The Effect of Snail-Associated *Chaetogaster* (Annelida: Naididae) on Host Behaviour and Fitness.

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Abstract

Members of the genus *Chaetogaster* (Annelida: Clitellata: Naididae) are small oligochaete worms found in freshwater habitats around the world. Most are free living predators or omnivores, but members of one species group are symbionts of molluscs, particularly snails. Despite being a common symbiotic association, little research has investigated the ecological aspects of this relationship. In particular, where the *Chaetogaster*-snail association falls on the mutualism-to-parasitism spectrum is still in question, especially since genetically distinct worm-host species pairings might result in different ecological relationships.

The broad purpose of this thesis is to investigate the relationship between symbiotic *Chaetogaster* and host snails from the family Physidae in central Alberta, including how these worms affect snail fitness and behaviour, as well as the potential for *Chaetogaster* to play a role in host defence against trematode parasites. To address these aims, I conducted a comprehensive survey that explored the host associations and population dynamics of *Chaetogaster* in the field, and several manipulative laboratory experiments.

Chapter 2 details my field-based research, conducting experiments and sampling in water bodies near Morinville and Fort Saskatchewan. My survey determined that abundance of *Chaetogaster* varies depending on season and snail host size. Sequencing of the 'barcode' region of the mitochondrial COI genes of hosts and worms revealed that the ponds surveyed each had two or more species of physid snail present, but only one species of *Chaetogaster*. My manipulative field experiments involved placing lab-reared *Physella acuta* snails in small cages in two ponds, half with *Chaetogaster* and half without. Unfortunately, due to destruction of field cages by wildlife and high mortality of experimental snails, the field experiments did not result in statistically useful data. A correlation analysis on data from field surveys indicated that there

is no association between high numbers of external *Chaetogaster* and trematode metacercariae within snails, suggesting that *Chaetogaster* may not prevent metacercariae from infecting freshwater snails.

Chapter 3 discusses two lab experiments regarding the effect of *Chaetogaster* colonization on physid fitness. In these experiments, one set of lab-bred *Physella acuta* were artificially colonized with worms and another set was not. The snails were individually monitored for 5 weeks. Using egg production as a proxy for host fitness, I found in both experiments that snails without *Chaetogaster* produced significantly more eggs than those with *Chaetogaster* over the five-week time frame. These results suggest that worm presence decreases host fitness.

Chapter 4 includes two experiments examining the behaviour of snails with and without symbiont worms. In the first, I observed snails collected from the field with naturally varying *Chaetogaster* abundances to quantify the distance that each snail moved over the course of an hour. I found no difference in the movement behaviour of snails regardless of *Chaetogaster* number. In the second experiment, I exposed lab-bred *Physella acuta* to conspecifics with and without *Chaetogaster* to determine if *Chaetogaster* presence attracts or repels nearby snails, and whether attraction/repulsion is influenced by whether the focal snail itself was carrying *Chaetogaster*. I did not find a pattern of focal snails preferring or avoiding snails with symbionts, which could either indicate that the snails are indifferent to *Chaetogaster* or that they are unable to detect the chemical signature of the worms in the water.

Although the results of my experiments cannot be used to definitively determine if *Chaetogaster* symbionts positively or negatively affect the snail species that I studied, for the lab-bred *Physella acuta*, they do suggest a general negative influence of symbiont worms on host

snails. It may be that after generations of living in captivity without *Chaetogaster*, members of this population of *P. acuta* have become more sensitive to these worms than snails in wild populations. Future research, particularly into other worm-snail species pairs, will be necessary to further elucidate this complex relationship.

Wisdom comes from experience. Experience is often a result of lack of wisdom.

-Terry Pratchett

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Table of Contents

Abstract ii

Acknowledgements vi

Table of Contents ix

List of Tables xii

List of Figures xv

List of Appendices xvii

CHAPTER 1. INTRODUCTION 1

Background 1

Terminology Overview 1

Why Study Symbioses? 2

Evolution 4

Context Dependency 6

Host Manipulation 8

Chaetogaster-snail symbiosis 9

Thesis Aims 12

Figures 14

CHAPTER 2. *CHAETOGASTER* IN THE FIELD AND MOLECULAR DIAGNOSTICS 15

Introduction 15

Overview 15

Field Surveys of *Chaetogaster* Dynamics 15

Does *Chaetogaster* Protect its Hosts from Trematodes? 18

Species Identification 21

Methods 22

Chaetogaster Survey 22
Cage Experiments 25
Molecular Analysis 31
Gut Contents 34
Results 35
Chaetogaster Survey 35
Cage Experiments 40
Molecular Analysis 42
Gut Contents 44
Discussion 45
Genetic Diversity 45
Chaetogaster Surveys 46
Water Quality 50
Host Defence 51
Gut Contents 52
Tables 54
Figures 64
CHAPTER 3. FITNESS EXPERIMENTS 84
Introduction 84
Methods 86
Fitness Experiment 1 86
Fitness Experiment 2 87
Statistics 88
Results 91
Fitness Experiment 1 91
Fitness Experiment 2 92

Discussion 95

Tables 99

Figures 103

CHAPTER 4. BEHAVIOUR EXPERIMENTS 114

Introduction 114

Host Manipulation 114

Changes in Host Activity 116

Chaetogaster Dispersal 117

Methods 119

Movement Assays 119

Preference Experiment 121

Results 126

Movement Assays 126

Snail Preference Experiment 128

Discussion 134

Movement Assays 134

Preference Experiments 136

Tables 140

Figures 150

CHAPTER 5. SYNTHESIS 162

Summary and Conclusions 162

Future Directions 165

LITERATURE CITED 168

APPENDICES 189

List of Tables

Table 2-1. Summary of snail survey dates in 2021. 54

Table 2-2. Summary of snail survey dates in 2022. 54

Table 2-3. List of specimens used for DNA analysis. 55

Table 2-4. Snail sequences chosen from GenBank for tree creation. 57

Table 2-5. *Chaetogaster* sequences chosen from GenBank for tree creation. 58

Table 2-6. List of specimens used for *Chaetogaster* gut contents investigation. 59

Table 2-7. Summary of *Chaetogaster* survey over both years and locations. 59

Table 2-8. Model creation for Lafarge 2021 survey data. 60

Table 2-9. Analysis of deviance table for model 2, Lafarge 2021. 60

Table 2-10. Model creation for Morinville 2021 data. 60

Table 2-11. ANOVA table for Morinville 2021 model output. 60

Table 2-12. Model selection for *Chaetogaster* survey in Lafarge 2022. 61

Table 2-13. Summary of statistical results, Lafarge 2022. 61

Table 2-14. Model selection for *Chaetogaster* density in Morinville 2022. 62

Table 2-15. Analysis of deviance table for *Chaetogaster* density, Morinville 2022. 62

Table 2-16. Cage Experiment 3 dissection results. 63

Table 2-17. Table of water quality results from Lafarge and lab colony water samples. 63

Table 3-1. Snails used for and excluded from analysis in Fitness Experiment 1. 99

Table 3-2. Average number of eggs produced/week by snails in Fitness Experiment 1. 99

Table 3-3. Percent of snail eggs that hatched, Fitness Experiment 1. 99

Table 3-4. Model selection for Fitness Experiment 1. 100

Table 3-5. Statistical results for Fitness Experiment 1. 100

Table 3-6. Snails used for analysis for Fitness Experiment 2. 100

Table 3-7. Average number of eggs produced/week by snails in Fitness Experiment 2. 101

Table 3-8. Model selection for Fitness Experiment 2. 101

Table 3-9. Model summary for Fitness Experiment 2. 102

Table 4-1. Some implications of potential outcomes of the Preference Experiment. 140

Table 4-2. Average distance travelled Movement Assay 1. 140

Table 4-3. Model selection and creation for Movement Assay 1 141

Table 4-4. Analysis of Deviance table (Type II tests) for Model 6 (Assay 1). 141

Table 4-5. Average distance travelled Movement Assay 2. 142

Table 4-6. Model selection for Movement Experiment (Assay 2). 142

Table 4-7. ANOVA table (Type II tests) for model 4 (Assay 2). 143

Table 4-8. Summary of snail first choice by treatment. 143

Table 4-9. Model selection for first choice in Preference Experiment. 143

Table 4-10. Summary statistics table for Model 2 (First choice). 144

Table 4-11. Model selection for time to first choice (Preference Experiment). 144

Table 4-12. Summary statistics table for Model 2 (time to first choice). 144

Table 4-13. Summary table for first arrestment choice (Preference Experiment). 145

Table 4-14. Model selection for first arrestment choice (Preference Experiment). 145

Table 4-15. Summary Statistics for Model 3 (First arrestment choice). 145

Table 4-16. Model selection for time to first arrestment (Preference Experiment). 146

Table 4-17. Summary Statistics for Model 3 (time to first arrestment). 146

Table 4-18. Summary table of longest arrestment data. 146

Table 4-19. Model selection for longest arrestment choice (Preference Experiment). 147

Table 4-20. Summary statistics of Model 3 (longest arrestment choice). 147

Table 4-21. Model selection for duration of longest arrestment. 147

Table 4-22. Analysis of variance summary table (duration of longest arrestment). 147

Table 4-23. Mean number of time checks spent in each area of arena. 148

Table 4-24. Model selection for time spent in each area. 148

Table 4-25. Model 4 analysis of deviance table (time spent in each area). 148

Table 4-26. Summary table for the movement of tested snails (Preference Experiment). 149

Table 4-27. Model selection for movement analysis (Preference experiment). 149

Table 4-28. Model 2 analysis of deviance table (movement analysis). 149

List of Figures

Figure 1-1. Images of experimental organisms. 14

Figure 2-1. Morinville and Lafarge Canada collection locations. 64

Figure 2-2. Images of cage experiment setup. 65

Figure 2-3. Overall abundance of external *Chaetogaster* in 2021 and 2022. 66

Figure 2-4. Abundance of external *Chaetogaster* relative to snail size for all surveyed snails. 67

Figure 2-5. Abundance of external *Chaetogaster*/snail at Lafarge in 2021. 68

Figure 2-6. External *Chaetogaster* abundance by host snail size from Lafarge in 2021. 69

Figure 2-7. Abundance of external *Chaetogaster*/snail at Morinville in 2021. 70

Figure 2-8. External *Chaetogaster* abundance by snail length from Morinville 2021. 71

Figure 2-9. Metacercariae counts from 241 Lafarge snails in 2022. 72

Figure 2-10. Number of external *Chaetogaster*/snail in Lafarge per month in 2022. 73

Figure 2-11. *Chaetogaster*/snail numbers from 241 Lafarge snails over summer 2022. 74

Figure 2-12. Metacercariae counts from 243 Morinville snails in 2022. 75

Figure 2-13. The number of internal *Chaetogaster* by snail size for Morinville 2022. 76

Figure 2-14. Number of external *Chaetogaster*/snail in Morinville over summer 2022. 77

Figure 2-15. *Chaetogaster* abundance by snail size in Morinville over summer 2022. 78

Figure 2-16. Snail cages disturbed by local wildlife in 2021. 79

Figure 2-17. Physid neighbour-joining tree with p-distances. 80

Figure 2-18. *Chaetogaster* Neighbour Joining tree with p-distances. 81

Figure 2-19. Images of slide mounted external *Chaetogaster* from field-collected physids. 82

Figure 2-20. Images of slide mounted *Chaetogaster* from lab-bred snails. 83

Figure 3-1. Image of Fitness Experiment set up. 103

Figure 3-2. Frequency of total egg production/snail in each group in Fitness Experiment 1..... 104

Figure 3-3. Total number eggs produced/snail in Fitness Experiment 1 averaged per group. ... 105

Figure 3-4. Egg production over the 6-week period of Fitness Experiment 1, by group. 106

Figure 3-5. The total number of eggs/snail in Fitness Experiment 1 arranged by initial length. 107

Figure 3-6. Size comparison between treatment considering initial treatment and end groups. 108

Figure 3-7. Frequency of total egg production/snail in each group in Fitness Experiment 2..... 109

Figure 3-8. Total number of eggs produced/snail in Fitness Experiment 2 averaged per group. 110

Figure 3-9. Snail status over time by group in Fitness Experiment 2. 111

Figure 3-10. Total number of eggs/snail in Fitness Experiment 2 arranged by initial length. ... 112

Figure 3-11. Size comparison between treatments and end groups. 113

Figure 4-1. Diagram of Preference Experiment arena. 150

Figure 4-2. Total distance travelled by physid snails in Movement Assay 1. 151

Figure 4-3. Total distance travelled by physid snails in Movement Assay 2. 152

Figure 4-4. First choice made by tested snails. 153

Figure 4-5. Time to first choice made by tested snails. 154

Figure 4-6. First arrestment made by tested snails. 155

Figure 4-7. Tested snails from each treatment according to area of first arrestment. 156

Figure 4-8. Longest arrestment choice made by tested snails. 157

Figure 4-9. Time spent in longest arrestment. 158

Figure 4-10. Average snail position in arena at each time check. 159

Figure 4-11. Number of time checks tested snails spent in each section of arena. 160

Figure 4-12. Number of switches by tested snails between sections of arena. 161

List of Appendices

Appendix 1-1. A list of scientific literature focused on snail-associated *Chaetogaster limnaei*. 189

Appendix 2-1. Formula for Artificial Spring Water (ASW). 193

Appendix 2-2. Methods for water quality analyses. 194

Appendix 2-3. List of GenBank sample depositors for tree construction (snail subset). 196

Appendix 2-4. List of GenBank sample depositors for tree creation (*Chaetogaster* subset). 198

Appendix 2-5. Raw survey data from Lafarge ponds 2021. 200

Appendix 2-6. Raw survey data from Morinville (Heritage Lake) in 2021. 203

Appendix 2-7. Raw survey data from Lafarge Pond 1 in 2022. 205

Appendix 2-8. Raw survey data from Morinville (Heritage Lake) in 2022. 212

Appendix 2-9. Correlation analysis from 241 snails from Lafarge in 2022. 219

Appendix 2-10. Correlation analysis from 243 snails from Morinville in 2022. 220

Appendix 2-11. CO1 sequences from snail and worm specimens sequenced in this thesis. 221

Appendix 2-12. Neighbor-joining distance matrix (snail subset). 228

Appendix 2-13. Neighbor-joining distance matrix (*Chaetogaster* subset). 232

Appendix 2-14. The number of *Chaetogaster*/snail according to trematode infection status. 236

Appendix 3-1. Test of snail length measurements repeatability in ImageJ. 237

Appendix 3-2. Change in length of snails used in Fitness Experiment 1 (original data). 238

Appendix 3-3. Egg production by lab-bred snails in Fitness Experiment 1. 240

Appendix 3-4. Egg production by lab-bred snails in Fitness Experiment 2. 242

Appendix 4-1. Raw data from Movement Assay 1 for field-caught snails from Lafarge. 244

Appendix 4-2. Raw data from Movement Assay 2 for field-caught snails from Morinville. 245

Appendix 4-3. Raw Preference Experiment data. 246

Chapter 1. Introduction

Background

Terminology Overview

Studying the interactions between living organisms is one of the most fascinating and complex facets of biology. Understanding the nature of symbioses, which from the point of view of the host can range from mutualistic to parasitic (Ewald 1987; Bronstein 1994; Leung and Poulin 2008; Skelton et al. 2016), is of increasing importance given recent molecular evidence that few multicellular organisms exist without at least prokaryotic symbionts (Li et al. 2023; Mousa et al. 2022). Etymologically, 'symbiosis' comes from the Greek word for 'living together' (Dimijian 2000). The term symbiosis was originally used to describe a reciprocally positive relationship between two organisms (= mutualism)(Leung and Poulin 2008); however more recently, this term has become more general. In this thesis, I will follow the definition given by Leung and Poulin (2008) "…an intimate interaction between different organisms, where at least one of the parties is obligatorily dependent on the association as a part of its life history". Predation, in which one organism kills and consumes another, is not considered under this definition, since the two organisms involved do not live intimately together even temporarily.

Under this broad definition, symbioses may range from mutualistic, in which both organisms benefit from the relationship, to parasitic, where one party benefits and the other is negatively affected, or commensal, in which one party benefits and the other is neither harmed nor benefited. Since symbiotic relationships are complex and often don't fit neatly under one of these three labels, I will consider symbioses on a continuum (the mutualism-to-parasitism continuum) (Ewald 1987; Bronstein 1994; Leung and Poulin 2008; Skelton et al. 2016) with the

understanding that benefits and costs to either partner may vary depending on context. Symbiotic relationships have evolved countless times throughout history in all kingdoms and domains of life, between closely related organisms and evolutionarily distant ones (Dimijian 2000; Leung and Poulin 2008; Joy 2013).

Why Study Symbioses?

Symbiosis is likely one of the most common interactions between organisms, with parasitism in particular being one of the most successful life-history strategies (Poulin and Morand 2000; Dobson et al. 2008; Leung and Poulin 2008). Symbioses have evolved countless times in all groups of life and are likely one of the reasons for extremely rapid diversification in some clades (Poulin and Morand 2000; Sapp 2004; Hechinger and Lafferty 2005; Brucker and Bordenstein 2012; Joy 2013; Horká et al. 2016; Chow et al. 2021). These relationships are often highly context dependent and difficult to detangle (Karst et al. 2008; Leung and Poulin 2008; Chamberlain and Nathaniel Holland 2009; Brown et al. 2012; Shantz and Burkepile 2014). Scientists often argue over how symbionts affect or manipulate their host as well as how to define such behaviour (Poulin 2000; Thomas et al. 2005; Poulin 2010; Herbison et al. 2018; Poulin 2019).

