as a function of hours post consumption. Inverse prediction using logistic regression analysis (N= 150 observations) determined that the half-life of detection (hours post consumption at which predicted probability of detection approaches 50%) was 30 hrs for Lepidoptera ($\chi^2=20.36, p<0.0001$), and 86 hrs for aphids ($\chi^2=9.91, p=0.0016$). Mean proportion data shows that the positive rate of detection peaked for both aphids and Lepidoptera at 24 hrs post consumption and declined to a minimum at 72 hrs, with 90% of earwigs testing positive for aphids or lepidoptera at the peak time interval of 24 hrs (Figure 2-6). Less than 50% of earwigs tested positive for aphid DNA after 72 hours, whereas less than 10% of earwigs tested positive for Lepidoptera DNA after 72 hrs. Positive rate of detection was lower at time = 0 hrs and continued to rise up to the peak at 24 hours.

2.3.4. PCR screening of field-collected earwigs

Q2. If they are both present at the same time, are earwigs consuming the target pests in the field?

Overall, 14% of field collected earwigs in 2015 tested positive for aphid DNA, whereas in 2016, less than 2% tested positive (Table 2-2). There was no observable correlation between the total number of earwigs tested and positive instances of detection
(Figure 2-7). PCR screening for Lepidoptera was 100% negative in 2016 and were inconclusive in 2015, with only one individual earwig testing positive for lepidopteran DNA (Table 2-2).

2.3.5. **Effect of earwigs on pest prevalence**

**Q3. Is there a detectable pattern of correlation between earwig abundance and pest prevalence?**

Earwig density was negatively correlated with the proportion of trees occupied by Rosy Apple Aphid, Apple Grain Aphid and all three Lepidopteran pest species (Table 2-3). Pairwise correlations, using unstructured REML multivariate factor analysis of mean number of earwigs on proportion of trees occupied by target pest groups (pooled data for both field seasons). However, no detectable effect was observed for earwig density on presence of Woolly Apple Aphid, or Green Apple Aphid.

2.3.6. **Logistic regression**

Logistic regression analyses revealed a significant negative effect of earwig number on overall prevalence of aphids and Lepidoptera for both the 2015 and 2016 field seasons (Table 2-8). A high degree of statistical clarity for this effect was observed for overall lepidopteran species across both years (p <0.0001*). This effect was strongest on Apple Clearwing Moth (odds-ratio = 0.5976, p<0.0001 in 2015; odds-ratio = 0.7379, p<0.0001 in 2016). In other words, the model predictions show a 40% (2015) and 26% (2016) decrease in probability of finding a tree occupied by Apple Clearwing Moth with each unit increase (single earwig) found in the same tree. A strong effect was also observed for leafrollers in 2015 (odds-ratio = 0.3127, p<0.0001). However, no statistical inference could be derived for leafrollers in 2016 due to the high degree of variance and low number of observations. In contrast, the effect of earwig number differed greatly for aphids at the species level between the two field seasons. While Rosy Apple Aphid and Apple Grain Aphid prevalence was significantly affected by earwig number in 2015, no statistical effect was observed for any individual aphid species in the following year, despite the fact that a marginal effect was observed on overall aphids in 2016 (p =
Neither Woolly Apple Aphid nor Green Apple Aphid showed a response to earwig number across both years. Overall, these trends found in the logistic regression tests are consistent with the correlations observed in the pairwise REML multivariate analysis (Table 2-3).

2.4. Discussion

2.4.1. Seasonal prevalence of earwigs and pests

Q1. Are earwigs present in the field at the same time the target pests?

Consistent with our expectations, we found that earwigs are active and detectable at highly observable levels of activity throughout the growing season and that both their population density and occupancy on trees increased over time with season. This agrees with past field studies in apple orchard systems in western North America (Caroll et al., 1984; Orpet et al., 2019; Unruh et al., 2016). We were also able to monitor and identify trends in tree-level occupancy of key target pests and track them alongside the earwigs. These results demonstrate that earwigs overlap across multiple target pests and that they co-occupy the same trees when earwigs are at both low and high population densities across the season. This allows us to confirm that earwigs have the potential to consume multiple target pest species within a given growing season, a trait that is desirable among generalist biocontrol candidates.

2.4.2. DNA gut content analyses

Q2. Are earwigs consuming the target pests in the field?

The results from the DNA gut-content screening of field collected earwigs across the two seasons confirm that earwigs are consuming aphids under natural field conditions. However, only one earwig screened positive for Lepidoptera DNA across the two years, so it is unclear as to whether earwigs are consuming lepidopteran larvae in the field. In neither case could any statistical inference be made in relation pest prevalence and the probability of detection. The few studies in which earwig gut contents have been analyzed using similar PCR-based methods have yielded diverse results, which makes it
difficult to compare the results from our gut-content analysis. For example, a study by Orpet et al. (2019), showed that up to 40% of earwigs screened in apple orchards in Washington, USA tested positive for woolly apple aphid DNA, yet no correlation was found between woolly apple aphid abundance and rate of detection. This is consistent with our observations, even though we observed a positive relationship between earwig density and pest prevalence. In contrast, Romeu-Dalmau et al. (2012), working in Mediterranean citrus orchards, found that up to 50% of earwigs tested positive for aphid DNA, and this detection rate was positively correlated with aphid population density. Several factors associated with differences in methodology and screening protocol could account for these ostensibly disparate observations. One feasible explanation is that there may be population-level differences in earwig feeding behaviour between these study systems (Cross et al., 2015; Dib et al., 2010). In our study, numerical data were not recorded for the various groups of pests. Ultimately, earwig feeding behaviour, and thus, the likelihood that we would detect target DNA in an earwig gut, will depend on the density threshold at which the earwigs are likely to encounter or feed on a given prey item. Thus, we need to know what the minimum population density of the target pest is before earwigs are likely to feed on them and whether this above or below the economic threshold of the target pest. We also need to consider whether we can even reliably detect them at these density thresholds with our screening techniques. These gaps of knowledge remain unanswered in the literature and may be worth further study. Functional response data (feeding rate as a function of density of prey) may help to elucidate this relationship between earwig feeding rate and minimum density thresholds (See Chapter 3).

The PCR screening methods used here attempted to replicate the same protocol as in the study conducted by Romeu-Dalmau et al. (2012), using the identical aphid-specific primers. The results for both studies are consistent in that the rate of detection was lower at the time immediately post consumption (t = 0), peaking at a later time point (100% detection at t = 6 hrs for the Romeu-Dalmau et al. study, and 90% detection at t = 24 hrs in our study). However, results for our half-life tests showed vastly different decay rates. Romeu-Dalmau et al. (2012) extrapolated a 23.8 hrs half-life using a fitted exponential curve. In contrast, our study used inverse predictions of a logistic regression to
extrapolate a half-life of detection of 86 hrs. Although this appears to be a significantly large difference, there are several factors that can account for this pattern such as:

physiological condition of the earwigs tested, life history of the earwig, abiotic rearing conditions which may account for differences in enzymatic activity, and digestion rate of the prey consumed (Hosseini et al., 2009; Lövei et al., 1990). Another potentially important difference may be that earwigs tested in the gut retention time experiment were lab reared rather than tested using field-caught individuals which would have had unknown histories. This was done to account for differences in feeding history, age, or cohort-level which may introduce bias or statistical noise if they affected enzymatic activity, digestion, or other unaccounted for factors which may affect rate of DNA decay or if the rate of decay is sensitive to rate of consumption (Hosseini et al., 2009). The lab-reared earwigs were also naïve to aphids and were allowed to consume up to a higher maximum number of aphids (up to 8) or until satiation, rather than a fixed number of aphids in contrast to the previous study on citrus aphids, although the size and species of the prey consumed differed as well. Secondly, the half-life of DNA decay is almost certainly expected to be different under field conditions. For example, temperature, pH, and ultraviolet light, are all conventionally accepted factors that will affect the rate of DNA degradation and will vary with environment and time (Agustí et al. 2003; Harwood et al. 2007; Hosseini et al., 2009). One can also speculate that the composition of the gut content such as other prey items, gut microbes, plant material and inorganic contaminants may all affect rate of digestion, or DNAse activity. Additional considerations can also include quality of DNA produced by the extraction protocol, calibration of PCR techniques, and other sources of human error, making it difficult to not only troubleshoot potential problems, but also limit our ability to appropriately explain these differences. All the aforementioned factors in the context of different biological systems are likely to produce different molecular yields, and thus different results through PCR-based screening techniques. This makes it difficult, if not impractical, to corroborate and compare these results with similar gut content screening studies done in other arthropod predator-prey systems (Agustí et al. 2003; Harper et al. 2005; Harwood et al. 2007; Juen & Traugott 2007; Ma et al. 2005; Read et al. 2006; Zhang et al. 2007).
2.4.3. **Half-life of detection and reliability of detection**