With symbioses playing such a large role in ecosystems (Leung and Poulin 2008; Dunne et al. 2013; Thieltges et al. 2013; Frainer et al. 2018; Friesen et al. 2020; Timi and Poulin 2020), it is crucial to examine these relationships to determine how they work with special consideration as to how they may react to anthropogenic activities that are changing the environment. For example, a symbiosis of critical concern is that between corals (Cnidaria) and dinoflagellates (Dinoflagellata) commonly from the genius *Symbiodinium*, known as zooxanthellae (Lesser et al.

2013; Baker et al. 2018). This symbiosis is posited to benefit both parties in nutrient-poor but light-rich environments, where the corals provide carbon dioxide and base nutrients (e.g., ammonium) to the photosynthetic dinoflagellate symbionts, which in turn provide the coral with energy for growth (Wooldridge 2010; Lesser et al. 2013; Baker et al. 2018). This mutualism, however, is under stress due to increased ocean temperatures and other anthropogenic factors (Wooldridge 2010; Lesser et al. 2013; Baker et al. 2018). Research suggests that increased temperatures and eutrophication shift this relationship in favour of dinoflagellate symbionts that provide fewer benefits to coral and act more like parasites, although others suggest that the relationship was never mutualism to start, but rather a forced domestication of dinoflagellates (Wooldridge 2010; Lesser et al. 2013; Shantz and Burkepile 2014; Baker et al. 2018). In extreme, but increasingly common cases, anthropogenic effects can cause 'coral bleaching' where corals lose their zooxanthellae (Wooldridge 2010; Lesser et al. 2013; Baker et al. 2018). Coral bleaching is often followed by decreased coral fitness and increased mortality (Wooldridge 2010; Lesser et al. 2013; Baker et al. 2018). With anthropogenic effects tipping the balance between coral and zooxanthellae, research examining the delicate mutualism-to-parasitism balance may be the key to protecting this ecologically important relationship.

Attention to symbioses is also vital when conducting research that isn't directly focused on symbiotic interactions. Despite the ubiquitous nature of symbiosis, many general ecologists do not regularly consider symbionts when conducting their research (Lafferty et al. 2006; Timi and Poulin 2020). Timi and Poulin (2020) argue that ignoring parasites when studying the ecology, behaviour, and physiology of any organism can lead to grievous errors in interpretation. As examples, they present multiple instances of fish parasites altering fish weight, behaviour, stable isotope composition and community structure. The first example, relating to fish weight, is

especially compelling. Body mass index is a common measurement used as a proxy for body condition for fish (i.e., heavier fish relative to length are assumed to be in better condition); however, parasite biomass is rarely considered in this measurement (Timi and Poulin 2020). It was found fish condition was overestimated when parasite biomass was not accounted for (Lagrue and Poulin 2015; Timi and Poulin 2020). Since fish can be parasitized in high density, and therefore have a high parasite mass (Santoro et al. 2013; Timi and Poulin 2020) it would be a mistake to not include this metric when considering fish body condition. Although this example only discussed fish and their symbiont parasites, it would be remiss to think that this problem does not extend to research conducted on other free-living organisms. Going forward, it would be prudent to not only study symbioses directly, but to also consider these relationships and their effects in other branches of biology.

Evolution

Symbiosis has played a vital role in the history of the entire Domain Eukaryota, as the origin of the first eukaryotic cell involved endosymbiosis between one or more prokaryotic cells (Margulis and Bermudes 1985; López-García et al. 2017; López-García and Moreira 2020; Speijer 2020). Subsequent symbioses between eukaryotes and prokaryotes, and between eukaryotes and eukaryotes, produced diverse lineages of algae and other photoautotrophs (Krings et al. 2009; Ponce-Toledo et al. 2017; de Vries and Archibald 2017; de Vries and de Vries 2022). With advances in molecular techniques, we are becoming increasingly aware of how microbiomes of multicellular eukaryotes vary among taxa and individuals within a species, and how they may influence host fitness (Akbar et al. 2022; Bai et al. 2022; Li et al. 2023; Mousa et al. 2022).

It has been repeatedly argued that symbioses are a core reason for rapid evolution in some clades (Poulin and Morand 2000; Sapp 2004; Hechinger and Lafferty 2005; Brucker and Bordenstein 2012; Joy 2013; Horká et al. 2016; Chow et al. 2021). This includes the bacteria-eukaryote symbiosis, protective symbioses with palaemonid shrimp, trematode parasites and their diversity of hosts, and many more. Palaemonids represent a particularly convincing example. The Palaemonidae is a diverse family of decapod shrimp, with over 700 described species (Chow et al. 2021). In this group there are a few instances of cleaner-client symbioses in which the shrimp remove parasites or detritus from their hosts; however, these are in the minority compared to symbioses where the shrimp resides on a larger organism for their own protection (e.g., to hide from predators) and may neither benefit nor harm the host (Chow et al. 2021). Of all the species described in this family, it is estimated that over 60% of them are part of a symbiotic relationship (de Grave 2001; Chow et al. 2021). It has been suggested that a great portion of the diversity found in this group is due to evolution through new symbioses (e.g., host switching – in which a new species evolves after the symbiont switches to a new host) (Horká et al. 2016; Chow et al. 2021), although some have argued to the contrary (Davis et al. 2018). Playing host to this family of shrimp are a variety of taxa including Cnidaria, Echinodermata, Mollusca, Tunicata, Porifera and other Decapoda (Bruce et al. 2006; Horká et al. 2016; Chow et al. 2021). Generally, palaemonid shrimp symbioses are considered to lean towards the commensalism and mutualism end of the spectrum, however there is also evidence of parasitic behaviour in this group (de Grave 2001; de Grave et al. 2021; Ďuriš et al. 2011; Horká et al. 2016) and I am sure our understanding of symbiosis in this clade will continue to change as the body of knowledge continues to grow. Overall, palaemonid shrimp are a good example of how symbiosis can promote rapid evolution, although there are undoubtedly other factors at play.

Context Dependency

Although we would like to give each symbiosis a clear label as either mutualism, parasitism, or commensalism, this is seldom possible in reality. Symbioses are rarely, if ever, so clear cut and will often change depending on context (e.g., other organisms in the community, surrounding abiotic factors, life history phase, etc.) and individuals involved (Karst et al. 2008; Leung and Poulin 2008; Chamberlain and Holland 2009; Brown et al. 2012; Shantz and Burkepile 2014).

A clade with abundant symbioses is Hymenoptera, which includes sawflies, bees, wasps, and ants. While already a fascinating group of eusocial organisms on their own, ants are even more intriguing when one considers their roles in different symbioses. Many ant species have symbioses with bacteria, fungi, other insects (e.g., Hemiptera, Lepidoptera) and plants (Currie 2001; Defossez et al. 2009; Mayer et al. 2014; Sanchez and Bellota 2015; Chomicki and Renner 2017; Pérez-Lachaud et al. 2021). These symbioses can fall anywhere on the mutualism-to-parasitism spectrum and may move along this spectrum depending on the variables at play. One such symbiosis which purportedly falls on the mutualism end of the spectrum includes ant colonies that live in the highly modified leaves and stems of myrmecophytic plants. Here, the ants provide their hosts with different combinations of the following: protection from herbivory or fungal infection, protection from competition with other plants, and extra nutrients like nitrogen (Heil et al. 2001; Mayer et al. 2014; Chomicki and Renner 2015; Sanchez and Bellota 2015). In return, the myrmecophyte provides shelter in the form of hollow spaces called ‘domatia’ and sometimes food for its symbiotic partners (Mayer et al. 2014; Chomicki and Renner 2015; Sanchez and Bellota 2015). While at first glance this appears to be a clearly mutualistic relationship, the benefit to each party is not always equal. For instance, if few

herbivores are present or if the ant mutualists are not efficient herbivore/competitor deterrents, then the host plant may be providing the ant colony with shelter and food while receiving little in return (Bronstein et al. 2006; Okabe and Makino 2008).

Although sometimes a temporary association, cleaning symbioses are another example that highlights the context dependent nature of symbioses. Cleaning symbioses are a subset of short-term symbiotic relationships that involve smaller organisms removing detrimental material (e.g., dead tissues, parasites, wastes) from the body or nests of larger individuals. Cleaner-client interactions are common across oceans globally, and usually consist of small cleaner fish or crustaceans in one locale being visited by larger fish for 'cleaning' (Losey 1987; Poulin and Grutter 1996; Grutter 1999; Arnal et al. 2000; Arnal et al. 2001; Freckleton and Côté 2003; Vaughan et al. 2017). Approximately 259 species of marine and freshwater fish and crustaceans are known to participate in cleaner symbioses; however, it is likely that many more have yet to be discovered (Vaughan et al. 2017). During these temporary symbiotic interactions, cleaner fish move about the client removing ectoparasites, mucus and scales from the body, mouth, and gills of the client fish (Grutter 1997; Grutter 1999; Grutter and Bshary 2003; Côté and Mills 2020). Cleaner-client fish symbioses have been shown to improve not only the health of the host, but also to increase local fish abundance and diversity (Bshary 2003; Grutter et al. 2003; Waldie et al. 2011). They are of particular interest to fisheries as a potential method of parasite biocontrol (e.g., to reduce numbers of sea lice on farmed salmon); however, this research is still on-going (Overton et al. 2020). These relationships are usually considered mutualistic ones, since the client gains improved health from the removal of ectoparasites and the cleaners gain food (Grutter 1999; Waldie et al. 2011), but this classification can change depending on the context. Both cleaner and client fish have both been shown to 'cheat' in their interactions with the other

(Bshary and Grutter 2002; Grutter and Bshary 2003; Côté and Mills 2020). For example, cleaner fish may choose to feed on fish mucus or tissue rather than on ectoparasites due to preference or availability, which is not beneficial to the client fish (Bshary and Grutter 2002; Grutter and Bshary 2003; Cheney and Côté 2005). Although less common, there are also examples of predatory client fish 'cheating' by eating or attempting to eat cleaner fish (Trivers 1971; Bshary and Grutter 2006; Côté and Mills 2020). These instances of 'cheating' cleaner and client fish can cause a predominantly mutualistic symbioses to skew towards commensalism or parasitism.

Host Manipulation

Host manipulation is a trendy but difficult to pin down subtopic of symbioses on the parasitism end of the continuum. This phenomenon occurs when parasites are able to change the behaviour or physiology of their hosts (Thomas et al. 2005; Poulin 2010; Poulin and Maure 2015; Hughes and Libersat 2019). The caveat here however, that is often overlooked, is that this change must confer some sort of advantage to the fitness of the parasite, generally in terms of transmission from intermediate hosts to final hosts. Although there has been an up-tick in the number of papers considering this phenomena, multiple articles have argued that these examples of host manipulation are incomplete or exaggerated (Poulin 2000; Poulin 2010; Poulin and Maure 2015; Herbison et al. 2018; Doherty 2020). With this is mind, it is important to conduct thorough research when assessing possible cases of host manipulation and to take any affirmative reports with a grain of caution. Here I give an example of a possible instance of host manipulation between ants and butterflies, to illustrate the complexity of this phenomenon.

There are numerous reports of caterpillars from the family Lycaenidae (Lepidoptera) manipulating ant colonies into caring for the larval stage of the species (Als et al. 2001; Nash et

al. 2011; Tartally et al. 2019). One such example is the Alcon Blue butterfly, *Phengaris alcon* (Denis & Schiffermüller), which is an obligate brood parasite of *Myrmica* ant colonies (de Vries and Cocroft 1993; Nash et al. 2011; Tartally et al. 2019). The larva of this species of butterfly will initially feed on a specific host plant before using mimicry to trick an ant colony into adopting it (Akino et al. 1999; Nash et al. 2011; Tartally et al. 2019). The butterfly larva uses chemical and acoustic cues to impersonate a larval ant and will be carried back to the nest and placed in the brood chamber (de Vries and Cocroft 1993; Akino et al. 1999; Nash et al. 2011; Tartally et al. 2019). There the larva will either act as a predator on larval ants or be fed directly by ants in the colony until it pupates and undergoes metamorphosis. In order for the Alcon Blue to reach maturity, a complex series of events must occur, the most important of which is the successful manipulation of an appropriate host ant colony. Although this is by far my favourite example of butterfly-ant symbiosis, there are many others, some of which lean closer to the symbiosis end of the continuum (Maschwitz et al. 1984; Thomas et al. 1989; Fiedler 1998; Als et al. 2002).

Superficially, the idea of host manipulation is quite simple, the devil however is in the details as conducting experiment rigorous enough to prove such a phenomena can be difficult. As it is, most examples of parasite manipulation are likely still in the discovery phase rather than a statement of fact.

Chaetogaster-snail symbiosis

Members of the genus *Chaetogaster* (Annelida: Clitellata: Naididae) are freshwater oligochaete worms that, depending on the species, may be free-living or symbiotic. Hosts of symbiotic *Chaetogaster* are mostly snails (Mollusca: Gastropoda) although some clams may also

be colonized (Mollusca: Bivalvia) (Buse 1974; Gruffydd 1965; Liquin et al. 2021; Mack et al. 2023; Smythe et al. 2015). The focus of my thesis is the symbiosis between *Chaetogaster* and physid snails in Alberta (Figure 1-1).

Historically, *Chaetogaster* consisted of three species with type localities in Europe (Mack et al. 2023): two free-living species *Chaetogaster diaphanus* (Gruithuisen) and *C. diastrophus* (Gruithuisen), and the symbiotic species *C. limnaei* von Baer. *Chaetogaster limnaei* was subsequently divided into two subspecies: *C. limnaei limnaei* von Baer and *C. limnaei vaghini* Gruffydd (Gruffydd 1965a; Gruffydd 1965b). The rationale for Gruffyd's establishment of the two subspecies was mostly based on the worm's location on the host. *Chaetogaster limnaei limnaei* individuals were distinguished by being ectosymbionts living on the outside of their snail host, eating items from the surrounding water. In contrast, *C. limnaei vaghini* were distinguished by their being endosymbionts, inhabiting the kidneys of snail hosts and (presumably) feeding mostly on kidney cells (Gruffydd 1965a). In addition to microhabitat and diet, some morphological and behavioural differences between these two groups have been observed (Buse 1972; Gruffydd 1965a).

Ranging in length from 1-2 mm, *Chaetogaster limnaei* have a typical annelid body plan with some modifications, such as pharynx with increased muscularization for sucking in food items (Mack et al. 2023). These worms also lack chaetae (stiff bristles) on their dorsal side, although the ventral set is present and used for locomotion (Figure 1-1). Snail-associated *Chaetogaster* live on the head-foot region of their host snail. Based on observations of gut contents and behaviour of living worms, they feed on small invertebrates, algae, and debris found in the water column adjacent to the snail or on the snail's body (Buse 1972; Gruffydd 1965a). *Chaetogaster limnaei* primarily reproduces asexually by budding; although they also

appear to occasionally reproduce sexually as well, information on this is scarce (Buse 1972; Gruffydd 1965b; Mack et al. 2023). Worms in this group will rarely leave their host, unless a new host is readily available (i.e., two snails come into contact and a worm transfers from one to the other) and worm mortality is higher when it is not on a host snail (Gruffydd 1965b; Shaw 1992; Hopkins et al. 2015).

Chaetogaster limnaei has been considered a species with a nearly global distribution, and experiments from around the world have cited this species as their experimental organism (Mack et. al. 2023) (Appendix 1-1). A good half of these studies focus on assessing the role of *Chaetogaster* as a protective symbiont that reduces infection of host snails by intercepting and eating larvae of parasitic trematodes (Khalil 1961; Michelson 1964; Sankurathri and Holmes 1976; Fernandez et al. 1991; Ibrahim 2007). Other studies investigate the general ecology of *Chaetogaster* worms (Sankurathri & Holmes 1976; Young 1974) and the potential negative effects of *Chaetogaster* colonization on snails, including the possibility of *Chaetogaster* reducing the fitness of host snails (Rodgers et al. 2005; Stoll et al. 2013, 2017) or consuming host tissue (Gamble & Fried 1976).

However, recent molecular work has brought this three-species classification (*C. diaphanus*, *C. diastrophus*, and *C. limnaei*) into question and determined that species richness in the genus *Chaetogaster* is higher and the phylogeny much more complex than originally estimated (Mack et al. 2023). Instead of just a few species with global dominance, it is likely that *Chaetogaster* encompasses a wide range of species with smaller local distributions. For the purpose of this thesis, I will simply refer to the worm symbionts used in my experiments as '*Chaetogaster*'. A discussion of the identity of the species used in my experiments, based on comparison with sequence data from Mack et al. (2023), is in Chapter 2. The primary focus of

this thesis is on *Chaetogaster* that live externally to their hosts as ectosymbionts, therefore '*Chaetogaster*' will be in reference to an external worm, unless otherwise specified.

Thesis Aims

Where the symbiotic relationship between *Chaetogaster* and their snail hosts falls on the mutualism-to-parasitism continuum is still not well understood. Relatively few published papers have examined the relationship directly, and those that do generally consider only one small aspect of it (Appendix 1-1). Over time, researchers have described the relationship between *Chaetogaster* and their hosts as parasitism, commensalism, mutualism, and 'epizoic antibiosis' (Stoll et al. 2013), which considered altogether can create a confusing picture for the average reader. One main focus of my thesis is to investigate where this symbiotic relationship falls on the mutualism-to-parasitism spectrum for *Chaetogaster* and physid snails from Alberta. I conducted multiple field and laboratory experiments to explore how the presence of *Chaetogaster* worms affects the fitness and behaviour of their physid hosts. Another aim was to determine how time of year and host size were related to *Chaetogaster* abundances in the field.