While we were able to confirm that earwigs are consuming aphids in the field, we were unable to do the same with the Lepidoptera. While the half-life curve for Lepidoptera decays at a faster rate than for aphids (Figure 2-6), there appears to be a lower probability of detection overall for Lepidoptera as well. The half-life curves were established from lab-reared earwigs with different prey species. It is conceivable that, due to the lack of other potential contaminants or background DNA, the estimated half-life of decay curves are likely to be an overestimate relative to the true half-life of detection under field conditions. Although the proportion of trees occupied by Lepidoptera did not differ much from the proportion of trees occupied by aphids in our overall observations (Figure 2-1, Figure 2-2), the actual population densities of the two pest groups were not collected in our data set. However, it would not be unreasonable to assume that the number of aphids available for consumption would be a lot higher than the number of Lepidoptera, since typically their reproductive rate and fecundity are intrinsically higher than that of the target Lepidoptera species. Secondly, the three Lepidoptera species in our study are more cryptic in their behaviour compared to the aphids. Aphids typically reproduce and feed in clusters of clonal colonies on the undersides or tips of leaves, whereas oblique-banded leafrollers, apple clearwing moth, and eye-spotted budmoths are all solitary feeders (Dixon, 1973; Oatman, 1963; Sanderson, 1909). Leafrollers, as their name would suggest, would typically hide by rolling themselves in leaves, whereas apple clearwing moth larvae are tree-boring feeders, both potentially more defensive strategies than non-hiding aphids against foraging predators like earwigs (Judd, 2016; Sanderson, 1909). Secondly, we don’t know how frequently earwigs feed, making it difficult to establish an optimal timing window for field collections, given the information that we have from the half-life of detection curves. Earwigs are nocturnal feeders, making their predation habits difficult to study. Depending on population density and prey availability, it is also conceivable that earwigs may not feed every night. It has been reported that earwigs can survive for many weeks, or even months without food (Beall, 1932; Fulton, 1924). Earwigs, in addition to being generalist predators, are also facultative omnivores. Thus, they have the capacity to be opportunistic when particular species of prey population densities are low, allowing them
to feed on alternative prey that may be more readily available or even plant material (Crumb, 1941; Orpet et al., 2019; Symondson et al., 2002). Our field observations also confirm the presence of other potential prey groups such as thrips, mites, and apple leaf curl midge throughout our study season (data not presented). Finally, with the exception of the early season collections from May – late June, we were only able to screen a small proportion (20 earwigs per field site per collection date) out of all the possible earwigs collected. In low density pest populations, only a small subset of the total earwigs would be able to feed on the target prey species, which means it would be less likely that a random earwig sampled from the total population to test positive, even if earwigs were reliably able to find and consume the target pests at low population densities. Therefore, we cannot preclude the role earwigs may have in Lepidoptera predation even though the results from our Lepidoptera DNA screening were inconclusive.

2.4.4. Are earwigs having a measurable effect on pest prevalence in the field? 

Q3. Is there a detectable pattern of correlation between earwig abundance and pest prevalence?

We showed a strong negative correlation between the density of earwigs and tree occupancy by rosy apple aphid, apple grain aphid, and eye-spotted budmoth, whereas a weaker negative correlation was observed for apple clearwing moth and leafroller spp. (Table 2-3). These data suggest that earwigs are having an observable impact on apple tree occupancy by these pest groups in the field. Our logistic regressions allowed us to examine this pattern in closer detail, showing that earwigs reduce the likelihood of finding the target pest on the same tree and that this trend was more prominent in 2015 when pest prevalence was higher than in 2016. If earwigs were having a direct effect on pest prevalence, this would be consistent with our observations, as earwigs would be less likely to encounter pests when there are less pests present on the trees. However, we cannot necessarily infer a commensurate relationship between pest prevalence on trees and overall pest population. For example, we would expect a more disparate relationship between aphid occupancy and population density than we would for the Lepidoptera species. These have clear implications when trying to infer a causal relationship between earwigs and pest prevalence. If earwig predation efficiency is highly sensitive to prey
density, we would expect a weaker correlation between earwig density and prey occupancy for the Lepidopteran pests in our study which occupy trees at much lower densities than the aphid species. We must therefore acknowledge the limitations of inferences we can make based on the pest prevalence data alone and highlight the importance of further study the relationship between pest prevalence and pest density. Ultimately this relationship will depend on the natural configuration of spatial and temporal distribution of these pests and their dispersal patterns. The earwigs in this study, although known to be naturally gregarious (Beall, 1932; Fulton, 1924), were observed in laboratory conditions to self-regulate their population density via cannibalistic behaviour (personal observations; data not shown). This is one potential explanation for density limiting trends observed in the field data where there was a regular distribution of earwig density at the tree level throughout the season and would consistent with observations in the literature where generalist predators are more to be affected by inter and intraguild interactions in the field (Hassell, 1978; Kassen, 2002; Symondson et al., 2002). This can be a potential reason why the profile of earwig tree occupancy closely matches (and scales accordingly) with the trends observed for earwig density (Figure 2-3, Figure 2-4, Figure 2-5).

In order to minimize the seasonal effect on the earwigs and pests, which would violate the assumption of non-independence in the model, our logistic regression analyses were truncated by date. Nevertheless, we still cannot conclusively preclude the possibility that these correlational relationships between pest prevalence and earwig density were merely an effect of seasonal growth and decline. Because earwig density starts low and continues to increase throughout the field season, this would naturally overestimate the effect of earwigs on pest occupancy later in the season for pest populations that naturally decline while earwig populations increase (such as the Lepidoptera species – see Figure 2-2, and Figure 2-5). Conversely, this effect would also be underestimated earlier in the season when earwig numbers are naturally low and pest prevalence naturally high, as was the case for species such as the Rosy Apple Aphid (see Figure 2-1, and Figure 2-4). The dates chosen for truncation were one collection date after the last observation of a given pest by season and, thus, do not necessarily represent the typical phenology of a given pest. This was done in order to remain conservative with our estimates on the effect of
earwigs on pest occupancy and, would therefore not capture any potential effects earwigs may have on preventing reintroductions of pests later in the season. However, the results from the REML model need not assume non-independence, nor normality of the data and has been historically used in agricultural field studies where a seasonality factor cannot be separated from explanatory variables (Harville, 1977). Conjunctively, these data reasonably suggest that earwig number and presence appear to have an observable effect on the aforementioned target pests and that these patterns are more prominently observed during periods in the field season when earwigs have a greater opportunity to encounter these pests. This is consistent with trends observed between earwigs and woolly apple aphids found in orchards in Washington, USA (Orpet et al. 2019; Unruh et al., 2016).