With these goals in mind, I created five hypotheses that are tested and discussed in my thesis chapters:

1. *Chaetogaster* abundance on individual snails is affected by host size and seasonal factors (Chapter 2).
2. *Chaetogaster* presence is minimizes trematode infection rates in host snails (Chapter 2).
3. *Chaetogaster* presence affects host fitness (Chapter 3).
4. *Chaetogaster* presence changes the activity (movement vs rest) of host snails (Chapter 4).

5. *Chaetogaster* symbionts change the snail preference for associating with conspecifics of certain symbiont status. (Chapter 4).

Figures

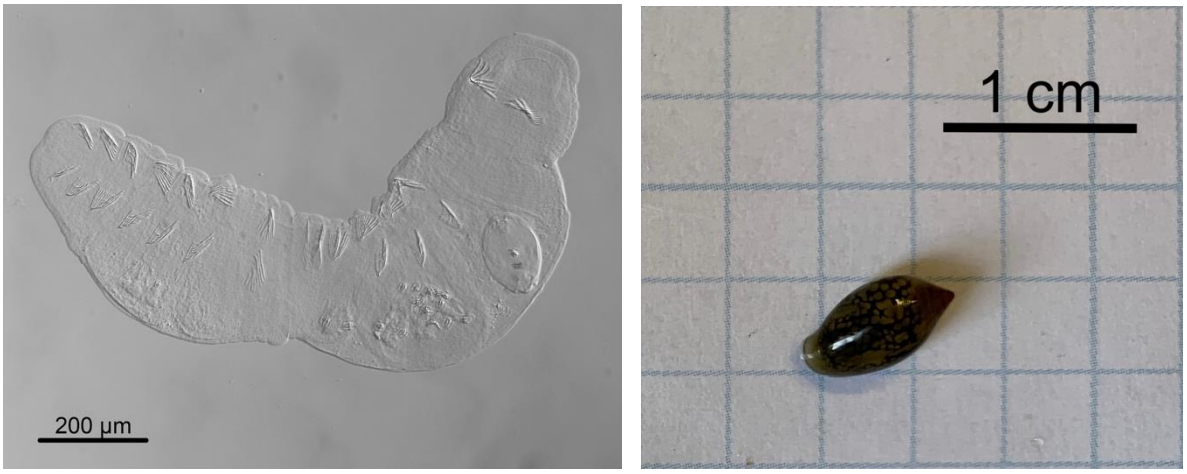

Figure 1-1. Images of experimental organisms. Left image is of an individual *Chaetogaster* under a compound microscope (Slide mount and photo credit to Heather Proctor). The *Chaetogaster* was from a line of organisms originally collected from Morinville. Right image of a physid snail from Morinville collected in July of 2022.

Chapter 2. *Chaetogaster* in the Field and Molecular Diagnostics

Introduction

Overview

Although there have been several studies investigating interactions between *Chaetogaster* and their hosts in the lab (Appendix 1-1), there has been little supporting data collected in the field. In this chapter, I present the results of two projects conducted in standing water bodies near Edmonton, Alberta. The first is a survey I completed to investigate the abundance of *Chaetogaster* in the field and relationship between snail size, parasitism of snails by trematodes and *Chaetogaster* abundance. I used some of the field-collected specimens for DNA barcoding to determine the identities of *Chaetogaster* and hosts. The second project was an experiment in which I placed caged snails with and without *Chaetogaster* into ponds to investigate if there is evidence of *Chaetogaster* protecting their hosts from trematodes.

As a note, in this thesis I use 'prevalence' to refer to the percentage or proportion of examined hosts in a population with a given symbiont. 'Intensity' is the number of *Chaetogaster* per snail excluding snails that have no *Chaetogaster*, while abundance is the same, but includes snails without *Chaetogaster*.

Field Surveys of *Chaetogaster* Dynamics

Seasonal dynamics of *Chaetogaster* in the field has been described in six articles to date, most from non-Canadian sites (USA: Fernandez et al., 1991; United Kingdom: Gruffydd, 1965b; Egypt: Ibrahim, 2007; Germany: Stoll et al., 2017; United Kingdom: Young, 1974) and from one

Lake Wabamun in Alberta (Sankurathri and Holmes 1976). Below I describe these surveys in temporal order.

A survey of the snail *Lymnaea peregra* (Müller) (Lymnaeidae) in Wales, U.K., showed that *Chaetogaster* mean abundance changed seasonally corresponding to snail population changes (i.e., births, deaths) with the largest peaks occurring in May and some evidence of a smaller peak happening later in the summer (Gruffydd 1965b). The author also noticed a trend of *Chaetogaster* abundance depending on snail size, with larger snails harbouring higher numbers of symbionts (Gruffydd 1965b). A two-year survey in the Worcester-Birmingham canal in the United Kingdom, found that seasonal changes in *Chaetogaster* prevalence depended on the identity of the host snail, with worms on *Physella frontinalis* (Linnaeus) (Physidae) and *Lymnaea peregra* having the highest prevalence in fall and lowest in summer (Young 1974). Conversely, *Chaetogaster* populations hosted by *Bithynia tentaculata* (Linnaeus) (Bithyniiidae) in the same survey had the highest prevalence in spring and lowest in winter. Because of these differences in phenology and host, Young (1974) suggested that there might be two species of *Chaetogaster* but found no physical differences to justify this. At the time, molecular diagnostic methods were not readily available, and one must wonder if that same conclusion would be found with current methods of genetic analysis.

There are two published seasonal surveys of *Chaetogaster* from North American sites. Sankurathri & Holmes (1976) found seasonal changes in *Chaetogaster* populations on snails while studying thermal effluents in Lake Wabamun, Alberta. Generally, *Chaetogaster* prevalence on *Physella gyrina* (Say) decreased once temperature reached above 24ºC causing yearly lows in summer. Conversely, a two-year study involving monthly surveys of a small pond in North Carolina, USA, revealed that the local *Chaetogaster* prevalence on *Heliosoma anceps*

Menke (Planorbidae) increased with temperature and so had the lowest frequencies in winter (Fernandez et al. 1991). The authors also observed that *Chaetogaster* population cycles were influenced by host reproductive cycles, with decreased *Chaetogaster* prevalence after the die off of large old snails.

Ibrahim (2007) assessed monthly populations of *Chaetogaster* in North Sinai, Egypt over a one-year period. Out of thirteen species of snail collected during the survey, five from the families Lymnaeidae, Planorbidae, Physidae, and Viviparidae were found to host *Chaetogaster* and within these, host size exhibited a positive trend with *Chaetogaster* prevalence and intensity. There was also evidence of population seasonality, generally with *Chaetogaster* prevalence and intensity reaching peaks in spring or summer depending on host species (Ibrahim 2007).

Most recently, a study aiming to investigate the effect of *Chaetogaster* colonization on reproductive success of their snail hosts (see Chapter 3 for full discussion on this topic) sampled seven streams in Germany three times over a seven-month period, once each in November, April, and June, (Stoll et al. 2017). This study found that snail size and species were two of several important variables influencing *Chaetogaster* abundance. *Chaetogaster* abundance increased with host size and different snail species had peak symbiont levels at different times of the year. For example, *Chaetogaster* abundance on *Physella acuta* (Draparnaud) peaked in April with a low in June, while *Biomphalaria tentaculata* (Say) (Planorbidae) had highest levels in June and lowest in November and April.

Overall evidence suggests that *Chaetogaster* populations fluctuate seasonally, and that host species and size influence this pattern (Gruffydd 1965b; Young 1974; Sankurathri and Holmes 1976; Fernandez et al. 1991; Ibrahim 2007; Stoll et al. 2017). In 2021, my casual observations of *Chaetogaster* populations in ponds near Edmonton suggested that they also

fluctuate seasonally. In 2022 I conducted a survey of two ponds from July to September to investigate this phenomenon more thoroughly. I hypothesized that *Chaetogaster* abundance on individual snails is affected by host size and seasonal factors. Given the studies described above, with particular weight given to the results of Sankurathri and Holmes (1976) given that the research was completed in Alberta, I predicted that *Chaetogaster* abunadnce would be highest at the start of summer and lowest during mid-to-late summer. I also predicted that larger-bodied snails should host greater numbers of *Chaetogaster*.

Does *Chaetogaster* Protect its Hosts from Trematodes?

Currently, it is still up for debate whether *Chaetogaster* presence positively or negatively affects snail hosts, although it is likely that any possible effect depends on *Chaetogaster* density and external environmental factors (Stoll et al. 2013). One of the potential positives of *Chaetogaster* colonization is that the worms may protect snails from trematode infection (Khalil 1961; Michelson 1964; Sankurathri and Holmes 1976; Fernandez et al. 1991; Ibrahim 2007). This potential defence mechanism has fascinated biologists for decades and has been the focus of many, if not most, *Chaetogaster* studies to date (Appendix 1-1).

Trematodes (Platyhelminthes: Trematoda) are a diverse group of parasitic flatworms found globally that play important roles in both marine and freshwater communities (Poulin and Morand 2000; Esch et al. 2001; Galaktionov and Dobrovolskij 2003; Gordy et al. 2016; Franzova et al. 2019). Trematodes have complex life cycles, usually infecting three different hosts through the course of their life cycle (Galaktionov and Dobrovolskij 2003). Sexual reproduction occurs in the definitive (a.k.a., final) host, which is usually a vertebrate, where adult trematodes produce eggs that are then released into the environment with the feces of the host.

These eggs may remain dormant until consumed by the next host or they may hatch into ciliated miracidia larvae that actively seek out the next host. This life stage, depending on the species, therefore may actively or passively disperse, which has implications for which species of host they may infect. In this first intermediate host, which is almost always a snail, asexual reproduction occurs in which larvae (rediae or sporocysts), produce the next life stage, called cercariae. As a side effect of infection, the host snail almost invariably becomes castrated and are unable to reproduce (Sorensen and Minchella 2001). Cercariae are small swimming larvae that are released from the first intermediate host into the water to seek out the next host of the life cycle (Galaktionov and Dobrovolskij 2003). Upon contact with (or consumption by) an appropriate second intermediate host, a cercaria transforms into a dormant encysted stage called a metacercaria. Second intermediate hosts are much more taxonomically diverse that first intermediate hosts, and include fish, annelids, arthropods and even other snails (Cribb et al. 2003; Galaktionov and Dobrovolskij 2003). When the second intermediate host is consumed by the definitive host, the metacercariae transform into sexually reproducing adults within the new host and complete the cycle. The life cycle here is a general example, so it is important to note that there are diverse exceptions to this pattern (Cribb et al. 2003; Galaktionov et al. 2012). For example, some trematode life cycles may use the same host for the first and second intermediate hosts or may skip the second intermediate host altogether.

In Alberta, freshwater snails are intermediate hosts for many species of trematodes (Cribb et al. 2003; Gordy et al. 2016), which typically infect snails via their first free-living life stage, miracidia. A study of six water bodies in central Alberta determined the average prevalence of trematode infections in freshwater snails to be 13-14% ; however, there were differences

according to snail family and season (Gordy et al. 2016). For example, one lake reached as high as 64% prevalence of trematode parasites in the snail community in July 2013.

Chaetogaster limnaei have been observed eating or blocking miracidia and cercariae from host snails, which suggests that *Chaetogaster* presence may reduce the frequency of colonized snails becoming first or second intermediate hosts of trematode parasites (Khalil 1961; Michelson 1964; Sankurathri and Holmes 1976; Fernandez et al. 1991; Ibrahim 2007; Zimmermann et al. 2011; Muñiz-Pareja and Iturbe-Espinoza 2018; Hobart et al. 2022).

The results of a recent study found *Chaetogaster* to be a significantly effective natural preventative for trematode infection in the lab by reducing infection rate from 70% to 13.3% in *Galba trunculata* Müller (Lymnaeidae) (Muñiz-Pareja and Iturbe-Espinoza 2018). The benefit of *Chaetogaster* preventing trematode infection (assuming that such a benefit does exist), should be inversely proportional to the risk of trematode infection, which in turn would likely vary in space and time. Although there are some lab experiments manipulating *Chaetogaster* presence to determine their efficacy in preventing trematode infection of their hosts (Michelson 1964; Rodgers et al. 2005; Hopkins et al. 2013; Hopkins et al. 2016; Muñiz-Pareja and Iturbe-Espinoza 2018), no similar experiments have been run under field conditions.

Although fascinating, this phenomenon is difficult to study either in the lab or in the field. In the lab, it can be difficult to create conditions that are an accurate representation of the field (i.e., in terms of parasite numbers, appropriate parasite species, etc.) while conditions in the field can be unpredictable and difficult to quantify. Another aspect that makes researching this phenomenon so difficult is that a pattern of higher *Chaetogaster* abundance on heavily infected snails does not actually negate the possibility of these worms preventing trematode infection as one might expect. Rather, it may be that *Chaetogaster* may prefer hosts with a trematode

infection, as the parasite larvae released from the host act as a steady food supply to the worm (Hobart et al. 2022). With this in mind, results from observations and experiments attempting to associate *Chaetogaster* presence and trematode infection rates must be carefully interpreted.

In the second study discussed in this chapter, I put caged snails with and without *Chaetogaster* into local ponds to determine if *Chaetogaster* presence affects trematode infection rates. I hypothesized that *Chaetogaster* presence minimizes host infection rates by trematode parasites and predicted that snails with *Chaetogaster* would have a lower probability of trematode infection than those without these potentially protective worms.

Species Identification

Most lab and field studies to date have considered snail-associated *Chaetogaster* to belong to the single species *Chaetogaster limnaei* von Baer, with two subspecies *C. l. limnaei* and *C. l. vaghini* Gruffydd, but molecular studies have recently called the status of the species and subspecies into question (Smyth et al. 2015; Mack et al. 2023). Similarly, taxonomy of snails in the family Physidae, my focal family, is in considerable flux with many contradictory classifications (e.g., Young et al. 2021). Therefore, I submitted specimens of *Chaetogaster* and physid snails from my field collections, and from a laboratory colony maintained in the lab of Patrick Hanington (School of Public Health, University of Alberta), for sequencing of the ‘barcode’ segment of the mitochondrial COI gene.

Methods

Chaetogaster Survey

Collection and Dissection

At the end of summer 2021 (August and September) I made four collection trips, two to Heritage Lake in Morinville (53.800, -113.666) and two to the ponds on Lafarge Canada properties located between Edmonton and Fort Saskatchewan (precise location not available due to a privacy agreement with the company) (Figure 2-1and Table 2-1).

On these trips, between 27 and 40 snails were collected for use in an experiment detailed in Chapter 4 and as preliminary survey data. Collected snails were brought back to the lab in large clean yoghurt containers and dissected within 30 h of being collected. Since the snails were not kept in separate containers after collection it is possible that worms moved between snails, so the results from this preliminary survey should be interpreted with caution. During dissection, snails were assessed for external *Chaetogaster* and trematode infection (i.e., rediae or sporocysts). Information on internal *Chaetogaster* abundance and encysted metacercariae was not reliably gathered for these specimens and are therefore not presented. Maximum shell length of snails from these 2021 collections was measured using electronic calipers (Fischer Scientific digital calipers, Model: 14-648-17).

In 2022 I made 13 trips from the beginning of July to the end of September to Heritage Lake in Morinville and the ponds on the Lafarge Properties (Table 2-2). On each of these trips I used hand nets to collect ~20 physid snails at each location (see Table 2-2 for exceptions). Each snail was immediately placed in an individual 100 mL screw top plastic vial with some pond water and returned to the lab, where the vials were left with their lids tipped open to allow gas exchange.

I dissected the snails within 30 h of collection and counted the number of external and internal *Chaetogaster* colonizing each snail. I also collected data on internal trematode infection (as presence/absence) and number of metacercarial cysts. Snails from these surveys were photographed and measured using ImageJ software (1.51m9). Unfortunately, photographs of collected snails from week 12 were lost due to a backup error, and only nine of the 40 snails from this collection date have associated size data (five from Lafarge and four from Morinville). The snails without size measurements were excluded from all analyses.

The protocol for photographing and dissecting snails in this experiment and throughout this thesis is as follows. Soft tweezers were used to gently pick up the snail from the water and place it on a clean paper towel. The snail was left on the towel for less than a minute to remove excess water drops and then the snail was placed on a piece of graph paper that had been put inside of a Ziploc bag. The snail was placed aperture down and the graph paper flattened to remove bumps. An iPhone was placed at 7 cm above the snail on a ring stand. Lastly, a small piece of paper with the snail's identification number was placed next to the snail and the picture was taken (3024 pixels by 4032 pixels). I used ImageJ ([https://imagej.net/ij/index.html], version 1.51m9) to calculate the length of each snail as the longest straight-line distance along the long axis of the snail shell. Further discussion on ImageJ and the accuracy of snail measurements using this tool are discussed in Chapter 3.

Once I was ready to dissect a snail, I used soft forceps to place it in a small plastic petri dish with just enough water to cover the snail. Next, I used fine-tipped forceps to carefully break and remove the snail shell without damaging the soft tissues of the snail. Once the shell was removed, I counted all of the external *Chaetogaster*, using the soft tweezers to reposition the snail as needed. I then euthanized the snail by quickly removing the head from the body using

the fine tip tweezers. I counted the number of internal *Chaetogaster* and metacercariae by gently opening the snail body to reveal the kidney. Once the kidney had been examined, I dissected the rest of the snail to check for further evidence of trematodes in the form of metacercariae, rediae or sporocysts.

Statistics

All statistical analyses and graphs in this thesis were created in R version 4.2.2. (R Core Team 2022). Survey data were examined separately by year and location. A linear model (stats package: R Core Team 2022) or generalized linear model (MASS package: Venables & Ripley 2022) was created for each dataset to examine which variables affect number of exterior *Chaetogaster* per host. When available, information on internal *Chaetogaster* is presented. Independent variables included a combination of collection week, snail length, trematode infection status, number of encysted metacercariae and number of internal *Chaetogaster* as well as interaction terms. Regardless of significance, collection date and snail length were kept as predictor variables in all models since they were the primary variables of interest in this survey. Internal *Chaetogaster*, trematode infection, and metacercariae were kept in the models only for those present in more than 10% of the collected snails, although if multiple variables were viable and the model became too complex for reliable interpretation then variables only the variables of most interest were considered. To simplify creation, selection, and interpretation of models only one interaction was included per model, and interactions were only between two predictors at a time (i.e., higher level interactions of three or more variables were not included). After model creation, the best model was selected through a comparison of AICc.

If two models were within 2 AICc, then both models were examined for fit and assumptions. Model assumptions were then checked using qqplots or the plot() function in the package DHARMa (Hartig 2022). Additionally, multicollinearity between predictor variables was also tested using the vif() function in the car package (Fox & Weisberg 2019). If both models met the assumptions, then they were compared using an ANOVA test for significance; if they were not found to be significantly different, then the simplest model was chosen.

In addition to the above tests, the correlation between the number of external *Chaetogaster* per snail and the number of metacercariae per snail was examined in both the Lafarge and Morinville datasets from 2022. This was done using the ggpubr package (Kassambara 2023). Since the two variables did not have a normal distribution, the non-parametric Kendall method was used for the correlation analysis.

Cage Experiments

The cage experiment was attempted three times over the course of my master's degree (twice in 2021 and once in 2022). Methods for the three attempts were very similar and are described below, with differences between attempts pointed out.

Experimental organisms

The snails used in this experiment were chosen from a colony of physid snails started and maintained by previous graduate students of Dr. Patrick Hanington (Michelle Gordy and Jacob Hambrook) in the University of Alberta School of Public Health. The original snail colony was found in water-supply pipes of the aquatic animal care unit in Biological Sciences at the University of Alberta, where they had likely arrived as hangers-on from commercially produced fish or aquarium supplies. These snails were raised in the Hanington lab prior to my degree. Over

the course of my master's care of these snails fell on Jacob Hambrook and myself. While some snails had rotifer colonies present on their shells, I found no evidence of trematode infections or *Chaetogaster* presence, and proceeded to use these lab snails in experiments where I required control over the presence or absence of symbionts.

The lab-snail colony was maintained in several medium sized plastic containers (L: 40 cm, W: 25 cm, and H: 15 cm, with a water depth of ~5 cm) in a room with a 12:12 light:dark schedule. About 30 snails were maintained in each container and when new snails hatched the larger snails in the colony were moved to a new container. The room was maintained at 23ºC and the snails were regularly fed with lettuce and algae wafers (Hikari Algae Wafers). Water changes were done frequently with artificial spring water (ASW) (formula in Appendix 2-1) to maintain acceptable water quality. The bottom of the containers was lined with a thin layer of sand as well as autoclaved empty snail shells to provide calcium. This colony of snails was used for several experiments over the course of my thesis and will from here on be denoted as 'lab-bred snails'. A discussion of the species and molecular diagnostics can be found later in this chapter.

Preparation of snails for the field experiment

I randomly selected 60 lab-bred snails for the cage experiment. The snails were measured prior to treatment assignation and sorted into three size categories: small (≤ 5 mm), medium (5 mm > x ≤ 6 mm), and large (> 6 mm). From within each size class, half of the snails were randomly assigned to the *Chaetogaster*(-) treatment, and the other half were assigned to the *Chaetogaster*(+) treatment so that there was a total of 30 variously sized snails in each treatment. At this point each treatment group was kept in a separate container and each of the two containers was treated identically, excepting the addition of *Chaetogaster* to one treatment.

Chaetogaster removed from field-collected snails from the experimental water bodies (described below) were added to the snails of the *Chaetogaster*(+) treatment 1-3 weeks prior to the deployment of the experiment. A total of ~70 *Chaetogaster* were gently added via small pipette to the container of snails and allowed to colonize and potentially reproduce on the snails. Because of the risk of damaging snails or *Chaetogaster*, no attempt was made to standardize number of worms per *Chaetogaster*(+) host; however, snails in this treatment were checked prior to deployment to ensure that each had at least one (although most had more) *Chaetogaster*. With this method of colonization, it was not possible to determine exactly how many *Chaetogaster* were present on each snail at the start of the experiment since an exact count requires dissection of the snail in question.

Collection of *Chaetogaster* from field snails for this experiment (and all experiments in this thesis) are as follows: I placed a snail using soft tweezers into a small plastic petri dish with just enough water to cover the snail. Next, I used fine tip tweezers to carefully break and remove the snail shell without harming the snail. Once the shell was removed, I euthanized the snail by quickly removing the head from the body using the fine tip tweezers. Next, I used fine tip tweezers to gently brush *Chaetogaster* from the snail to be sucked up by a pipette or I directly suctioned the worm from the snail with the pipette. The collected *Chaetogaster* were then transferred to another container for holding before being transferred to a container with new snails.

Cage Creation

For this experiment, a total of 60 small cages were created (Figure 2-2). The containers used as the base of the cage were small (120 mL) plastic screw-top vials. First, two rectangular

windows (4 cm x 2.5 cm) were cut into the cups using a utility knife. Next about 10 small holes encircling each window were pierced through the plastic about half a centimeter away from the hole and were used to securely sew a rectangle of plastic mesh with 1 mm (i.e., mesh generally fine enough to prevent transfer of *Chaetogaster* worms but not too small to be easily clogged by debris) mesh openings over the windows. The mesh was then further secured to the vials using a hot glue gun. These mesh-covered windows were positioned directly opposite each other to allow water to flow through each cage. The lids of the containers had drilled holes 2.5 cm in diameter. These holes were covered in mesh using hot glue since the plastic of the lids was too thick to pierce for the sewing method. Since the physid snails used in this experiment are air-breathing pulmonates, small Styrofoam flotation blocks were attached to each cage, which ensured 1 or 2 cm of air-filled 'head room' per cage. The Styrofoam blocks were arranged in a triangular configuration to keep the cage floating mostly straight up and were attached by threading fishing line through small holes pierced through the body of the plastic vial.

Site Selection

The design of this experiment required leaving the experimental units unattended for a long stretch of time, so it was decided that to minimize the chance of curious humans interfering with the cages it would be best to do the experiment on private property. Through contacts in the Hanington lab, the Lafarge Canada property near Fort Saskatchewan was found to be suitable and permissions were obtained from Lafarge for use of their land for this experiment.

The Lafarge ponds are located on the site of a previous gravel mine and are intended to serve an ecological restoration function (P. Hanington, pers. comm.). They contain several freshwater snail species from the families Physidae, Lymnaeidae and Planorbidae. My own

dissections as well as previous evidence gathered by Ph.D. students (Monica Ayala-Diaz and Brooke McPhail, pers. comm.) indicated that towards the end of summer there was relatively high prevalence of trematode infections among the snails of these ponds. The Lafarge property contains three similarly sized ponds (approximately 200 m by 100 m). Two of the ponds were chosen for use since they were known to join at times of high water and therefore would be expected to have similar taxa present. Prior to deployment of the cages in late summer the water level had fallen enough so that each pond could be considered independent at that point.

Cage Deployment and Collection

To efficiently arrange and position the cages within the experiment ponds, 10 cages were positioned on a fishing line (Stealth-Braid, SpiderWire) using knots to keep them about a 10 cm apart. Both ends of the fishing line were then attached to 1.8 m tall metal stakes that were designed as signposts and had pre-existing perforations through which the lines could be tied. The stakes were driven into the mud bottom of the Lafarge ponds so that the cages floated along near the surface of the water in a row, like clothes on a clothesline (Figure 2-2). One snail was put into each cage, and a random coin toss decided which treatment would be placed in the first cage on each line. After the first cage, the remaining cages were assigned one of the two treatments (+/- *Chaetogaster*) in an alternating pattern. After each cage was assigned a treatment, a random snail from the appropriate treatment group was placed into each cage along with a small piece of an algae pellet or lettuce. A permanent marker was used to label the lid and bottom of each cage.

A total of 6 lines of 10 cages each (for a total of 60 cages) were deployed in each of the three attempted experiments. The first experiment was set up on August 9th, 2021, the second on

August 30th, 2021, and the third on July 27th, 2022. For the third experiment an additional chicken wire fence was erected around each line of cages to prevent interference from wildlife (Figure 2-2).

In both the first and second attempt of the cage experiment the cages were collected after being in the field for one week. The cages of the third attempt were collected after 16 days. Any snails still alive at the point of collection were brought back to the lab, briefly checked for *Chaetogaster* presence under the dissecting microscope and then put into individual small containers. Snails were left for three weeks in the lab with regular water changes and lettuce *ad libitum* to allow any trematode infections to develop to the patent (visible to humans) stage. After the three weeks, the snails were measured and dissected. During dissection, information on the presence and number of *Chaetogaster* and metacercarial cysts was collected as well as the presence of trematode infection.

Water Quality Investigation

Two water samples from Lafarge (one from each pond) and a sample of the water from the lab-snail colony were collected for chemical analysis on August 17th, 2022, the samples from Lafarge were collected in the morning in one litre plastic bottles and promptly wrapped in tinfoil to prevent changes in Chlorophyll a. The samples were taken back to the lab in a cooler with ice packs and delivered to the Biogeochemical Analytical Service Laboratory (BASL) at the University of Alberta for processing as soon as possible. The water sample from the lab reared snail colony was taken on the same day and given the same treatment. This water sample was taken from a long-standing colony container with approximately 30 snails that received monthly water changes. The chemical analyses completed on the water samples determined the following:

total phosphorous, total nitrogen, pH, alkalinity, conductivity, total dissolved solids, and amount of chlorophyll a (Appendix 2-2).

Following the collection of samples for water quality analysis, I investigated the effect of water chemistry of Lafarge water on lab-bred snails. Water was collected from one Lafarge Pond and used in a small trial, wherein 14 lab-bred snails were placed into small containers with Lafarge water and monitored over the course of 54 days (August 19th – October 12th). Snails were given lettuce *ad libidum* and received Lafarge pondwater changes approximately once a week.

Statistics

As discussed in the Results section, due to paucity of data no statistical analyses could be performed for the cage experiments.

Molecular Analysis

Sample Collection

Samples used for sequencing were collected in both summer of 2021 and 2022 (Table 2-3). Snail samples were prepared by pinching a small portion of the foot region from the snail body with forceps (remainder of the snail was immediately placed in ethanol to completely euthanize the snail). The collected snail tissue was then briefly rinsed once in water and twice in 95% ethanol to remove *Chaetogaster* and any other organisms present. Following rinsing the snail tissue was placed in a screw top Eppendorf tube filled with absolute ethanol and sealed. A label was added to the tube with permanent marker and then the sample was placed in a -20ºC

freezer. *Chaetogaster* samples were collected from selected snails using the gentle application of forceps or a pipette and then preserved in the same way described above.

DNA Extraction, amplification, and Replication

Samples were processed either by Sophie Dang from the Molecular Biology Service Unit (MBSU) at the University of Alberta or by Ph.D. student Brooke McPhail from the Hanington Lab at the University of Alberta (Table 2-3).

DNA extractions conducted by Sophie Dang used Qiagen's DNeasy blood and tissue kit (Cat# 69504) following the manufacturer's protocol. One change was made to the described protocol, as snail samples were eluted into 100 uL of buffer AE and *Chaetogaster* samples were eluted into 50 uL of buffer AE (standard protocol elutes into 200 uL of buffer AE). Following extraction, primers used were LCO1490 and HCO2198 (Folmer et al. 1994). PCR cleanup was done with Cytiva SeraMag Select beads (Fisher Scientific Cat# 09928106) at a 0.8 to 1 ratio. (0.8x beads, 1x PCR product) and purified PCR products were sequenced using BigDye 3.1 following manufacturer's instructions (Applied Biosystems) and resolved on a 3730 DNA Analyzer (Applied Biosystems).

DNA extractions conducted by Brooke McPhail DNA was extracted using the Qiagen DNEasy Blood and Tissue kit following manufacturer protocols. PCR primers LCO1490 and HCO2198 were used with 0.5 uL of each primer (Folmer et al. 1994), 10 uL of AccuStart II GelTrack PCR SuperMix and 9 uL of sample per reaction. The samples were then cleaned using Truin Science PCR clean up kit and sent for sequencing at Macrogen (South Korea). Samples were edited and aligned using Geneious software.

Molecular Diagnostics

COI sequences from my samples were provided to me by Sophie Dang and Brooke McPhail. The small size of the *Chaetogaster* specimens in addition the difficulty of cleaning such samples fully from snail mucus, resulted in some these samples not running properly. A total of 4 snail samples and 7 *Chaetogaster* samples did not result in useable sequences (VAF-1S, VAF-4S, VAF-15S, VAF-16S, VAF-17W, VAF-18W, VAF-21W, VAF-23W, VAF-25W, VAF-32W, VAF-42W)(Table 2-3). A total of 24 physid and 17 *Chaetogaster* sequences from the original samples were of sufficient quality to be used for analyses. With the useable sequences, I first aligned them using MUSCLE software in MEGA11 (https://www.megasoftware.net/), and then trimmed the sequences so that they were all the same length according to the shortest sample (snail samples: 612 base pairs, *Chaetogaster* samples: 626 base pairs). Additionally, missing base pairs were called manually using the original trace files.

Each individual sequence was put into the NCBI standard nucleotide BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find published sequences close to those of my specimens. I chose the top match for each sequence; if the top match had already been chosen for a previously BLASTed sequence, I chose the next-best sequence. Only sequences that had 95% similarity or higher were chosen. Additionally, only sequences that had been identified more finely than genus were considered. Three extra snail sequences from GenBank for a physid species belonging to a genus different from my specimens, *Aplexa elongata* (Say) were chosen as an outgroup to root the snail neighbour-joining tree. Similarly, I chose two sequences from *Chaetogaster diaphanus* (Gruithuisen) to root the worm tree. A total of 24 physid (Table 2-4) and 17 *Chaetogaster* (Table 2-5) sequences were pulled from GenBank with the resulting BLAST searches.

My sequences, along with those acquired from the BLAST search were compiled into two separate files (snails sequences and *Chaetogaster* sequences) and aligned using MUSCLE software in MEGA11 before tree creation. After alignment, the sequences were again trimmed to be the same length according to the shortest sequence (Snail sequences: 570 base pairs, *Chaetogaster* sequences: 557 base pairs). Each group was used to create a Neighbour Joining tree using the p-distances method (MEGA11). I edited the following tree in iTOL: interactive Tree Of Life (a phylogenetic tree editor: https://itol.embl.de/) and Inkscape (an .svg file editor: https://inkscape.org/).

Snail species clusters were differentiated using branch lengths. Groups with 5% or higher p-distances (i.e., branch length of 0.05 or higher) were considered different as suggested by previous molecular work completed in Physidae (Young et al. 2021; do Espirito Santo et al. 2022). A distance matrix was also created using p-distances in MEGA11 software. Mack et al. (2023) found interspecific p-distance between putative *Chaetogaster* species to range from 5-18.5%, therefore I also used a 5% cut-off for the worm sequences.

Gut Contents

Considering the lack of agreement over whether *Chaetogaster* feed exclusively on debris and non-snail organisms or if they also ingest snail organic matter, I examined gut contents of a small subset of the worms used in my thesis.

A total of 11 snails from various locations were sampled for *Chaetogaster* and preserved in 95% ethanol in microtubes (Table 2-6). From each snail, one to four *Chaetogaster* were selected, and slide mounted by Heather Proctor. Slide mounts were then photographed by Heather Proctor, with myself assisting, and the gut contents of each *Chaetogaster* coarsely identified.

Results

Chaetogaster Survey

Overarching trends

Here I briefly discuss the graphical trends and summary statistics of the field-survey data collected in 2021 and 2022. In the next sections I will discuss data from each location and year separately and the results of my formal statistical analyses. I chose to run these analyses separately since the data from 2021 was less reliable (i.e., snails weren't kept in separate containers before dissection which potentially allowed worms to move between hosts) and due to the differences between ponds (both abiotic and biotic variation).

The overall average number of external *Chaetogaster*/snail was consistent in samples collected from Lafarge in 2021 and 2022 with 5.71 and 5.27 *Chaetogaster*/snail respectively (Table 2-7). The average abundance in Morinville was quite different between years, with 13.6 *Chaetogaster*/snail in 2021 and only 3.88 on average in 2022. The variation in *Chaetogaster* abundance was similar in all four groups, except in Morinville snails collected in 2022 (Table 2-7, Figure 2-3). Shell lengths of the physid snails were generally consistent between the different groups, although snails in Lafarge in 2021 were generally the smallest (mean = 8.33 mm) while snails collected in Morinville that year were the largest (mean = 12.85 mm, Table 2-7). When comparing the size of host snail to the number of *Chaetogaster* present, there is a visual trend of worm abundance increasing with host size (Figure 2-4).