It is important to acknowledge the limitations when making inferences based on correlational data (Langford et al., 2001). For example, frequency and timing of sampling can greatly affect patterns observed in our data set depending on predation behaviour by the earwigs. If earwigs are able to quickly track prey to trees, but are limited by density thresholds, we would be less likely to detect any correlational pattern. However, interpreting these trends can be extremely difficult as we don’t know how earwigs respond to density in the context of multiple species over a two-week sampling interval of time. Past studies have postulated that negative correlations can be associated with top-down processes, whereas positive correlations are likely to indicate with bottom-up processes (Dib et al., 2010; Hassell 1978; Kindlmann & Dixon, 2001; Rusch et al., 2010), however, many of these predator-prey models rely on more simple single predator- single prey systems and are unlikely to be extrapolated to generalists like earwigs. Additionally, when earwigs are found ubiquitously in the orchard, as was the case later in the field season, this could potentially mask correlational patterns based on tree occupancy by certain pests such as the woolly apple aphid (Caroll & Hoyt, 1984; Dib et al., 2011). Numerical data based on pest population density will be an important first step in answering these questions. However, more work will need to be done in order to determine the efficacy of earwigs as biocontrol agents based on density and economic thresholds which have not yet been established for pests such as the woolly apple aphid (Orpet et al., 2019).
2.4.5. **Earwigs as an ecological indicator for pest prevalence?**

Although the logistic regression analyses alone cannot be used to infer a causal link between earwig density and pest occupancy, the trends observed demonstrate a potential proof of concept for a diagnostic tool in pest management monitoring. The presence/absence data captures a representative picture of the predator-prey apple orchard ecosystem, although low in resolution as it gives us no population-level pest data across time. However, the pragmatic implementation of this method should not be overlooked, as it has allowed us to collect a much larger data set of pest group and species composition, was less labor intensive, and less prone to human error associated with count data. By collecting data across multiple field seasons, it might be feasible to reliably use this method as a predictive pest management tool to allow growers to gauge the probability that a tree has a given pest from earwig count data alone. This can be done relatively easily, as corrugated cardboard traps allow us to non-destructively monitor earwig abundance, while at the same time, provide shelter and refuge for earwigs as a conservation biocontrol tool.

Non-consumptive effects may account for the incongruent patterns observed between the field survey data and our gut content analysis. Traditionally, non-consumptive effects have examined predator impact on prey due to perceived predation risk and that this has been shown to have trophic-level effects in some insect systems (Hermann & Landis, 2017). However, there are many other possible ways to investigate non-consumptive effects of predators. For example, generalist predators have a higher degree of dietary plasticity, and thus are likely to have more food web interactions (Dib et al., 2010; Dib et al., 2011; Schmidt et al., 2003; Symondson et al., 2002). For example, past studies have demonstrated significant interaction effects between earwigs and ants and their ability to suppress aphid pests (Piñol, 2009; Romeu-Dalmau et al., 2012). Presence/absence data may underrepresent non-consumptive effects such as displacement of other natural enemies, or remediation of resource competition between pest groups—although these interactions are rarely studied in biocontrol. A post-hoc analysis of natural enemies in our data set showed an overall positive correlation between the number of earwigs and...
certain natural enemy groups such as *Aphidius* (data not presented), suggesting the possibility that earwigs might play a role in facilitating food web interactions.

Although the findings in this study seem to provide some reasonable evidence to suggest that earwigs are playing an important role in apple orchard pest occupancy, suffice to say, more work must be done in the field to draw causal links beyond what can be speculated on correlational data. Numerical data for pests at population level, non-disruptive methods of exclusion studies, and further research on natural enemy intraguild interactions and their correlates with pest dynamics are all steps that need to be taken in order to elucidate the efficacy of European earwig as a biocontrol asset in agroecosystems and to remediate its current reputation in the public eye.
2.5. Figures and Tables

Table 2-1. Aphid-specific and Lepidoptera-specific PCR primers and target DNA species tested for specificity against a background of earwig DNA. Aphid primer sequences were obtained from Romeu-Dalmau et al. (2012), and Lepidoptera primer sequences were obtained from Herbert et al. (2004).

<table>
<thead>
<tr>
<th>Primer</th>
<th>PCR product size</th>
<th>Species</th>
<th>Test Positive (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AphF1 - 5′-ATTTGGTATTTGATCAGG-3'</td>
<td>224 bp</td>
<td>Rosy Apple Aphid</td>
<td>+</td>
</tr>
<tr>
<td>AphR3 - 5′-CGTGGAAAGATATATCTGGAC-3'</td>
<td></td>
<td>Green Apple Aphid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Woolly Apple Aphid</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apple Grain Aphid</td>
<td>N/A</td>
</tr>
<tr>
<td>LepF1- 5′-ATTCAACCAATCATAAAGATATTG-3'</td>
<td>648 bp</td>
<td>Apple Clearwing Moth</td>
<td>N/A</td>
</tr>
<tr>
<td>LepR1- 5′-TAAACTTCTGGATGCCAAAAATC-3'</td>
<td></td>
<td>Eye-spotted Budmoth</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oblique-banded leafroller</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 2-1. Mean proportion of trees occupied by different aphid species in 2015 (left) and 2016 (right). (N = 30 trees per time point in 2015; N = 30-50 trees per time point 2016)
Figure 2-2. Mean proportion of trees occupied by different Lepidoptera species in 2015 (left) and 2016 (right). (N = 30 trees per time point in 2015; N = 30-50 trees per time point in 2016).
Figure 2-3. Earwig density (mean number of earwigs per tree) across the field season for 2015 (left) and 2016 (right). Error bars correspond to standard error of mean number of earwigs sampled per date across 3 field sites. Total N = 626 trees. Red dashed line represents theoretical maximum limit on mean number of earwigs collected per date, as maximum number of earwigs collected for gut content analysis was capped off at 50 earwigs per site, per date.
Figure 2-4. Mean proportion of trees occupied by earwigs (green), aphids (red), or both earwigs + aphids on the same tree (purple dotted-line) across the two field seasons. (N = 30 trees per time point for 2015; N=30-50 trees per time point for 2016)
Figure 2-5. Mean proportion of trees occupied by earwigs (blue), Lepidoptera (red), or both earwigs + Lepidoptera on the same tree (purple dotted-line) across the two field seasons. (N = 30 trees per time point for 2015; N=30-50 trees per time point for 2016)
Figure 2-6. Half-life of detection results, shows mean proportion of earwigs testing positive for the Aphid DNA (A), and Lepidoptera DNA (B) at 0, 8, 24, 42, and 72 hours post consumption of Rosy Apple Aphid (Aphid), or Oblique-banded-leafroller (Lepidoptera), using COI mitochondrial aphid and lepidoptera-specific DNA primers. (n = 30, per treatment group, per time point)
Figure 2-7. Total number of individual earwigs testing positive for Aphids (red) or Lepidoptera (green) across the two field seasons. The stack bar graphs show the proportion of total tested relative to the number of individuals that test negative (grey).
Table 2-2. Summary of total number of individual earwigs testing positive for either aphid or lepidopteran DNA via PCR screening in 2015 and 2016.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Earwigs Tested</th>
<th># Individuals Tested Positive for Aphid</th>
<th># Individuals Tested Positive for Lepidoptera</th>
<th>% Earwigs Positive</th>
<th>% Positive Aphids</th>
<th>% Positive Lepidoptera</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>257±1.64</td>
<td>37±0.41</td>
<td>1±.04</td>
<td>14%</td>
<td>14%</td>
<td>0%</td>
</tr>
<tr>
<td>2016</td>
<td>415±1.27</td>
<td>8±0.21</td>
<td>0±0.00</td>
<td>1.9%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 2-3. Pairwise correlations, using unstructured REML multivariate factor analysis of mean number of earwigs on proportion of trees occupied by target pest groups (pooled data for both field seasons)

<table>
<thead>
<tr>
<th>Factor 1 (Mean Earwigs/Tree)</th>
<th>Factor 2 (Proportion of Trees Occupied By)</th>
<th>Correlation</th>
<th>N</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Earwigs</td>
<td>Aphids</td>
<td>-0.5671</td>
<td>51</td>
<td>-0.7288</td>
<td>-0.3455</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Rosy Apple Aphid</td>
<td>-0.4886</td>
<td>51</td>
<td>-0.6735</td>
<td>-0.2462</td>
<td>0.0003*</td>
</tr>
<tr>
<td></td>
<td>Apple Grain Aphid</td>
<td>-0.0144</td>
<td>51</td>
<td>-0.2888</td>
<td>0.2623</td>
<td>0.9203</td>
</tr>
<tr>
<td></td>
<td>Woolly Apple Aphid</td>
<td>-0.1870</td>
<td>51</td>
<td>-0.4399</td>
<td>0.0934</td>
<td>0.1888</td>
</tr>
<tr>
<td></td>
<td>Green Apple Aphid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>Leafroller spp.</td>
<td>-0.3939</td>
<td>51</td>
<td>-0.6039</td>
<td>-0.1327</td>
<td>0.0042*</td>
</tr>
<tr>
<td></td>
<td>Eye-spotted Budmoth</td>
<td>-0.4387</td>
<td>51</td>
<td>-0.6373</td>
<td>-0.1856</td>
<td>0.0013*</td>
</tr>
<tr>
<td></td>
<td>Apple Clearwing Moth</td>
<td>-0.3998</td>
<td>51</td>
<td>-0.6084</td>
<td>-0.1397</td>
<td>0.0036*</td>
</tr>
</tbody>
</table>
Table 2-4. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (A = At least 1 or more Aphid species, B = Rosy Apple Aphid) as a function of number of earwigs present. The logistic curves are fitted using maximum likelihood ratio tests. Y axis shows the probability of the outcomes (1 = positive for occupancy, 0 = negative for occupancy). The total population of points are representative of the distribution of data points that determined the fitted curve. NS = non-significance, * = significant effect of # earwigs on probability of specified pest occupancy.