2021 Lafarge Collections

In total, 76 physid snails were dissected over 2 sampling trips in 2021 (raw data: Appendix 2-5). Of these, only 2 snails had no external *Chaetogaster*. The number of worms/snail

ranged from 0 to 21, with an average abundance of 5.7 ± 0.58 (SE). Data on other snail-associated organisms were not consistently gathered and therefore are not discussed here.

Data were not normally distributed and so a generalized linear model (MASS package: Venables & Ripley 2022) with a negative binomial regression was created. The variables considered as potential predictors of number of external *Chaetogaster* per snail (abundance) were collection date, host snail length and their interaction (Table 2-8). Selection via AICc determined that both models explained the data equally well, however Model 1 (with interaction) was found to have very high multicollinearity (i.e., collection date and the interaction term were correlated) which would reduce the reliability of model output. Therefore, Model 2 was chosen.

Here, the effect of collection date was not found to have a significant effect on the number of external *Chaetogaster*/snail ($X^2 = 3.408$, $p = 0.065$, Table 2-9 and Figure 2-5); however, with only two time points just two weeks apart that is not surprising. Conversely, the effect of snail shell length was very significant ($X^2 = 84.858$, $p < 2.0^{-16}$, Table 2-9 and Figure 2-6), with larger snails generally having higher worm numbers.

2021 Morinville Collections

Over 2 collection dates in 2021 (August 23rd and Sept 26th) a total of 57 snails were collected and dissected (raw data: Appendix 2-6). All had external *Chaetogaster* present. Worm abundance ranged from 2 to 29 worms/snail, with an average of 13.60 ± 0.80 (SE) worms. Data on other snail-associated symbionts were not consistently gathered over both trips and therefore are not discussed here.

The data was determined to be normal with a Shapiro-Wilks normality test ($W = 0.969$, $p = 0.155$) and so two linear models were created to analyze the data. The models included only collection date, snail host length and their interaction (Table 2-10). Model selection via AICc

concluded Model 2 (without interaction) to fit the data best. Model 2 was checked for multicollinearity and model assumptions, and it was concluded that the model did not violate any assumptions.

The effect of collection date was not significant ($F_{1,54} = 2.552$, $p = 0.116$, Table 2-11), although again, with only two collection dates this is not unexpected, despite the dates being farther apart for this dataset (Figure 2-7). The effect of host length was significant ($F_{1,54} = 8.613$, $p = 0.005$), again with larger snails carrying more worms (Figure 2-8).

2022 Lafarge Survey

A total of 260 snails were collected in this survey, 19 of which were omitted from analysis due to missing data (raw data: Appendix 2-7). Of the 241 snails remaining, 198 of them had external *Chaetogaster* with an average abundance of 5.27 ± 0.37 (SE) worms/snail. Internal *Chaetogaster* and trematode infection were both relatively rare, or close to non-existent, with only one snail having internal *Chaetogaster* and only 15 having internal trematode parasites (sporocysts or rediae). Encysted metacercariae were found in 36 snail hosts, ranging from 1 to 39 metacercariae per snail. There was a slight visual trend of metacercarial abundance increasing with snail size and the number of external *Chaetogaster* (Figure 2-9), however the two variables were not found to be correlated ($R = -0.066$, $p = 0.21$, Appendix 2-9).

A visual investigation and calculation of summary statistics indicated the response variable was not normally distributed and exhibited signs of overdispersion. Therefore, I analyzed this data using a generalized linear model (MASS package: Venables & Ripley 2022) with a negative binomial distribution. Models considered only collection week, snail host length, metacercarial abundance and their interaction (Table 2-12). Since both internal *Chaetogaster* and

trematode infection (sporocysts/rediae) were in found in less than 10% of the population they were not included in model formation.

Model selection indicated that both Models 2 (Collection week, host snail host, number of metacercariae) and 6 (Collection week, host snail length) explained the data equally well, however model 2 was found to have high multicollinearity between snail size and the number of encysted metacercariae/snail (likely because bigger snails have had more time to accumulate cysts and are larger targets for infection, Figure 2-9). Since multicollinearity reduces confidence in p-values and model outputs, model 6 (with metacercariae removed) was chosen as the best (Table 2-12). Model 6 was found to meet all model assumptions.

The effect of date the snails were collected had a strongly significant effect (X^2 = 181.285, $p < 2.2^{-16}$, Table 2-13). In general, snails had the highest number of external *Chaetogaster* at the end of summer in September, and the lowest number in August (Figure 2-10). There was also a smaller secondary peak in *Chaetogaster* numbers in mid-July. Snail length also had a significant effect on *Chaetogaster* abundance (X^2 = 43.076, $p = 5.265^{-11}$, Table 2-13), with larger snails generally having a higher number of symbionts (Figure 2-11).

2022 Morinville Survey

A total of 260 snails were collected in this survey, 17 of which were omitted from the final analysis due to missing data (raw data: Appendix 2-8). Of the remaining 243 snails, 209 had external *Chaetogaster* with an average abundance of 3.88 ± 0.23 (SE) *Chaetogaster*/snail. Approximately half of all sampled snails (143 snails) also carried internal *Chaetogaster*, with an average abundance of about 1.3 ± 0.12 (SE) symbionts/snail. In this dataset, snails had a maximum of 10 internal *Chaetogaster*. Additionally, 7 snails were found to contain infections of trematode rediae/sporocysts and 15 had metacercariae. The number of metacercariae/snail

ranged from 1 to 6 (Figure 2-12). A visual inspection suggests that the number of metacercariae may decrease with increased numbers of *Chaetogaster*/snail (Figure 2-12) however no association was found between the two variables ($R = 0.035$, $p = 0.52$, Appendix 2-10)

A visual investigation and calculation of summary statistics indicated *Chaetogaster* abundance was not normally distributed and exhibited signs of overdispersion. Therefore, I analyzed this data using a generalized linear model (MASS package: Venables & Ripley 2022) with a negative binomial distribution. Models were created using collection week, snail host length and internal *Chaetogaster* (Table 2-14). Internal trematode infection and metacercarial abundance was excluded from model creation as less than 10% of the population carried these parasites.

Model selection indicated that Model 2 (collection week, host snail length, number of internal *Chaetogaster*) explained the variation in the response variable the best; however this model was found to have very high multicollinearity between snail size and the number of internal *Chaetogaster*/snail (likely because larger snails have more space for internal symbionts, Figure 2-13). Since multicollinearity reduces confidence in p-values and model outputs, Model 6 (with internal *Chaetogaster* removed) was chosen as the best (Table 2-14). Model 6 was found to meet all model assumptions.

The effect of date the snails were collected had a strongly significant effect on external *Chaetogaster* abundance ($X^2 = 184.322$, $p < 2.2^{-16}$, Table 2-15 and Figure 2-14). Generally, snails collected in Morinville had a similar pattern of external *Chaetogaster* abundance over time as those collected in Lafarge. *Chaetogaster* numbers peaked in both early July and mid-to-late September, with a seasonal low at the end of July (Figure 2-14). Snail host length also had a

significant effect ($X^2 = 6.363$, p = 0.012), with larger snails generally having more external *Chaetogaster* (Figure 2-15).

Cage Experiments

Cage Experiments 1, 2 and 3

In 2021, cage experiments 1 and 2 were damaged by unidentified wildlife during their first week in the field which resulted in some of the lines of cages being found at the edge of the pond after having been torn from their supporting posts (Figure 2-16). Both experiments were therefore ended at this point and the cages collected at the one-week mark. A total of 21 and 16 snails were still alive at collection in the first experiment from the *Chaetogaster*(-) and *Chaetogaster*(+) treatments, respectively. A total of 16 live snails from each treatment were collected in the second attempt at the cage experiment in 2021. Once the snails were dissected it was observed that none from either attempt had gained a trematode infection, and therefore the effect of *Chaetogaster* presence on the infection could not be assessed.

In the first attempt of the cage experiment the retrieved snails were not checked for *Chaetogaster* upon arrival to the lab, so exact data on how many snails retained or gained *Chaetogaster* was not collected. These data were collected in attempts 2 and 3. For cage experiment 2, the retrieved snails were examined in the lab the day following collection and each snail was checked for obvious signs of *Chaetogaster*. Of the 16 remaining snails, three of the 16 from the *Chaetogaster*(+) treatment had lost *Chaetogaster*, while none of the 16 *Chaetogaster*(-) had gained them.

In the third attempt of the cage experiment in 2022, when the lines of cages were surrounded by chicken wire fences, the cages were not damaged by wildlife; however, despite

the success of this modification, when the cages were opened at the 2-week mark most of the snails had perished. The cages were retrieved at this point and the number of living snails counted. A total of 9 *Chaetogaster*(+) and 8 *Chaetogaster*(-) cages contained live snails at collection. However, of these cages, 7 had more than one snail (either living or as an empty shell). At the time of deployment, only ~2 extra lab snails were brought into the field, and both were accounted for back in the lab after the cage experiment had been set up, so it was assumed that these second snails must be from the pond itself. The pairs of snails were given close inspection, however due to similar shape, size and colouring it was not possible to confidently determine which snails were the experimental snails originally placed in the cage and so those cages were not considered further. After excluding these snails, none of the final dissected snails contained an infection of rediae or cercariae; however, two snails of the *Chaetogaster*(+) treatment contained one metacercaria each (Table 2-16). No snails from the *Chaetogaster*(+) treatment had lost their *Chaetogaster*, but 3 of the 8 *Chaetogaster*(-) snails had gained *Chaetogaster* during the experiment.

Water Quality Investigation

Death of so many lab-bred snails after two weeks in the Lafarge ponds in 2022 suggested that the Lafarge habitat might be poor for the lab-bred snails. This prompted the comparison of water quality from the lab colonies with that of the Lafarge ponds and a test of survival of lab-bred snails in Lafarge Pond water. The water quality analysis completed by BASL at the University of Alberta determined that the water from the lab colony generally had very different properties than that collected from Lafarge (Table 2-17). The water from the lab colony had

much higher total phosphorous and nitrogen content than water from Lafarge as well as lower pH, calcium carbonate, conductivity, dissolve solids and chlorophyll a.

Of the 14 lab bred snails put into Lafarge water for 54 days, none perished. All snails lived to the end of the pilot experiment when they were euthanized. The choice to euthanize them at this point was made due to the logistics and timing of experimental work in my thesis and I am under the impression that these snails could have survived longer in Lafarge water if I had continued the experiment. Some snails produced eggs during this time; however, eggs were not counted.

Considering the impromptu nature of my water quality investigation, it is not possible to say if water quality was the cause of death for the lab snails used in Cage Experiment 3. However, judging by the results in Table 2-17, the extreme change in water quality from the lab to the field is likely to have been a contributing factor along with changes in temperature, food availability, etc.

Molecular Analysis

Snail Molecular Diagnostics

The 24 physid snail sequences acquired for tree creation (Appendix 2-11) separated into approximately three clades (Figure 2-17 and Appendix 2-12). Calculated branch distances support a species-level differentiation between the three. The first group includes a mixture of field-collected snails from Lafarge and Morinville as well as several different species from the GenBank samples: *Physella ancillaria* Say, *Physella wrighti* (Te & Clarke), and *Physella gyrina* (Say). The second group includes two snails from Lafarge and two from GenBank, both of which were identified as *Physella jennessi* Dall. The last clade is composed of *Physella acuta*

(Draparnaud) sequences from GenBank along with sequences from lab-bred snails, which were originally retrieved from the pipes of the aquaculture facility at the University of Alberta. The hypothesis that these snails were likely brought in through purchases of fish stock is supported by the created tree, as the *P. acuta* samples grouped with them come from a wide variety of locations around the world (Table 2-4 and Figure 2-17). The overall topology of this tree suggests that Lafarge has a greater diversity of physids than Morinville with at least two lineages present, while the lab-bred snails are completely different from either Lafarge or Morinville snails.

Worm Molecular Diagnostics

The 17 *Chaetogaster* sequences (Appendix 2-11) acquired for tree creation separated into approximately three clades, although the calculated branch distances are less than 5% and so do not support them belonging to different species (Figure 2-18 and Appendix 2-13). Two of the groups are a mix of Lafarge and Morinville *Chaetogaster* sequences, while the third only includes the two internal *Chaetogaster* samples from Morinville. Most of the sequences pulled from GenBank originated from Smythe et al. (2015) (accession numbers with the following codes: KF952298 – KF952346) and a comparison of the trees in that paper with the one below reveals a similar arrangement of clades. The other samples from GenBank that are close matches to my *Chaetogaster* sequences are primarily from Mack et al. (2023) and correspond to an as yet undescribed species of North American *Chaetogaster* (*Chaetogaster* sp. 22). Additionally, some of the samples from Smythe et al. (2015) fall into the group classified as *Chaetogaster* sp. 22 by Mack et al. (2023). These include two samples from New York, USA (accession numbers: KF952333 and KF952336). Overall, the results of this analysis indicate that all *Chaetogaster*

used in my experiments or collected in surveys were likely of the same species, corresponding to *Chaetogaster* sp. 22 in Mack et al. (2023).

Gut Contents

Multiple food items were found in the gut contents of the slide-mounted *Chaetogaster*. Of the samples taken some were found not to have any food in their stomachs and are not considered further in this section. In *Chaetogaster* collected from field snails, identifiable gut contents included: diatoms, pollen, and rotifers (Figure 2-19). In *Chaetogaster* collected from lab bred snails (*Chaetogaster* strain originally from Morinville), primarily rotifers and pollen food items were found in the stomachs (Figure 2-20).

Discussion

Genetic Diversity

Although presented towards the end of Results, I will start by discussing the outcomes of molecular analyses first, as they will add context to the other results discussed in this and subsequent chapters. The most recent and most thorough exploration of genetic diversity in the genus *Chaetogaster* indicate that there are at least 24 species in this genus and that '*Chaetogaster limnaei*' is at least three distinct species, two of which occur in North America (*Chaetogaster* sp. 22 and sp. 24). All 17 sequences of *Chaetogaster* from Lafarge and Morinville grouped with Mack et al.'s (2023) *Chaetogaster* sp. 22 (Figure 2-18**)**. Since the two North American species have both been found in Alberta (Mack et al. 2023), it is possible that both could exist at my sampling locations, and species 24 was by chance not included in my sequenced samples. However, for simplicity, until a more thorough investigation of the *Chaetogaster* species at these locations is conducted I will assume that only one species is present.

The physids sampled at Lafarge and Morinville represent a more complicated situation, with at least two species present at the Lafarge ponds. This uncertainty is exacerbated by the number of samples that have been entered into GenBank under incorrect or out of date species names. One clade of snail samples from Lafarge and Morinville grouped with snails under the names *Physella ancillaria*, *P. wrighti*, and *P. gyrina* although based on branch distances there was no indication of actual species level difference between all of the samples within this clade (Figure 2-17). Attempts to create a stable classification of the Physidae has continually resulted in complicated and generally incomplete species groupings (see do Espirito Santo et al., 2022; Young et al., 2021), in part due to an abundance of cryptic species and consistent mislabelling,

and possibly also hybridization. With the limited number of specimens sequenced from Lafarge and Morinville, it is quite possible that more physid species are present at these sites, so I will generally assume physid populations from Lafarge and Morinville to consist of two or more species. The snails that I bred in the lab were clearly identified as a different species from those from Lafarge and Morinville. These samples grouped with *Physella acuta* from around the world. Note that *Physella acuta* is often still identified in current publications as '*Physa acuta'*, but I am using the most up to date name '*Physella acuta'* throughout this thesis. *Physella acuta* is known for its near global invasion of freshwater habitats (Bousset et al. 2004; Albrecht et al. 2009; Zukowski and Walker 2009; Ng et al. 2015; Ng et al. 2018; Saha et al. 2019; Collado et al. 2020; Ansari et al. 2023; Miyahira et al. 2023). Therefore, it is not surprising that snails found in the pipes of the aquatics department at the University of Alberta members of this species.

Chaetogaster Surveys

Sampling at Lafarge and Morinville revealed a seasonal pattern of *Chaetogaster* abundance over the summer months (Figure 2-10 and Figure 2-14). *Chaetogaster* abundance was generally low at the start of summer, followed by a peak in July. Abundance dipped again in August, followed by another peak in September. This two peak pattern was seen at both Lafarge and Morinville, although the magnitudes and timings varied. This pattern did not match my initial predictions, as I had expected *Chaetogaster* populations would be highest at the start of summer and lowest during mid-to-late summer.

To my knowledge, seasonal dynamics of *Chaetogaster* has been monitored and published in five articles to date, most from abroad (Gruffydd 1965b; Buse 1974; Young 1974; Fernandez et al. 1991; Ibrahim 2007; Stoll et al. 2017) and from one Lake Wabamun in Alberta

(Sankurathri and Holmes 1976). At a coarse level, the patterns reported by those publications agree with my own, that *Chaetogaster* abundance varies with time of year, potentially due to temperature. While all of these studies claim to have investigated *Chaetogaster limnaei*, with the results from Mack et al. (2023) it can only be assumed that these studies include a mix of the three species that fall under this old name, and possibly more, as Mack et al. (2023) had sequences from snail-associated *Chaetogaster* from only two continents.

Possible explanations for the two peak pattern found here are numerous and include abiotic variation (particularly temperature), snail population dynamics, and differences in snail or worm species present. It has been reported that *Chaetogaster* populations are sensitive to temperature, particularly high temperatures that result in increased worm mortality (Sankurathri and Holmes 1976). It is reasonable to suppose that the two peak pattern I observed may be driven by *Chaetogaster* thermal tolerance, with worm populations thriving at warm temperatures at the start and end of summer but declining at the prolonged high temperatures at the peak of summer.

Another possible driver of this pattern may be the reproductive cycle of the host snails. Physid snails lay most of their eggs at the start of summer, after which point older snails will perish which leads to a sharp transition from mostly large older snails directly to a population made up of primarily juvenile snails in early-to-mid summer (DeWitt 1955; Stoll et al. 2017; pers. obs. VAF). As the season progresses, the juvenile snails would mature to adults. As discussed later in this section, larger snails are generally host to more worm symbionts, so it is possible that worm populations are driven by this cyclic change in snail populations. Here the pattern would follow that *Chaetogaster* numbers are high at the start of summer (when large snails are still abundant), low in mid-summer (when most snails are juveniles) and then high again at the end of summer (once the juvenile snails have grown).

Due to recent reports of '*Chaetogaster limnaei*' consisting of at least three species (Mack et al. 2023), it is becoming increasingly apparent that the inconsistent results between papers investigating these worm symbionts and various hosts may be caused by these authors unknowingly investigating different species. This is applicable here, since due to small sample size in my molecular analysis and most samples coming from the same collection date, I may be unintentionally collecting survey data on multiple snail or worm species without being aware of it. So, the two peak pattern I have observed in my survey may be an artifact of two or more worm (and/or snail) species exhibiting different abiotic or biotic preferences throughout the season. It is also possible that a mix of the above described mechanisms may in combination be creating the pattern that I have observed here.

I observed a consistently positive relationship between snail body size and number of worms/snail. This is not unexpected, as some previous investigations reported the same pattern (Gruffydd 1965b; Ibrahim 2007). This observation likely indicates that a part of the seasonal change in *Chaetogaster* abundance can be attributed to the snail population cycle (i.e., there are more *Chaetogaster* when there are more mature, large snails), which is supported by observations made by Fernandez et al. (1991). It would be interesting to investigate the mechanism that acts to create this pattern and it is intriguing to speculate if there is a maximum worm threshold depending on snail size. Do *Chaetogaster* limit their own reproduction at a certain density on a host? Or is there perhaps active competition for space and food that acts to remove worms above a maximum worms/mm^2 threshold?

Although the *Chaetogaster* survey completed in 2022 was generally quite thorough, I would like to note that the survey could have been extended both at the start and end of the July-September collection period by several weeks. Collecting earlier than June or later than October

would not have been practical (i.e., the weather was not conducive to field work and snails were scarce), but data from June and October would have given valuable insight into how low *Chaetogaster* populations might get in winter. Additionally, completing a second (or more) full summer of weekly surveys would have been invaluable for information on how yearly differences (e.g., in temperature and snail population densities) affect *Chaetogaster* populations. Snail population sizes in the Lafarge and Morinville water bodies are variable from year to year (pers. obs. VAF) and so it's possible that *Chaetogaster* populations change drastically in response.

Internal *Chaetogaster* were found almost exclusively in Heritage Lake in Morinville, while only a handful of internal *Chaetogaster* were found in snails from the Lafarge ponds (Appendix 2-7 and Appendix 2-8). Since the molecular analysis indicated that all *Chaetogaster* likely belong to the same species, it is possible that this difference can be credited to some attribute of the host snails or pond environment. However, with the exact number of host physid species in Lafarge and Morinville still up for debate, it is difficult to make suggestions as to why certain snail species might have more internal *Chaetogaster* than others.

Relatively few snails collected in the 2022 survey contained evidence of trematode infection (metacercariae/rediae/sporocysts). Due to low numbers, these variables were not included in the main analysis but were briefly discussed separately. In terms of infection by rediae and/or sporocysts, I did not find a large enough number of infections to conduct a formal analysis of the data. Hobart et al. (2022) found that snails with *Chaetogaster* are generally less likely to have a trematode infection if the infectious mode is active (parasite enters host body through own actions), and more likely to have a trematode infection if the infectious mode is passive (via ingestion of the parasite by the snail). A visual inspection of my data suggest that it

does not align with the results from Hobart et al. (2022) as the abundance of *Chaetogaster* did not appear to be affected by redia/sporocyst infection status of the host snail (Appendix 2-14). Since the number of snails with metacercariae was larger than that of other trematode infections (rediae/sporocysts), I examined these data using a correlation analysis. I found no correlation between the number of *Chaetogaster*/snail and the number of metacercariae/snail (Appendix 2-9 and Appendix 2-10). This result is surprising as one would expect snails with more *Chaetogaster* to have fewer metacercariae if these worms do indeed protect their hosts from infectious parasites. These results perhaps suggests that the protective effect of these worms is slight overall or in this species pair (or species pairs) in particular. Future research may consider comparing the infection rates between host species, as *Chaetogaster* may protect certain species more effectively.

Water Quality

Many water quality variables were strongly different between the artificially composed water use for lab snails and those in the water bodies that were the site of the cage experiments (Table 2-17). This suggests that changes in water quality may have negatively affected snails when being moved from the lab to the field or vice versa. Water samples taken from the Lafarge ponds indicate that summer 2022 levels of nutrients to be quite high, although not as high as that of the water used to house snails in the lab.

Although a water quality analysis was not performed on water from Heritage Lake from Morinville, personal observation indicated that the water quality at this location is quite poor. Over the summer months, Morinville often had high amounts of green algae which increased in intensity as the weeks progressed. Such high levels of algae were not observed in the Lafarge

ponds. On the edge of this lake, there is also an RV/Campground and so the lake was used recreationally for most of the summer. It is likely that water in Morinville oscillates in extremes of nutrient and oxygen concentrations as well as year-round accumulation of human pollutants (e.g., sunscreen and boat fuel/grease).

A report on Heritage Lake (Morinville) water quality collected in 2011 indicated that the lake exhibited low levels of total phosphorous, but high levels of total nitrogen relative to my measurements of Lafarge ponds (Clough et al. 2017). Chlorophyll levels were much higher in my Lafarge samples as well. The pH of Morinville ponds during this sampling in 2011 varied from 8.3 to 9.4 (1m depth) in spring and summer respectively. A more recent report suggests that total phosphorous levels have risen since 2011 and were at about 334 ± 173 ug/L in 2020/2021, which is comparable to that of the Lafarge ponds (Barret et al. 2021). Chlorophyll a had also increased, while pH had decreased to ~8.27. This report suggests that current water quality at Morinville is likely comparable to that of the Lafarge ponds.

Host Defence

Due to its popularity in scientific literature (Appendix 1-1), investigating the possibility of host defence from trematode parasites by *Chaetogaster* was one of my first goals when planning experiments; however, due to malfunctions of my cage experiments there are little to no results to discuss on this topic. I was, however, able to analyse the association between the number of *Chaetogaster*/snail and the number of metacercariae/snail in the snails collected in my field survey in 2022. I found no evidence of an association between the two variables (Appendix 2-9 and Appendix 2-10). This clearly does not support the hypothesis of a defensive role for

Chaetogaster; however, this interpretation is confounded by the fact that both *Chaetogaster* and metacercariae numbers are positively related to snail size, and so conclusion may be spurious.

Although I was unable to obtain data from my manipulative experiments on this aspect of *Chaetogaster*-snail symbioses, it is still an attractive avenue of research. In particular, I believe that future attempts to study this in a field setting as opposed to in the lab are vital, as *Chaetogaster* may only eat trematode larvae under situations that do not happen in the field (e.g., extremely high cercaria densities or where other food options are not available). I would also generally recommend using native snails and co-occurring *Chaetogaster* for future investigations, considering that *Chaetogaster*-snail symbioses may be affected by the worm-snail species pair (discussed more thoroughly in Chapters 3 and 4).

Gut Contents

The *Chaetogaster* samples used in this examination were found to have a variety of food items in their stomachs. There was no evidence of these worms eating snail host material (e.g., mucus), but mucus may not have been apparent due to the method of clearing prior to slide-mounting, and only a small number of worms were investigated, so the possibility that symbiont worms sometimes eat host tissues or exudates cannot be eliminated. Worms from snails in the lab colony primarily were found to be eating rotifers, which was not unexpected since rotifers were prevalent in colony containers. *Chaetogaster* from the field were found to have a variety of algae and diatoms food items in their gut contents, which aligned with what *Chaetogaster* are known to feed on in the field (Gruffydd 1965a; Stoll et al. 2013).

Worms from the lab and field were found to have eaten pollen. This was expected for *Chaetogaster* from the field, but not for the ones in the lab since it was unlikely for pollen to be

carried by wind into containers in a room with no openable windows. However, the lettuce fed to the lab snails occasionally came from Heather Proctor's personal garden, so it was assumed that the pollen in the *Chaetogaster* gut contents had been carried into the colony containers on the organic lettuce.

Tables

Table 2-1. Summary of snail survey dates in 2021. Table includes information on the number of physid snails collected over 4 sampling trips from August 16th to September 26th.

Collection Date	Location	Number of physid snails collected
Aug. 16. 2021	Lafarge	40
Aug. 23. 2021	Morinville	27
Aug. 30. 2021	Lafarge	36
Sept. 26. 2021	Morinville	30

Table 2-2. Summary of snail survey dates in 2022. Table includes information of the number of physid snails collected at each site over 13 sampling trips from July 6th to September 27th.

Week	Date	Number of physid snails collected	
		Lafarge	**Morinville**
1	July 6th	20	20
2	July 14th	20	20
3	July 21st	20	20
4	July 28th	18	20
5	August 5th	20	20
6	August 11th	20	20
7	August 18th	20	20
8	August 24th	20	19
9	September 2nd	18	20
10	September 10th	20	20
11	September 14th	20	20
12	September 21st	5	4
13	September 27th	20	20

Table 2-3. List of specimens used for DNA analysis. Physid snails and *Chaetogaster* worms used for phylogenetic analysis were collected from the Lafarge ponds and Heritage Lake in Morinville. Six lab-bred snails were also submitted. For some of the Lafarge samples the precise pond identity was not recorded. For most of the *Chaetogaster* specimens, the identity of its snail host is reported in the Notes column. Samples were processed either by Sophie Dang (S.D.) or Brooke McPhail (B.M.) with protocols outlined in Methods section of Chapter 2. Here, 'S' = a snail specimen and 'W' = a *Chaetogaster* worm specimen.

Specimen ID	Origin	Collection Date	Processed	Notes
VAF-1S	Morinville	June 15 2022	B.M.	
VAF-2S	Morinville	June 15 2022	B.M.	
VAF-3S	Morinville	June 15 2022	B.M.	
VAF-4S	Morinville	Sept 27 2021	B.M.	
VAF-5S	Morinville	Sept 27 2021	B.M.	
VAF-6S	Morinville	Sept 27 2021	B.M.	
VAF-7S	Lafarge	Aug 31 2021	B.M.	Pond 1 or 2
VAF-8S	Lafarge	Aug 31 2021	B.M.	Pond 1 or 2
VAF-9S	Lafarge	Aug 31 2021	B.M.	Pond 1 or 2
VAF-10S	Lafarge	Aug 31 2021	B.M.	Pond 1 or 2
VAF-11S	Lafarge	Aug 17 2022	B.M.	Pond 1
VAF-12S	Lafarge	Aug 17 2022	B.M.	Pond 1
VAF-13S	Lafarge	Aug 17 2022	B.M.	Pond 1
VAF-14S	Lafarge	Aug 17 2022	B.M.	Pond 2
VAF-15S	Lafarge	Aug 17 2022	B.M.	Pond 2
VAF-16S	Lafarge	Aug 17 2022	B.M.	Pond 2
VAF-17W	Lafarge	Aug 17 2022	B.M.	Pond 1 (from VAFS-11)
VAF-18W	Lafarge	Aug 17 2022	B.M.	Pond 1 (from VAFS-12)
VAF-19W	Lafarge	Aug 17 2022	B.M.	Pond 1 (from VAFS-13)
VAF-20W	Lafarge	Aug 17 2022	B.M.	Pond 2 (from VAFS-14)
VAF-21W	Lafarge	Aug 17 2022	B.M.	Pond 2 (from VAFS-15)
VAF-22W	Lafarge	Aug 17 2022	B.M.	Pond 2 (from VAFS-16)
VAF-23W	Morinville	Aug 17 2022	B.M.	Host ID not recorded
VAF-24W	Morinville	Aug 17 2022	B.M.	Host ID not recorded
VAF-25W	Morinville	Aug 17 2022	B.M.	Host ID not recorded

VAF-26S	Lafarge	Aug 17 2022	S.D.	Pond 1
VAF-27S	Lafarge	Aug 17 2022	S.D.	Pond 1
VAF-28S	Lafarge	Aug 17 2022	S.D.	Pond 1
VAF-29S	Lafarge	Aug 17 2022	S.D.	Pond 2
VAF-30S	Lafarge	Aug 17 2022	S.D.	Pond 2
VAF-31S	Lafarge	Aug 17 2022	S.D.	Pond 2
VAF-32W	Lafarge	Aug 17 2022	S.D.	Pond 1 (from VAFS-26)
VAF-33W	Lafarge	Aug 17 2022	S.D.	Pond 1 (from VAFS-27)
VAF-34W	Lafarge	Aug 17 2022	S.D.	Pond 1 (from VAFS-28)
VAF-35W	Lafarge	Aug 17 2022	S.D.	Pond 2 (from VAFS-29)
VAF-36W	Lafarge	Aug 17 2022	S.D.	Pond-2 (From VAFS-30)
VAF-37W	Lafarge	Aug 17 2022	S.D.	Pond 2 (from VAFS-31)
VAF-38W	Morinville	Aug 17 2022	S.D.	Host ID not recorded
VAF-39W	Morinville	Aug 17 2022	S.D.	Host ID not recorded
VAF-40W	Morinville	Aug 17 2022	S.D.	Host ID not recorded
VAF-41W	Morinville	Aug 17 2022	B.M.	Internal *Chaetogaster*
VAF-42W	Morinville	Aug 17 2022	B.M.	Internal *Chaetogaster*
VAF-43W	Morinville	Aug 17 2022	B.M.	Internal *Chaetogaster*
VAF-44S	Lab snails	NA	B.M.	
VAF-45S	Lab snails	NA	B.M.	
VAF-46S	Lab snails	NA	B.M.	
VAF-47S	Lab Snails	NA	S.D.	
VAF-48S	Lab Snails	NA	S.D.	
VAF-49S	Lab Snails	NA	S.D.	
VAF-50W	Morinville	NA	S.D.	Host ID not recorded
VAF-51W	Morinville	NA	S.D.	Host ID not recorded
VAF-52W	Morinville	NA	S.D.	Host ID not recorded

Table 2-4. Snail sequences chosen from GenBank for tree creation. Table includes sample ID of the BLASTed specimen, the GenBank accession number for the sequence selected for inclusion in the tree, locality of that specimen as reported in GenBank, species identification as reported in GenBank. A list of sequence depositors for each chosen sample is in Appendix 2-3.

Blasted Sample	GenBank Accession	Origin	Species ID
VAF-2S	KM612060	SK, Canada	*Physella ancillaria* Say
VAF-3S	MG421540	MB, Canada	*Physella ancillaria*
VAF-5S	MG423475	MB, Canada	*Physella ancillaria*
VAF-6S	MG422937	MB, Canada	*Physella ancillaria*
VAF-7S	GU680899	MB, Canada	*Physella jennessi* (Dall)
VAF-8S	GU680874	MB, Canada	*Physella jennessi*
VAF-9S	MG421606	MB, Canada	*Physella ancillaria*
VAF-10S	MG422342	MB, Canada	*Physella ancillaria*
VAF-11S	MG422145	MB, Canada	*Physella ancillaria*
VAF-12S	MG421380	MB, Canada	*Physella ancillaria*
VAF-13S	AF419323	Not given	*Physella wrighti* (Te & Clark)
VAF-14S	AF346745	Not given	*Physella wrighti*
VAF-26S	AY651179	CO, USA	*Physella wolfiana* (Lea)
VAF-27S	MK308008	MD, USA	*Physella gyrina* (Say)
VAF-28S	KT831388	AB, Canada	*Physella gyrina*
VAF-29S	AY651200	VA, USA	*Physella gyrina*
VAF-30S	AF346741	Not given	*Physella gyrina*
VAF-31S	MG421410	MB, Canada	*Physella ancillaria*
VAF-44S	KP182986	Singapore	*Physella acuta* (Draparnaud)
VAF-45S	OM970095	Vietnam	*Physella acuta*
VAF-46S	KF737921	Greece	*Physella acuta*
VAF-47S	MZ798294	Iran	*Physella acuta*
VAF-48S	OP566899	Not given	*Physella acuta*
VAF-49S	KM206699	Iraq	*Physella acuta*
NA – outgroup	KM612034	SK, Canada	*Aplexa elongata* Say
NA – outgroup	KM611811	SK, Canada	*Aplexa elongata*
NA – outgroup	MG421227	MB, Canada	*Aplexa elongata*

Table 2-5. *Chaetogaster* sequences chosen from GenBank for tree creation. Table includes sample ID of the BLASTed specimen, the GenBank accession number for the sequence selected for inclusion in the tree, locality of that specimen as reported in GenBank, species identification as reported in GenBank. A list of sequence depositors for each chosen sample can be found in Appendix 2-4.