A: Total Aphids (At least 1 or more Aphid Species)

B: Rosy Apple Aphid
Table 2-5. Mosaic plots for logistic regression analyses on the predicted probabilistic outcomes of a tree being occupied by a pest category (C = Green Apple Aphid, D = Apple Grain Aphid, E = Woolly Apple Aphid)
Table 2-6. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (F = At least 1 or more Lepidoptera Species, G = Apple Clearwing Moth) as a function of number of earwigs present. The logistic curves are fitted using maximum likelihood ratio tests. Y axis shows the probability of the outcomes (1= positive for occupancy, 0 = negative for occupancy). The total population of points are representative of the distribution of data points that determined the fitted curve. Parameter estimates of effects tests are found in Table 4. NS = non-significance, * = significant effect of # earwigs on probability of specified pest occupancy.
Table 2-7. Mosaic plots for logistic regression analyses for both field seasons on the predicted probabilistic outcomes of a tree being occupied by a pest category (H = Eye-spotted Budmoth, I = Leafroller spp.)
Table 2-8. Logistic regression analysis parameters, using a maximum likelihood ratio test on predicted probability of pest occupancy as a function of increasing number of earwigs per tree. Where $\beta$ is defined as the parameter estimate of the effect of number of earwigs on pest occupancy. Number of observations analyzed for each pest group were determined from date of first detection until 1 collection date after seasonal disappearance.

<table>
<thead>
<tr>
<th>Number(Earwig) x Pest Species (1 = presence, 0 = absence)</th>
<th>Observation s</th>
<th>$\beta$</th>
<th>SE $\beta$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>p</th>
<th>Odds-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2015</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 Aphid Species</td>
<td>303</td>
<td>-0.1372</td>
<td>0.0583</td>
<td>6.595</td>
<td>1</td>
<td>0.0102*</td>
<td>0.8717</td>
</tr>
<tr>
<td>Rosy Apple Aphid</td>
<td>153</td>
<td>-0.5086</td>
<td>0.1970</td>
<td>11.358</td>
<td>1</td>
<td>0.0008*</td>
<td>0.6013</td>
</tr>
<tr>
<td>Green Apple Aphid</td>
<td>93</td>
<td>0.6117</td>
<td>0.5798</td>
<td>0.8643</td>
<td>1</td>
<td>0.3525</td>
<td>1.8436</td>
</tr>
<tr>
<td>Apple Grain Aphid</td>
<td>303</td>
<td>-0.5787</td>
<td>0.1969</td>
<td>17.231</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.5606</td>
</tr>
<tr>
<td>Woolly Apple Aphid</td>
<td>303</td>
<td>-0.0532</td>
<td>0.0558</td>
<td>0.9751</td>
<td>1</td>
<td>0.3234</td>
<td>0.9482</td>
</tr>
<tr>
<td>≥ 1 Lepidopteran Species</td>
<td>303</td>
<td>-0.5292</td>
<td>0.1303</td>
<td>30.109</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.5891</td>
</tr>
<tr>
<td>Apple Clearwing Moth</td>
<td>303</td>
<td>-0.5148</td>
<td>0.1399</td>
<td>24.911</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.5976</td>
</tr>
<tr>
<td>Eye-spotted Budmoth</td>
<td>303</td>
<td>-0.5784</td>
<td>0.2830</td>
<td>8.575</td>
<td>1</td>
<td>0.0034*</td>
<td>0.5607</td>
</tr>
<tr>
<td>Leafroller spp.</td>
<td>303</td>
<td>-1.1624</td>
<td>0.3645</td>
<td>29.291</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.3127</td>
</tr>
<tr>
<td><strong>2016</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1 Aphid Species</td>
<td>296</td>
<td>-0.1063</td>
<td>0.0532</td>
<td>4.098</td>
<td>1</td>
<td>.0429*</td>
<td>0.8992</td>
</tr>
<tr>
<td>Rosy Apple Aphid</td>
<td>231</td>
<td>-0.1241</td>
<td>0.0933</td>
<td>1.822</td>
<td>1</td>
<td>0.177</td>
<td>0.8833</td>
</tr>
<tr>
<td>Green Apple Aphid</td>
<td>296</td>
<td>-0.0056</td>
<td>0.2583</td>
<td>0.0004</td>
<td>1</td>
<td>0.982</td>
<td>0.9943</td>
</tr>
<tr>
<td>Apple Grain Aphid</td>
<td>231</td>
<td>-0.1443</td>
<td>0.1737</td>
<td>0.7164</td>
<td>1</td>
<td>0.397</td>
<td>0.8656</td>
</tr>
<tr>
<td>Woolly Apple Aphid</td>
<td>296</td>
<td>-0.0748</td>
<td>0.0526</td>
<td>2.0479</td>
<td>1</td>
<td>0.152</td>
<td>0.9279</td>
</tr>
<tr>
<td>≥ 1 Lepidopteran Species</td>
<td>296</td>
<td>-0.2925</td>
<td>0.0588</td>
<td>27.344</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.7464</td>
</tr>
<tr>
<td>Apple Clearwing Moth</td>
<td>296</td>
<td>-0.3478</td>
<td>0.0592</td>
<td>29.310</td>
<td>1</td>
<td>&lt;0.0001*</td>
<td>0.7379</td>
</tr>
<tr>
<td>Eye-spotted Budmoth</td>
<td>170</td>
<td>-0.3369</td>
<td>0.2667</td>
<td>2.0355</td>
<td>1</td>
<td>0.1537</td>
<td>0.7140</td>
</tr>
<tr>
<td>Leafroller spp.</td>
<td>60</td>
<td>N/A</td>
<td>N/A</td>
<td>0.2809</td>
<td>1</td>
<td>0.5961</td>
<td>N/A</td>
</tr>
</tbody>
</table>
How does temperature affect the predation capacity of a generalist predator?

3.1. Introduction

3.1.1. Earwigs as generalist predators

European earwigs (*Forficula auricularia*) (EEWs) are generalist predators, and have been utilized in both augmentative and conservation biocontrol applications to target aphids in various fruit orchard systems (Mueller et al., 1988; Nicholas et al., 2005, Piñol et al., 2009) and leafrollers in vineyards (Frank et al., 2007). Earwigs have two interesting characteristics as biocontrol agents: (1) they are primarily nocturnally active, feeding and foraging only at night, while preferring to seek shelter and hide in cool places during the day (Albouy & Caussanel, 1990), and (2) they are purportedly omnivorous, which has contributed to them being perceived as suspected pests. There have been numerous anecdotal and historical accounts found in the literature where they are often presumed to feed on leaves, flowers, and some varieties of soft fruits (Fulton, 1924; Grafton-Cardwell et al., 2003; McLeod & Chant, 1952). However, many of these observations have been a point of contention (Nicholas et al., 2004; Orpet et al., 2019). These characteristics make them simultaneously mysterious and conspicuous, as they can be found in a wide range of behavioural and environmental contexts, making it difficult to elucidate their net role in agroecosystems.

3.1.2. Generalists in Biocontrol

Biological control efficacy has historically focused on analytical models and empirical observations based on population-level measurements of the natural enemies and their host or prey population (DeBach & Rosen, 1991; Gurr et al., 2000; Symondson et al., 2002). Although this approach can prove to be pragmatic in its ability to rule out biocontrol candidates by measuring the net-effect at the population level, these macro-
scale analyses do not provide an opportunity to investigate what individual traits are indicators of good natural enemy performance and how these traits interact with environmental factors. This can potentially provide insight as to the mechanisms and contexts in which biocontrol agents fail to perform and can allow us to approach biocontrol solutions which are more appropriate for each specific circumstance.