BLASTed sample	**GenBank Accession**	**Origin**	**Species**
VAF-19W	OQ281711	NB, Canada	*Chaetogaster sp. 22*
VAF-20W	OQ281726	NB, Canada	*Chaetogaster sp. 22*
VAF-22W	OQ281710	AB, Canada	*Chaetogaster sp. 22*
VAF-24W	OQ281729	MD, USA	*Chaetogaster sp. 22*
VAF-33W	KF952346	NY, USA	*Chaetogaster limnaei*
VAF-34W	KF952336	NY, USA	*Chaetogaster limnaei*
VAF-35W	KF952309	MA, USA	*Chaetogaster limnaei*
VAF-36W	KF952303	NY, USA	*Chaetogaster limnaei*
VAF-37W	KF952300	MA, USA	*Chaetogaster limnaei*
VAF-38W	OQ281712	AB, Canada	*Chaetogaster sp. 22*
VAF-39W	KF952313	MA, USA	*Chaetogaster limnaei*
VAF-40W	KF952333	NY, USA	*Chaetogaster limnaei*
VAF-41W	KF952340	MA, USA	*Chaetogaster limnaei*
VAF-43W	KF952323	MA, USA	*Chaetogaster limnaei*
VAF-50W	KF952311	NY, USA	*Chaetogaster limnaei*
VAF-51W	KF952298	NY, USA	*Chaetogaster limnaei*
VAF-52W	KF952326	NY, USA	*Chaetogaster limnaei*
NA – outgroup	LN810268	Switzerland	*Chaetogaster diaphanus*
NA – outgroup	JQ519897	Not given	*Chaetogaster diaphanus*

Table 2-6. List of specimens used for *Chaetogaster* gut contents investigation.

Sample	**Related experiment**	**Organism Origin**		**Preservation**
		Chaetogaster	**Snail**	**date (in 2022)**
Internal *Chaetogaster*	NA	Morinville	Morinville	August 29
External *Chaetogaster*	*Chaetogaster* Survey	Morinville	Morinville	July 14
External *Chaetogaster*	*Chaetogaster* Survey	Morinville	Morinville	July 14
External *Chaetogaster*	*Chaetogaster* Survey	Lafarge	Lafarge	July 14
External *Chaetogaster*	*Chaetogaster* Survey	Morinville	Morinville	July 28
External *Chaetogaster*	Fitness Experiment 1	Morinville	Lab Bred	June 13
External *Chaetogaster*	Fitness Experiment 1	Morinville	Lab Bred	June 13
External *Chaetogaster*	Fitness Experiment 1	Morinville	Lab Bred	June 13
External *Chaetogaster*	Fitness Experiment 1	Morinville	Lab Bred	July 8
External *Chaetogaster*	Fitness Experiment 2	Morinville	Lab Bred	Sept 20
External *Chaetogaster*	Fitness Experiment 2	Morinville	Lab Bred	Sept 20

Table 2-7. Summary of *Chaetogaster* survey over both years and locations. The mean abundance +/- SE, and range in number of external *Chaetogaster* found per dissected physid snail in 2021 and 2022 from Lafarge (LF) and Morinville (M) sites. Sample size of snails and mean +/-SE and range of snail size are also reported.

Year	**Location**	**N**	**Mean +/- SE Worms/Snail**	**Range Worms/Snail**	**Mean +/- SE Snail Length (mm)**	**Range Snail Length (mm)**
2021	LF	76	5.71 ± 0.58	0 – 21	8.33 ± 0.33	4.34 – 15.8
2021	M	57	13.60 ±0.80	2 – 29	12.85 ± 0.24	7.00 – 15.45
2022	LF	241	5.27 ± 0.37	0 – 46	11.70 ± 0.21	4.36 – 18.70
2022	M	243	3.88 ± 0.23	0 – 23	9.92 ± 0.15	5.27 – 17.85

Table 2-8. Model creation for Lafarge 2021 survey data. The chosen model is indicated with an asterisk '*'.

Model	Parameters	Interaction terms	AICc
1	Collection week, Snail host length	Between both variables	351.08
2*	Collection week, Snail host length	NA	349.53

Table 2-9. Analysis of deviance table for model 2, Lafarge 2021. Significant values are indicated with an asterisk '*'.

Predictor	LR X^2	DF	P Value
Collection Week	3.408	1	0.065
Snail Host Length	84.858	1	$< 2.0^{-16}$*

Table 2-10. Model creation for Morinville 2021 data. The chosen model is indicated with an asterisk '*'.

Model	Parameters	Interaction terms	AICc
1	Collection week, Snail host length	Between both variables	369.06
2*	Collection week, Snail host length	NA	366.06

Table 2-11. ANOVA table for Morinville 2021 model output. Significant values are indicated with an asterisk '*'.

Predictor	Sum of squares	DF	F value	P Value
Collection Week	84.10	1	2.552	0.116
Snail Host Length	283.82	1	8.613	0.005 *
Residuals	1779.45	54		

Table 2-12. Model selection for *Chaetogaster* survey in Lafarge 2022. The chosen model is indicated using an asterisk '*'.

Model	Parameters	Interaction terms	AICc
1	Collection week, Snail host length, number of metacercariae	Collection week: snail host length	1143.65
2	Collection week, snail host length, number of metacercariae	Snail host length: metacercariae	1135.58
3	Collection week, Snail host length, number of metacercariae	Collection week: metacercariae	1152.78
4	Collection week, Snail host length, number of metacercariae	NA	1139.70
5	Collection week, Snail host length	Between both variables	1141.26
6*	Collection week, Snail host length	NA	1137.42

Table 2-13. Summary of statistical results, Lafarge 2022. Significant values are indicated with an asterisk '*'.

Predictor	LR X^2	DF	P Value
Collection Week	181.285	12	$< 2.2^{-16}$*
Snail Host Length	43.076	1	5.265^{-11}*

Table 2-14. Model selection for *Chaetogaster* density in Morinville 2022. The chosen model is indicated with an asterisk '*'.

Model	Parameters	Interaction terms	AICc
1	Collection week, Snail host length, number of internal *Chaetogaster*	Collection week: snail host length	1071.08
2	Collection week, snail host length, number of internal *Chaetogaster*	Snail host length: internal *Chaetogaster*	1048.92
3	Date, Snail host length, number of internal *Chaetogaster*	Collection week: internal *Chaetogaster*	1072.23
4	Date, Snail host length, number of internal *Chaetogaster*	NA	1053.64
5	Date, Snail host length	Between both variables	1068.72
6*	Collection week, Snail host length	NA	1051.50

Table 2-15. Analysis of deviance table for *Chaetogaster* density, Morinville 2022. Results are from the selected model 3 (n=243). Significant values are indicated with an asterisk '*'.

Parameter	LR X^2	DF	p Value
Collection week	187.588	12	$< 2^{-16}$ *
Host snail length	6.052	1	0.014 *

Table 2-16. Cage Experiment 3 dissection results. Cages were deployed on July 27th, 2022, and collected 16 days later. All cages with live snails (*Chaetogaster*(+) = 9, *Chaetogaster*(-) = 8), excluding those found containing extra snails, were examined and the snails dissected for examination of symbionts.

Number of:	**Treatment**	
	***Chaetogaster*(+)**	***Chaetogaster*(-)**
Cages with live snails	9	8
Snails dissected (i.e., excludes cages with extra snails)	7	3
Snails with trematode infection	0	0
Snails with metacercariae	2	0

Table 2-17. Table of water quality results from Lafarge and lab colony water samples.

Water property	**Lafarge Pond 1**	**Lafarge Pond 2**	**Lab Colony**
Total P (µg/L as P)	375	235	13000
Total N (µg/L as N)	2000	1990	6050
pH	8.85	8.74	6.5
Alkalinity (mg/L as $CaCO_3$)	255.69	238.43	7.55
Conductivity (µS/cm)	956	953	223
Total Dissolved Solids	675	664	154
Chlorophyll-a (µg/L)	39.01	71.98	21.22

Figures

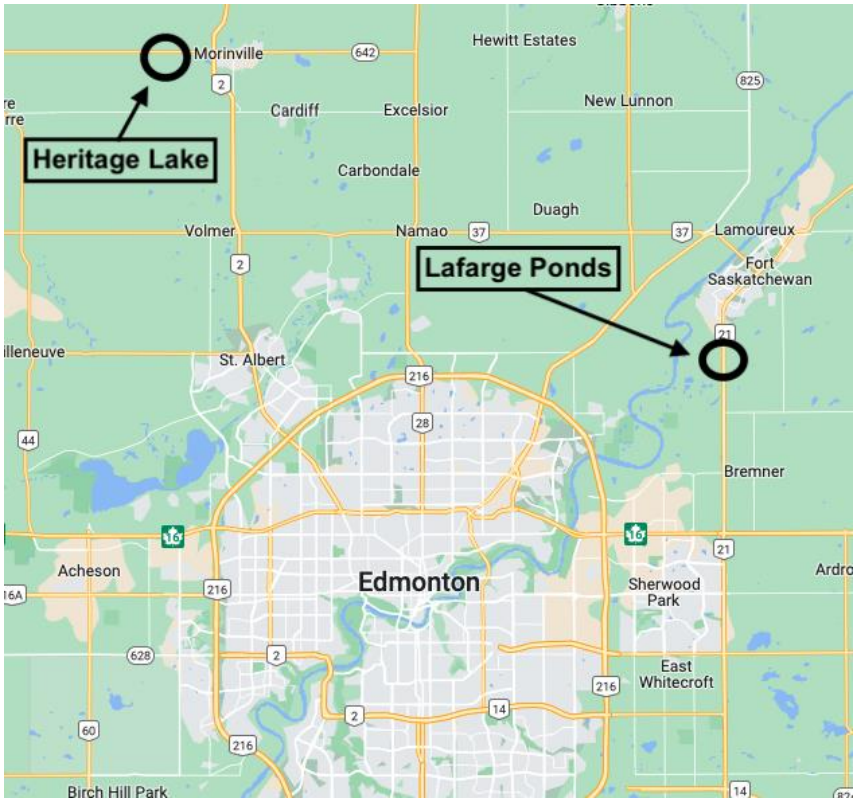

Figure 2-1. Morinville and Lafarge Canada collection locations. The chosen field sites were used in the snail and *Chaetogaster* surveys and field experiments described in this thesis.

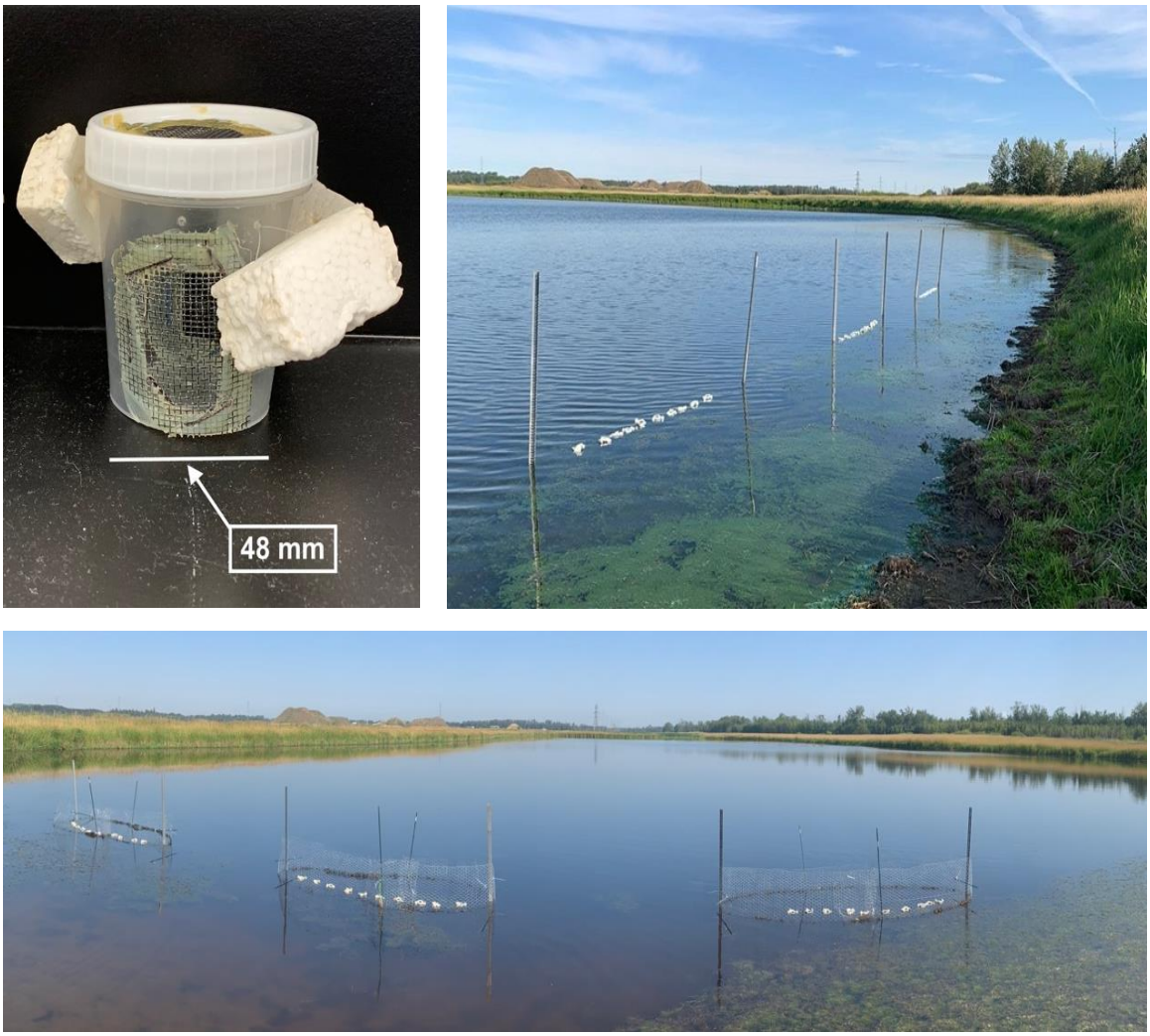

Figure 2-2. Images of cage experiment setup. Individual snail cage (top left) and lines of cages set up in one of the two Lafarge ponds in 2021 (top right). Lines of cages with chicken-wire fences used in 2022 (bottom).

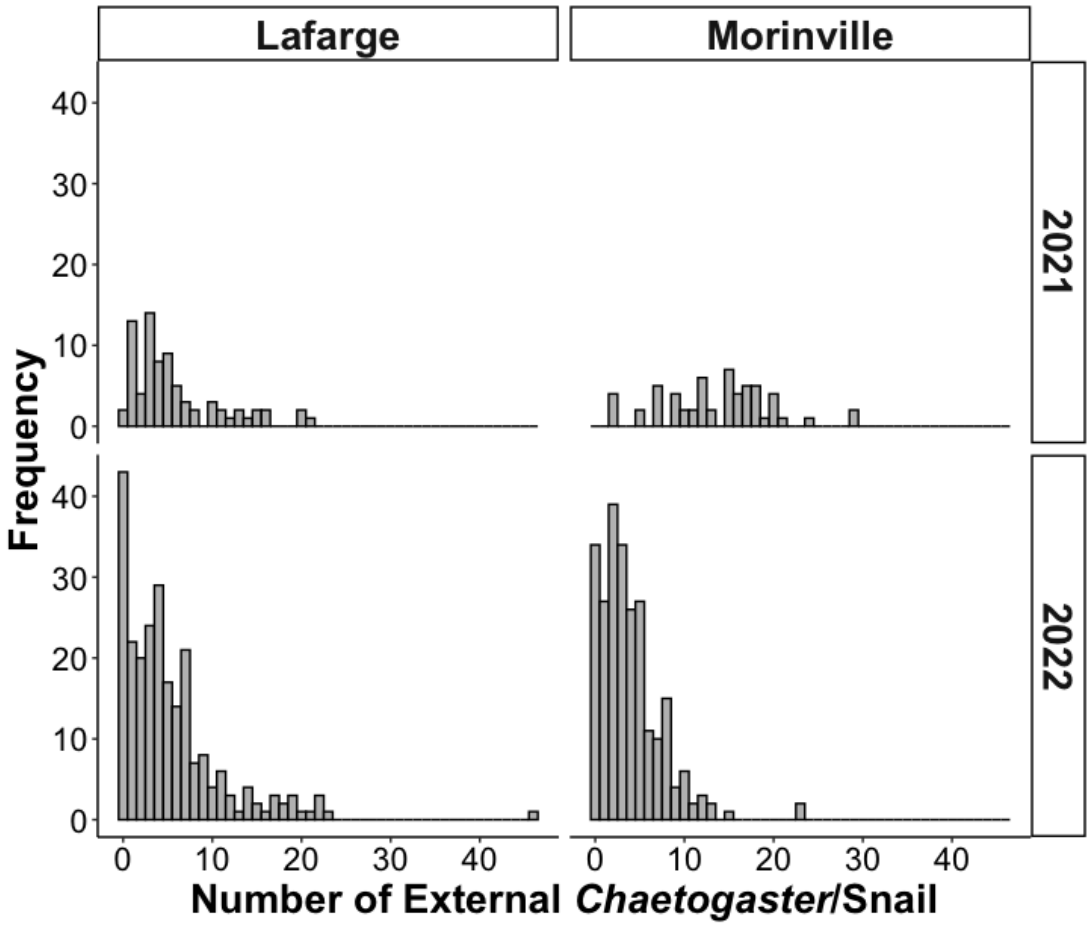

Figure 2-3. Overall abundance of external *Chaetogaster* in 2021 and 2022. A total of 617 snails from Lafarge and Morinville ponds were dissected over 2021 and 2022 to determine the number of external *Chaetogaster*/snail (see Table 2-1 and Table 2-2 for details).