The terms “generalism/specialism” can refer to a wide array of traits associated with resource use across multiple dimensions of analyses (see Chapter 1), and thus, can be a point of misunderstanding. To avoid this confusion and focus the aim of our study, we will use the “generalist/specialist” terminology to strictly refer to the dietary niche breadth of EEWs in this chapter. There is a consensus that speciation via natural selection will inevitably give rise to species across a continuum of niche breadth dimensions which vary in different environmental and ecological contexts (Kassen, 2002; Loxdale et al., 2011; Peers et al., 2012). Therefore, choosing successful biocontrol candidates that target specific pests within agricultural ecosystems may provide insight as to what character traits might be conducive to the aims of suppressing pest numbers.

There has been a long historical debate between the merits of generalists vs specialists in biocontrol as to what traits are most desirable when selecting an effective biocontrol agent (DeBach et al., 1991; Hassell, 1978; Huffaker, 1971; Symondson et al., 2002). Specialists such as parasitoids, necessarily have their reproductive fitness tightly linked with access to their host prey. For this reason, specialists tend to be better adapted when environmental conditions where there is a higher degree of resource homogeneity and less variance (Dennis et al., 2011; Peers et al., 2012; Rosenzweig, 1995; Tilman, 1982). In contrast, due to their presumed broader dietary breadth, generalist predators like earwigs are predicted to be more likely to exhibit plasticity in their capacity to adapt to intergenerational changes in quantitative and/or qualitative variance in prey availability (Agosta et al., 2010; Symondson et al., 2002). This is consistent with the many examples of when generalists have been used successfully in conservation biocontrol where endemic predators such as carabid beetles were used to prevent invasion or outbreak of pest as they are capable of persisting locally by feeding on alternative resources when pest numbers are scarce (DenBoer, 1982; Symondson et al., 2002). However, there are
trade-offs, as it is more difficult to elucidate the role of generalist predators, where the
total number of potential food web and ecological interactions are more likely to
outnumber than what one would expect from specialists, whom, by definition, are
presumed to have a narrower niche breadth (Peers et al., 2012; Rand & Tscharntke,
2007).

3.1.3. **Functional Response and Temperature**

Assessing suitable biocontrol agents requires the ability to estimate their efficacy.
One way to do this is to measure their potential voracity on target pests (Lucas et al.,
1997). A functional response, defined as a change in the number of prey attacked and
consumed as a function of prey density, has been a classic way to study predation
(Solomon, 1949). One of the most utilized predator-prey models that describes this
interaction strength incorporates the search rate (sometimes also referred to as the
encounter rate), handling time, and density into the Holling disc equation (Holling, 1959).
The type II functional response equation (Equation 1) describes a prey response which
increases non-linearly with density, decelerating into an asymptotic plateau. This upper
asymptote describes the theoretical maximum number of prey which can be consumed by
a given predator in a given time period and would only be limited by handling time
(defined by the time it takes to consume and digest prey). At high prey density, this
theoretical maximum consumption rate would approach \( \frac{1}{t} \). The speed of the
deceleration will depend upon, \( a \), the search rate of the predator (which can also be
interpreted as the rate at which the predator encounters and attacks prey).
**Equation 3-1.** Type II Holling disc equation

\[
y = \frac{x \cdot a}{(1 + x \cdot a \cdot h)}
\]

y = % of total available prey consumed per unit of time  
a = instantaneous search rate  
h = handling time  
x = density of available prey

Historically, the functional response of a predator has been assumed to be a reasonable tool to evaluate the efficacy of a natural enemy on important agricultural pests as a conventional method of biocontrol evaluation (Lucas et al., 1997; Latham & Mills, 2010). However, laboratory experiments and analytical techniques have rarely addressed the multitude of relevant factors and attributes that may affect parameters that determine predation rate or voracity in the field, and instead focus more on bridging the gap between phenomenological and mechanistic models with respect to density dependence (Bolker, 2008; Casas & Hulliger, 1994; Dib et al., 2010; Juliano, 2001; Rogers, 1972). There are many reasons that can account for this gap for generalist predators. For example, functional response models rely on a few key assumptions that are easier to approximate for specialists such as the tightly linked density-dependence between predator and prey, as the assumption that prey depletion will have a significant effect on predation rate over time and, indeed, variants of the Holling type II model have been utilized in order to account for this (Rogers, 1972; Symondson et al., 2002). However, this is an assumption that will almost never be met for a generalist predator in the field, where prey switching, or alternative resource seeking behaviour are effectively intractable in practice (Symondson et al., 2002; Van Lenteren & Bakker, 1976).

Herein lies the limitation of functional response models as representations of real-world biocontrol practice. Under laboratory conditions, several important factors are unaccounted for that are likely to have significant effects on generalist feeding habits in the field. These factors may include: prey switching, resource preferences, relative and
absolute prey densities across more than one prey resource (Beddington et al., 1978; Cock, 1978; Symondson et al. 2002). Therefore, measuring the functional response of a generalist is more likely to inform us about the physiological limits of prey consumption rather than predation efficiency in the field (Daugaard et al., 2019; Englund et al., 2011).

It is a daunting task to elucidate a causal connection between the results obtained through lab voracity trials and correlational data found in the field. However, functional response models can still be used as relevant tools for testing how external factors like temperature can impact physiological mechanisms of feeding behaviour for insects (Sentis et al., 2012; Symondson et al. 2002). For example, energy efficiency (defined as the ratio of gained through food consumption and energy lost through metabolic processes) has been shown to be significantly impacted by temperature (Vasseur & McCann, 2005; Vucic-Pestic et al., 2010). Obviously, as insects are ectotherms, the impact of temperature on the metabolism of earwigs can, thus, be expected to have a major effect on functional response parameters like search rate and handling time (Sentis et al., 2012). Testing functional responses of different species across different temperatures can aid us in identifying thermal thresholds as well as the biological limits of consumptive ability across more contexts. Previous research has shown that local temperatures can have drastic ecological and behavioural effects on ectotherms at the population level (Brown et al. 2004; Hoekman 2010; Petchey et al. 2010). Therefore, we can predict that because temperature necessarily affects the physiology of ectotherms, parameters in the functional response model which are tied to the physiology of the predator like search rate, and handling time are likely going to affect net performance of the predator. Examining physiological performance in closer detail in order to elucidate the net implications on biocontrol applicability is a difficult task and would require addressing multiple environmental factors. European earwigs, as nocturnal generalist predators, have unique traits that might explain why they are found ubiquitously across urban and agricultural ecosystems. Studying how temperature affects their consumptive ability in isolation may further reinforce the amounting evidence found in the literature demonstrating their utility as biocontrol agents in apple orchards (Mueller et al., 1988; Orpet et al., 2019; Quarrell et al., 2017)
3.1.4. **Objectives of this study**

The overall aims of this study were to examine how temperature might affect and interact with certain traits associated with generalist predators and test how well traditional predator-prey models describe this relationship. In order to accomplish this, I sought out the following objectives:

1. To investigate the effect of temperature on parameters of the basic type II Holling functional response model for earwigs using two target pest groups, the Rosy Apple Aphid (RAA) (*Dysaphis plantaginea*) and the Oblique-banded leafroller (OBLR) (*Choristoneura rosaceana*).
2. To determine thermal thresholds for earwig predation by investigating the effects of temperature on the voracity of the earwigs on the two target pests and potential interaction effects with density.

3.2. **Methods**

3.2.1. **Rearing protocol**

Adult earwigs were collected from organic apple orchard farms in Cawston, BC. Earwigs used for the Rosy Apple Aphid (RAA) study were collected during the 2015 field season (between the last week of June and the first week of September), whereas earwigs used for the OBLR study were collected in 2017 between the third week of August and second week of September. Approximately 5-10% of field caught adult earwigs were parasitized by tachinid fly parasitoids (*Triarthria sentipennis*), while 2-7% were parasitized by a nematode (*Mermis nigrescens*) (data not presented). The earwigs were kept in 16x10x7.5 cm polyethylene food storage containers at densities no greater than 60 adults per container. Each container had 4-8 rolled up corrugated cardboard shelters, dental wicks of distilled water and “Kibbles’n’Bits” branded dog food in 29.6 mL SOLO cups. The colonies were stored in growth chambers at 20°C and 40-70% RH, 16:8 day:night cycle, and monitored daily for dead earwigs and parasitoid emergence. After three consecutive weeks without parasite emergence, the adult earwigs were selected haphazardly and placed individually in 59.2 mL vented SOLO cups with water-
soaked dental wicks. They were maintained at 23°C and starved for 14 days prior to the experiments in order to standardize satiation level. During preliminary protocol development tests, there was no observable difference between male and female voracity level for both aphids and leafrollers for the 2015 and 2016 field-collected earwigs (data not shown), however, an effect was shown in the 2017 field-collected earwigs, and thus, only males were used in the oblique-banded leafroller functional response experiments that were conducted during this time.

Rosy Apple Aphids (RAA) were lab-reared on their secondary host plant, the broadleaf plantain, *Plantago major* throughout the time of the experiment. The same protocol for rearing was used as outlined in Chapter 2, where colonies were collected from trees from various apple orchards in Cawston, BC. The aphid colonies underwent daily inspection and removal of aphid mummies to completely rid the colonies of parasitoid wasps over 2 weeks. Initially the reproductive rate on the plantains was very low, producing a lot of alates, suggesting that many of the field collected aphids were still in their sexual phase, as they were collected near the end of the summer season. The field-collected colonies were reared for approximately three generations before a consistent parthenogenic colony was established. Once parthenogenesis was established the colonies were moved into room temperature conditions (approximately 22-23°C) in indoor growth room facilities with growth lights for the plants at 16:8 day:night cycle. All aphids used in each replicate of the feeding trials were 3rd instar apterous individuals from the same cohort and were of uniform age structure (± 12h).

The same protocol was used to rear oblique-banded leafroller (OBLR) as described in Chapter 2. Egg masses were obtained from a lab colony from the Pacific Agriculture Research Station in Summerland, BC. Colonies were set up at room temperature between 21-23°C with 16:8 day:night cycle. Leaf roller egg masses were housed in 29.6 mL SOLO cups and inspected daily for hatching of neonates (newborn larvae). Newly hatched individuals were placed in 59.2 mL SOLO cups at a density of 7-10 individuals per cup and reared for the remainder of their life cycle on artificial McNeil’s pinto bean diet (Shorey & Hale, 1965). All leafrollers used in each replicate of the experiment were
reared from a single cohort and were haphazardly chosen at the 2nd instar, assessed visually from head capsule size.

3.2.2. **Functional Response Experiments**

Functional response studies were carried out at four densities (10, 25, 35, 50 aphids per leaf, and 10, 15, 20, 25 leafrollers per Petri dish), and five temperatures (10°C, 15°C, 20°C, 25°C, 30°C). This temperature range spans well above and below the recorded dusk-dawn temperatures from local weather stations, when earwigs are expected to forage, and thus is assumed to adequately cover the ecological temperature range for earwig predation behaviour. OBLR arenas were 2-dimensional style arenas in Petri dishes (20mm x 100mm in size) with lids vented using mesh. RAA arenas were set up using the same Petri-dishes with broadleaf plantain leaves approximately 6 cm in diameter kept in 1.5ml microcentrifuge tubes containing water and 1% agar to ensure that the leaves do not dehydrate. Individual temperature readings were recorded using iButton™ data loggers in a subsample of the Petri plates (5 each treatment group) in each replicate of the experiment. This allowed us to measure single arena conditions inside the growth chamber to account for potential confounding effect of the growth chamber on the response variable for this experiment. All of the arenas were set up the night before (roughly 24-12 hours before the experiment) to allow the prey to establish at room temperature before commencing the experiment the next day.

Functional response experiments took place in incubators in complete darkness and at 40-70% RH. Three temporal replicates for the aphid experiments (16 each treatment group), and six temporal replicates for the leafroller experiments (20 each treatment group) took place in the two respective field seasons. A pseudo-random number generator using ‘dqrng’ package in R was used to randomly assign each incubator a specified temperature setting between each temporal replicate. Earwigs would forage for a full 24 hrs for the OBLR experiments and 8 hrs for the RAA experiments in complete darkness. When the experiment ended the number of remaining prey were counted. Prey were not replaced during the experiment. Partially consumed and missing prey were counted as consumed. A control group of arenas containing prey with no added earwigs were also set up at each
density to account for potential prey escape and mortality during the experiment. The data were then extrapolated to 24 hrs for aphids and raw values were transformed into proportions of total prey consumed in order to minimize heteroscedasticity in the data set.

3.2.3. Statistical analysis

In order to test the effect of temperature on search rate and handling time parameters, functional response experimental data for both RAA and OBLR were fitted to the type II Holling disc equation (Equation 1) using analytic Gauss-Newton method in order to provide estimates for the parameters. In order to test the effect of density, temperature, and interaction effects on predation, the data were analyzed using a generalized linear model (GLM) (binomial distribution) using the logit function to determine the effects density across the different temperature treatments. These data were transformed into proportions in order to reduce heteroscedasticity. If detected, confidence intervals and standard error were scaled via overdispersion parameter described in (McCullagh & Nelder, 1989). All statistical analyses were conducted using the statistics program JMP 14.1.0 and R 3.5.3 using ‘lme4’, ‘emdbook’ and ‘friar’ packages.

3.3. Results

3.3.1. Rosy Apple Aphid

Analysis of effects tests (Table 3-2) on the functional response data set showed a strong effect of both aphid density, ($\chi^2 = 19.80, p < 0.0001$), and temperature ($\chi^2 = 35.81, p < 0.0001$), but no observable interaction effect ($\chi^2 = 0.112, p = 0.738$).

A decelerating trend was observed with respect to density when the aphid data were fitted to the type II Holling disc equation (Figure 3-1). Consistent with the effect test results that showed an overall lack of interaction effect between temperature and density, the goodness of fit did not differ very much between temperatures except at 10°C where there was less variance in predation rate. Parameter estimates for $h$ (handling time), and $a$ (search rate) obtained from the fitted type II model for the aphid data set are shown in
Table 3-1. The handling time was estimated to be shortest at 15°C, showing a higher theoretical maximum consumption of aphids at this temperature, although search rate was highest at 20°C. A temperature effect was most strongly observed at 10°C, where density-dependent effects were severely dampened. However, at the other extreme, 30°C, this effect did not seem to differ in comparison to the effects observed at 25°C.

3.3.2. Oblique-banded leafroller

Effects test for the OBLR data set (Table 3-2) showed a strong effect of density ($\chi^2= 29.85$, p < 0.0001), and temperature ($\chi^2= 31.53$, p < 0.0001), but no interaction effects ($\chi^2= 1.02$, p = 0.3112). Parameter estimates for the OBLR data set could not be obtained at 10, 25, or 30°C from the fitted type II Holling model, as they crossed the lower and upper bounds for either $h$ (handling time = 0), and/or $a$ (search rate = 1), suggesting that the results from the functional response experiments were poorly explained by the non-linear model (Table 3-1). This effect can be corroborated with the lack of a decelerating effect of density observed and the poor fitting of the type II curves (Figure 3-2). However, the density-dependent effect on predation was not detected at 20 and 30°C (Table 3-3).

3.4. Discussion

3.4.1. Temperature and Density on Predation

The effects tests showed that both temperature and density, independently, had a measurable effect on earwig predation efficiency for both OBLR and RAA, while an interaction effect between the two was not detected (Table 3-3). However, the relative effect size was small for each factor, despite the fact that the observed effect with a high degree of statistical certainty (p< 0.0001 for both temperature and density).

Although a weak decelerating density-dependent effect was observed for RAA predation, this trend did not fit well with the OBLR data set (Figure 3-1, and Figure 3-2). For this reason, parameter estimates could not be extrapolated for the OBLR data set, as standard errors for the estimate crossed the minimum and maximum boundaries for search rate and
handling time. This is reflected in the uncharacteristically convex-shaped curves for the OBLR data set (Figure 3-2), as no solution for search rate or handling time could be reached by force-fitting the data to the type II equation. This is also consistent with the notion of energetic efficiency, whereby the rate of energy obtained through consumption of OBLR was relatively high compared to the rate energy expended due to increased metabolism (Sentis et al., 2012; Vucic-Pestic et al., 2010). If aphids are a poorer source of energy, this can also explain the difference in the shapes of the fitted curves between the two species. One way this can be interpreted is that individual variation in performance across all densities masked any potential decelerating trend, suggesting a lack of density-dependent effect on consumption when fitted to the type II Holling model. Past functional response studies have shown that when predation efficiency is too high at the lowest prey density tested, this can result in an inability to discriminate between type II and type III curves. Conversely, if prey predation efficiency is too high at the highest density, this can result in an inability to discriminate between type II and type I curves (Juliano, 1993; Symondson et al., 2002). The GLM results are consistent with the latter explanation, as the strength of the density-dependent effect of predation did not appear to drastically differ across temperatures (Figures and Tables 3-1). In other words, earwigs seemed to consistently be able scale their consumption of prey with their density. The profile of the RAA functional response, although very weakly matches the pattern of a type II curve, did not translate to an overall large effect of density on predation when temperature is taken into account. Overall, the proportional effect of density was three times lower than the effect of temperature on aphid predation (Table 3-3). This is consistent with the observation that RAA predation efficiency was very high across the board for all temperature treatments above 10°C (Table 3-1), suggesting that density had a relatively weak effect on predation voracity overall (Table 3-2). A similar pattern was observed for the OBLR data set, although the proportional difference of effective size between density and temperature on predation was not as large (Table 3-3). Although a trend was detected with a high degree of statistical certainty, the magnitude of the effect on proportional predation remained relatively minor when analyzed within a temperature treatment group (Table 3-2). Predation efficiency was very consistent across the treatment groups such that it appeared
to density-independent at 25°C for OBLR, and 20 and 30°C for RAA. This suggests that the ability for the earwig to capture and consume prey was too efficient to simulate normal foraging conditions. It should therefore come as no surprise that the Holling type II model did not accurately describe the predation behaviour of the earwigs within the constraints of the experimental environment. There are many reasons that can account for this. The earwigs were tested in a very homogenous environment with minimal environmental complexity (a quasi-2D environment in a petri dish) with prey that exhibited little to no capacity to escape predation. For this reason, we could not reliably extrapolate search rate or handling time parameters, as the earwigs, once above 10°C, were likely consuming prey at close to their physiological maximum. Past literature has shown that in laboratory experiments, where temperature is kept constant, and prey are provided ad libitum, tend to overestimate the thermal optimum for performance of predators in the field, especially in cases where temperature is more likely to have a stronger effect on digestion rate and handling time than search rate (Daugaard et al., 2019; Englund et al., 2011; Sentis et al., 2012).

These results, at first glance, appear to be inconsistent with previous functional response studies of earwigs and generalist predators (Asante, 1995; Dib et al., 2011; Sentis et al., 2012). Many of these studies include the assumption that density-dependent effects will be non-linear over time, as prey gets consumed throughout the course of the experiment. Typically this has been addressed by utilizing the Rogers random predator equation (Rogers, 1972) to account for the lack of replacement of prey items (and hence change of density factor across time) as they are consumed throughout the experiment (Bolker 2008; Casas & Hulliger 1994; Dib et al., 2010; Juliano 2001; Rogers 1972). I did not utilize this method of analysis for the data in this study, as the primary focus was to test the effects of temperature on the net consumptive capacity of the predator. While this can potentially change the shape of the curve to better fit a type II profile (Daugaard et al., 2019), this would only suggest that our reported effect size values are underestimates, as the true treatment densities would be lower than the starting point of the experiment. Thus, the results may be more accurately reflective of the earwig’s physiological consumptive capacity across density and temperature.
3.4.2. **Generalist predator traits**

Our results show that given sufficient resource abundance, generalist predators can exhibit an insensitivity to density-dependence. This is not a particularly insightful observation, as limiting factors such as search rate would be maximized, while handling time minimized, as the predator approaches the theoretical maximum consumptive capacity. However, we were able to successfully provide good evidence that temperature can significantly affect this physiological maximum. Past work has shown that temperature will affect digestion rate, movement speed, and metabolism; all of these factors have been historically been considered to be subsumed by search rate and handling time parameters in a typical type II Holling model, but rarely are these factors disentangled or discussed (Dib et al., 2010; Rosenbaum & Rall, 2018; Symondson et al., 2002). There has been some evidence in the literature that suggest that generalist predation dynamics are less likely to be closely linked with prey density relative to specialists, since their capacity to utilize alternative resources allow them to be more flexible with preferences (Beddington et al. 1978; Symondson et al., 2002; Toft & Wise; 1999). This is consistent with our observations that the earwigs exhibited a relatively weak response to density within the range purported temperature range where they exhibit the most seasonal activity (Dib et al., 2011; Dixon et al., 2005; Orpet et al., 2019). Despite using a very similar experimental arena and laboratory conditions as others have used in the past (Asante, 1995; Dib et al., 2011; Sentis et al., 2011), the disparate results observed do not necessarily contradict the data found in the literature when the aforementioned factors and behavioural traits are taken into consideration. Even if we can incorporate these complex interactions into a more realistic model (which is doubtful) (Van Lenteren & Bakker, 1976), under field conditions, the shape of the functional response curve for generalist predators can be significantly influenced by the quality (and relative density) of alternative resources as well as the nutritional state of the predator (Greenstone, 1979; Hopper et al., 1995; Symondson et al., 2002).
3.4.3. **Implications for biocontrol**

The results in this chapter illustrate the limitations of functional response models for providing explanatory relevance for biocontrol practice in the field. However, we were able to provide evidence that earwigs, as ubiquitous generalist predators in their ecosystem, might be less reliant on density-dependent effects for predation performance than what you would expect from traditional functional response experiments which are better suited to explain the predation efficiency of specialists (Beddington et al., 1978; May & Hassell, 1981; Nicholson & Bailey; 1935). Being able to be remain consistent during periods of low pest density, can be a desirable trait for a biocontrol agent, as it allows the predator to persist in the environment even when pest numbers are low (Luff, 1982; Murdoch & Oaten, 1975; Southwood & Commins; 1976). Past observations have shown that generalists are more likely than specialists to curtail establishment of immigrating pests in an ecosystem, even while their numbers are low (Chiverton, 1986; Landis et al., 1997; Menalled et al., 1999). We were also able to find evidence that other environmental factors, which may be independent of pest density, can have an equal, if not greater magnitude of an effect on predation performance (Table 3-2, Table 3-3), this suggests that earwigs may be more limited by their physiology or consumptive ability rather than resource accessibility. By extension, we can hypothesize that generalist predators that share similar characteristics may function in an ecologically similar way. The results presented in this chapter address some aspects of the generalist/specialist dichotomy in biocontrol in the context of a very traditional model with a long history of being used as a measure of predation performance. Although these data are by no means exhaustive nor necessarily representative of many of the trends observed for generalist predators in biocontrol, they can at least provide an example of how the same measurable factors (like density, and temperature) can affect the performance of a biocontrol agent in drastically different ways due to fundamental trait differences which can be taken for granted. In order to move beyond theoretical models and correlational field data, we should perhaps prioritize expanding the scope of inquiry beyond merely refining traditional methods of investigation. One way this can be achieved would be through more precise hypothesis testing by doing more laboratory experiments based on observational data in the field. By doing so, we might be able to better identify desirable
characteristics and traits when selecting an appropriate biocontrol agent for the appropriate system that can help us bridge the gap between theory and practice.
3.5. Figures and Tables

Table 3-1. Parameter estimates obtained from fitted type II Holling equations for rosy apple aphid *Dysaphis plantaginea* and oblique-banded leafroller (*Choristoneura rosaceana*) (N = 48 per group for RAA, N = 120 for OBLR) T= time = 1 day. Parameter estimates that crossed above 1 for ‘a’ or below 0 for ‘h’ within the SE were omitted. Upper asymptote = maximum % prey consumed in 1 day. Search rate = proportion of 1 day to encounter 1 prey item.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Search Rate (a) (Proportion of 1 day to find 1 prey item) ± SE</th>
<th>Handling time (h) ± SE</th>
<th>Upper Asymptote (theoretical maximum % of total attacked per 1 day) (1/h)</th>
<th>Mean % attacked ± SE</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oblique-banded leafroller</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>N/A</td>
<td>N/A</td>
<td>0.10 ± 0.01</td>
<td>0.0631</td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>0.0469 ± 0.010</td>
<td>2.11 ± 0.26</td>
<td>0.47</td>
<td>0.29 ± 0.01</td>
<td>0.0797</td>
</tr>
<tr>
<td>20°C</td>
<td>0.0654 ± 0.012</td>
<td>1.77 ± 0.16</td>
<td>0.56</td>
<td>0.37 ± 0.02</td>
<td>0.0787</td>
</tr>
<tr>
<td>25°C</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.49 ± 0.01</td>
<td>0.0886</td>
</tr>
<tr>
<td>30°C</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.14 ± 0.01</td>
<td>0.0792</td>
</tr>
<tr>
<td></td>
<td>Rosy Apple Aphid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>0.0130 ± 0.007</td>
<td>5.43 ± 1.31</td>
<td>0.18</td>
<td>0.12 ± 0.01</td>
<td>0.0652</td>
</tr>
<tr>
<td>15°C</td>
<td>0.0255 ± 0.007</td>
<td>1.08 ± 0.31</td>
<td>0.92</td>
<td>0.39 ± 0.03</td>
<td>0.1524</td>
</tr>
<tr>
<td>20°C</td>
<td>0.0524 ± 0.025</td>
<td>1.46 ± 0.30</td>
<td>0.68</td>
<td>0.45 ± 0.03</td>
<td>0.2212</td>
</tr>
<tr>
<td>25°C</td>
<td>0.0441 ± 0.012</td>
<td>1.12 ± 0.19</td>
<td>0.89</td>
<td>0.50 ± 0.03</td>
<td>0.1655</td>
</tr>
<tr>
<td>30°C</td>
<td>0.0276 ± 0.013</td>
<td>1.35 ± 0.40</td>
<td>0.75</td>
<td>0.36 ± 0.03</td>
<td>0.1766</td>
</tr>
</tbody>
</table>

Table 3-2. Results of the effects of density broken down by temperature on proportion of oblique-banded leafroller (2nd instar) (OBLR), and rosy apple aphid (RAA) consumed over time (24 h) by earwigs using a generalized linear model (binomial distribution) using a logit function. (N = 120 observations for OBLR and N= 48 per group for RAA)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>ß</th>
<th>SE ß</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oblique-banded leafroller</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>0.0654</td>
<td>0.0127</td>
<td>28.208</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>15°C</td>
<td>0.0249</td>
<td>0.0069</td>
<td>13.182</td>
<td>1</td>
<td>0.0003*</td>
</tr>
<tr>
<td>20°C</td>
<td>0.0361</td>
<td>0.0055</td>
<td>43.174</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>25°C</td>
<td>0.0099</td>
<td>0.0057</td>
<td>3.041</td>
<td>1</td>
<td>0.0812</td>
</tr>
<tr>
<td>30°C</td>
<td>0.1365</td>
<td>0.0141</td>
<td>114.796</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rosy Apple Aphid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>0.0239</td>
<td>0.0064</td>
<td>14.806</td>
<td>1</td>
<td>0.0001*</td>
</tr>
<tr>
<td>15°C</td>
<td>0.0205</td>
<td>0.0077</td>
<td>7.241</td>
<td>1</td>
<td>0.0071*</td>
</tr>
<tr>
<td>20°C</td>
<td>0.0168</td>
<td>0.0100</td>
<td>2.849</td>
<td>1</td>
<td>0.0915</td>
</tr>
<tr>
<td>25°C</td>
<td>0.0288</td>
<td>0.0073</td>
<td>16.125</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>30°C</td>
<td>0.0170</td>
<td>0.0091</td>
<td>3.568</td>
<td>1</td>
<td>0.0589</td>
</tr>
</tbody>
</table>
Table 3-3. Factor effect of temperature, density, and interaction effect on proportion of oblique-banded leafroller (2nd instar) (OBLR), and rosy apple aphid (RAA) consumed over time (24 h) by earwigs using a generalized linear model (binomial distribution) using a logit function. (N = 600 observations for OBLR and N= 240 for RAA)

<table>
<thead>
<tr>
<th>Factor</th>
<th>β</th>
<th>SE β</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oblique-banded leafroller</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>0.0326</td>
<td>0.0060</td>
<td>29.848</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.0268</td>
<td>0.0048</td>
<td>31.532</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Density*Temperature</td>
<td>0.0008</td>
<td>0.0009</td>
<td>1.023</td>
<td>1</td>
<td>0.3118</td>
</tr>
<tr>
<td><strong>Rosy Apple Aphid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>0.0199</td>
<td>0.0045</td>
<td>19.802</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.0558</td>
<td>0.0095</td>
<td>35.812</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Density*Temperature</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.1121</td>
<td>1</td>
<td>0.7377</td>
</tr>
</tbody>
</table>
Figure 3-1. Functional responses of *Forficula auricularia* adults at five densities of *Dysaphis plantaginea*. The type II Holling disc equation was fitted separately for each temperature (n = 48) for all temperature treatments. RMSE values are represented as units of dependent variable (proportion of total prey items consumed over time period).
Figure 3-2. Functional responses of *Forficula auricularia* adults at five densities of *Choristoneura rosaceana*. The type II Holling disc equation was fitted separately for each temperature (n = 48) for all temperature treatments. RMSE values are represented as units of dependent variable (proportion of total prey items consumed over time period).
Chapter 4.

Concluding Remarks

Evaluation of biocontrol efficacy has historically focused on two aspects; (1) the capacity of the biocontrol agent to target and consume specified prey, and (2) the ability for this consumption to be directly responsible for regulating or impacting the pest populations. Often, this assumes the need for dietary specificity, and closely tied population dynamics between the biocontrol agent and the host. These demands are neither necessary, nor sufficient conditions for making a useful biocontrol agent, and are often difficult to evaluate in generalists, like European earwigs. Despite this fact, their ubiquity in agroecosystems and their prevalence in relation to many agricultural pests suggests that they have an important role in predator-prey food web dynamics.

In Chapter 2, I provided evidence that European earwig abundance can reliably track pest prevalence of key apple orchard pests, that they co-occupy temporally and spatially with these pests throughout the season, and that they are consuming them under natural field conditions. Secondly, I was able to find a strong negative correlation between both earwig abundance and earwig prevalence and the presence of both aphid and Lepidoptera pest groups. These observations, in conjunction, provide a reasonable amount of evidence that earwigs are likely having an impact on apple orchard pests and that their presence can provide us with an approximate ecological indicator of the pest status in an apple orchard.

In Chapter 3, I have also demonstrated that earwigs, like other generalist predators studied in the literature, can have their consumptive capacity greatly altered by extrinsic factors like temperature. I evaluated their predation performance using a simplified traditional functional response model and found that, under artificial conditions, traditional functional response studies poorly emulate conditions relevant for studying the physiological consumptive limits of a generalist predator and how factors like temperature can affect their predation efficiency. I also observed that under optimal temperatures, predation became far less density dependent on prey density, suggesting
that generalist predators may respond more strongly to extrinsic factors that affect their physiology, like metabolic rate, or digestive ability. This is especially interesting in the context of a generalist predator that is capable of switching prey species in response to temperature and can have significant implications in what we consider to be desirable traits in biocontrol agents.

Although this evidence is not sufficient to conclusively make a recommendation to utilize earwigs as conservation biocontrol agents in the field, they can still be used to elucidate their role in pest regulation in apple orchard ecosystems. The research here suggests that earwigs can be used as an indicator species for pest species prevalence, and that they are resilient predators that can scale their consumptive capacity with heterogenous environmental factors like temperature. The European earwig has been unfairly maligned for most of the time that they have co-occupied with humans. I hope that the evidence that I have presented in my thesis can, in some degree, shift our attitudes by shedding light on their ecological role in agroecosystems and serve as a small part in a larger trend of how we ought to approach generalist predators in future biocontrol research.
References