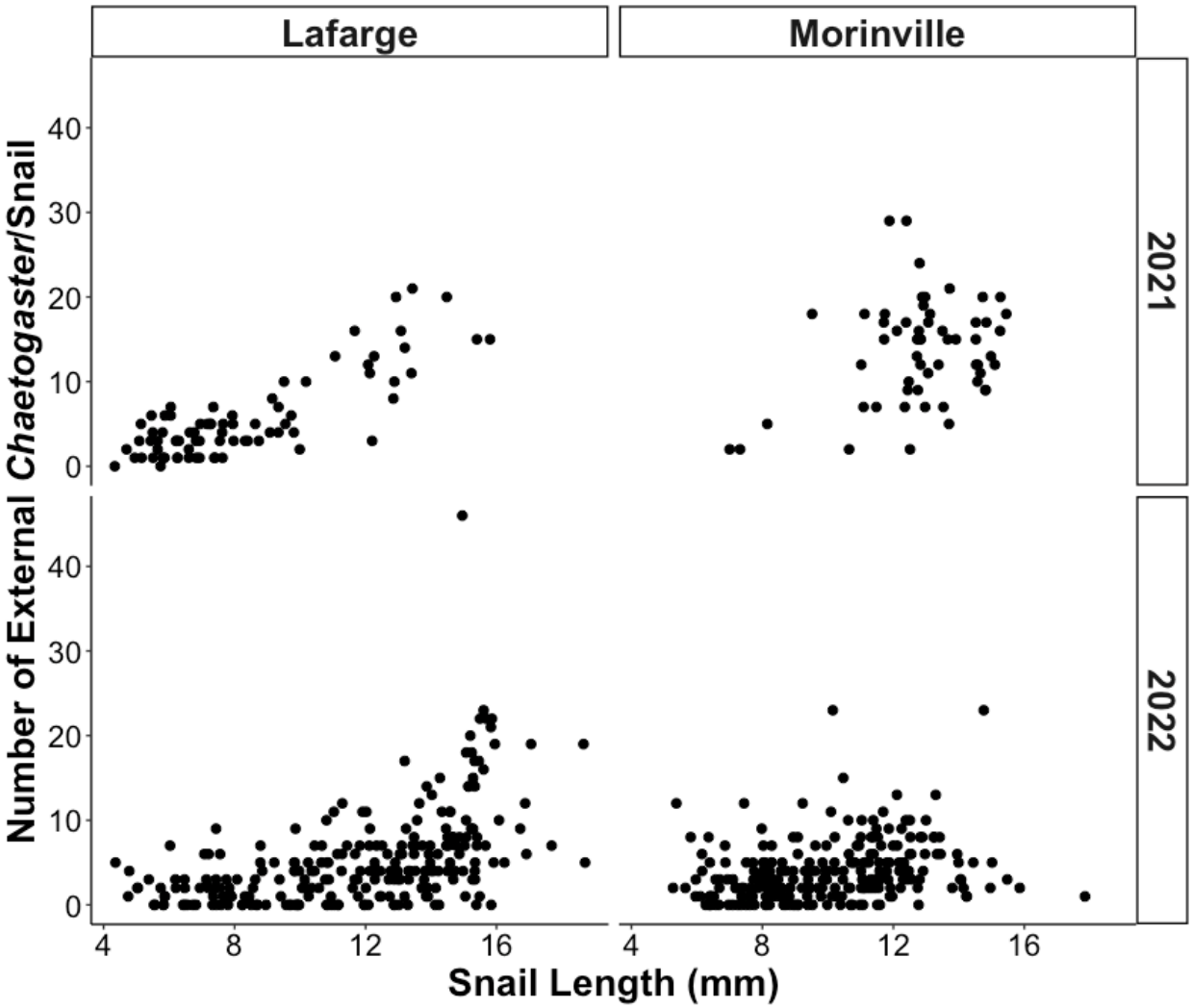

Figure 2-4. Abundance of external *Chaetogaster* relative to snail size for all surveyed snails. A total of 617 snails from Lafarge and Morinville ponds were dissected over 2021 and 2022 to determine the number of external *Chaetogaster*/snail (see Table 2-1 and Table 2-2 for details).

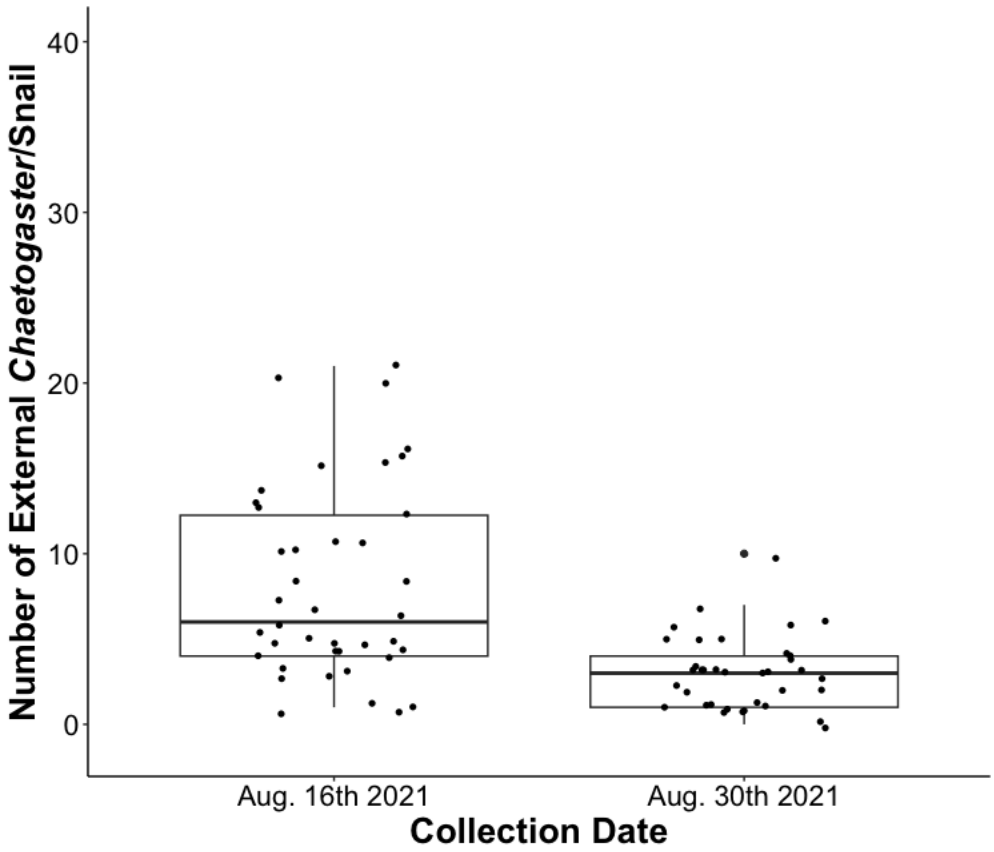

Figure 2-5. Abundance of external *Chaetogaster*/snail at Lafarge in 2021. In 2021, 76 snails collected on the 16th and 31st of August were dissected for external *Chaetogaster*. Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values. Data points have been jittered for clarity.

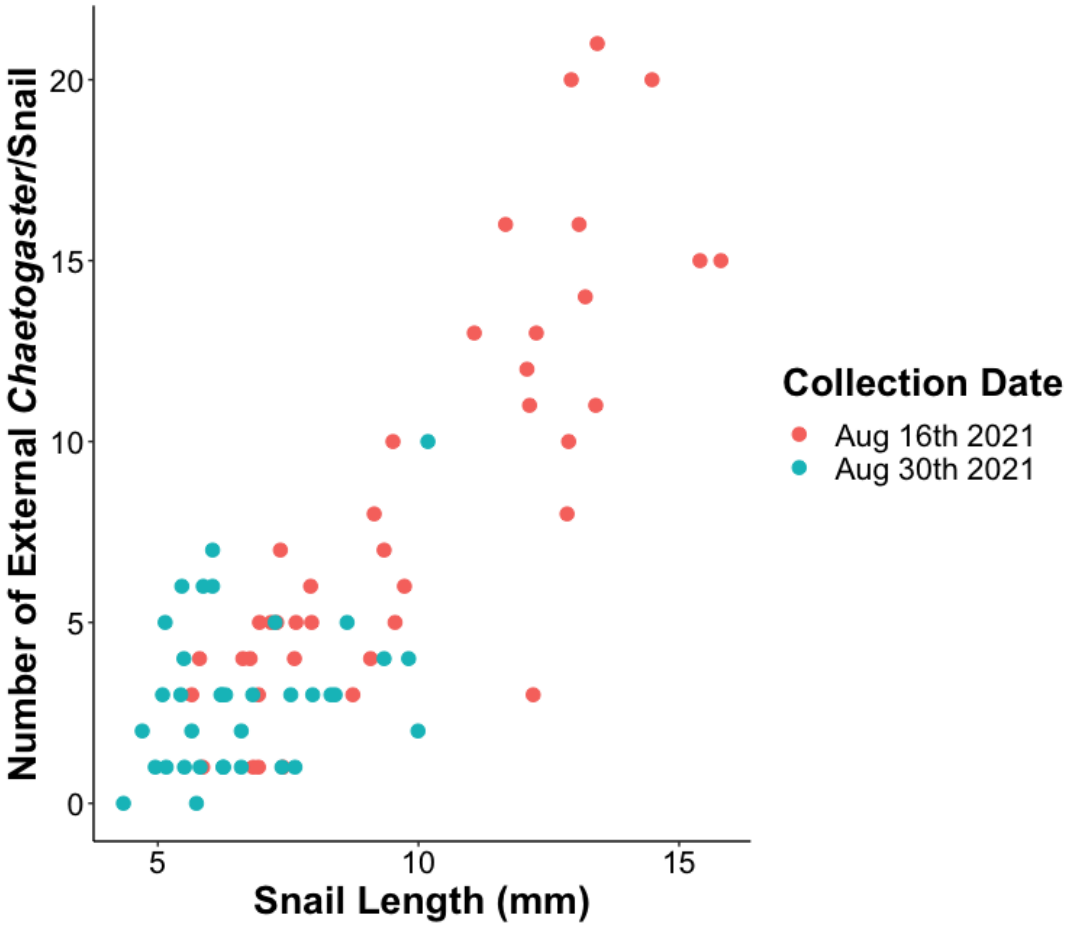

Figure 2-6. External *Chaetogaster* abundance by host snail size from Lafarge in 2021. 76 snails were dissected for external *Chaetogaster*. Snails were collected on August 16th and 30th in 2021.

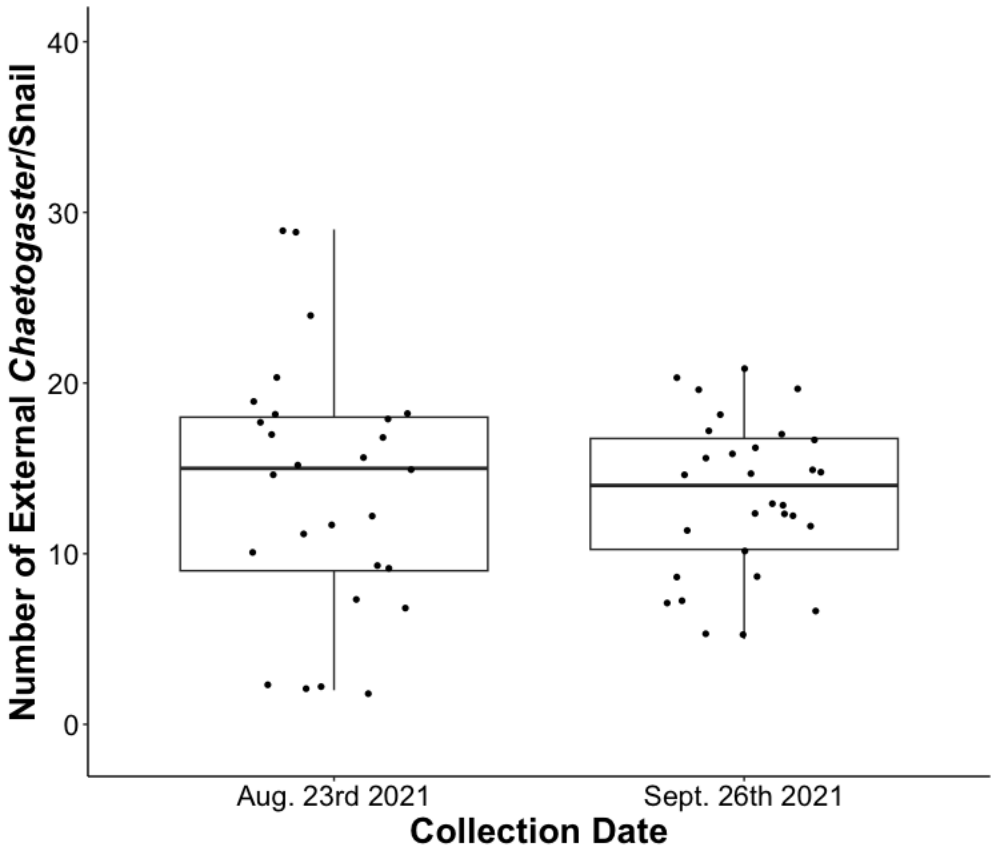

Figure 2-7. Abundance of external *Chaetogaster*/snail at Morinville in 2021. In 2021, 57 snails collected on the 23rd of August and 26th of September were dissected for external *Chaetogaster*. Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values. Data points have been jittered for clarity.

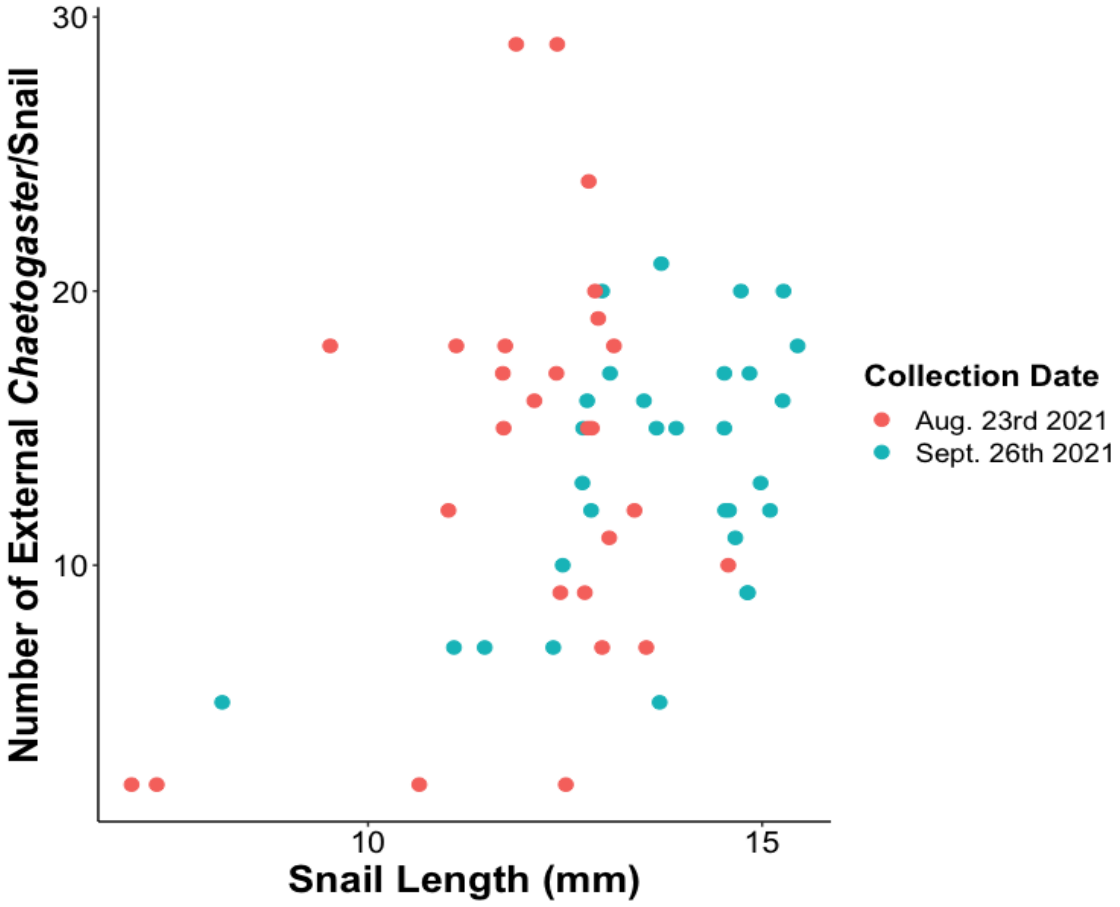

Figure 2-8. External *Chaetogaster* abundance by snail length from Morinville 2021. Fifty-seven snails from two sampling trips were dissected to collect data on the number of external *Chaetogaster*. Snails were collected on August 23rd and September 26th in 2021.

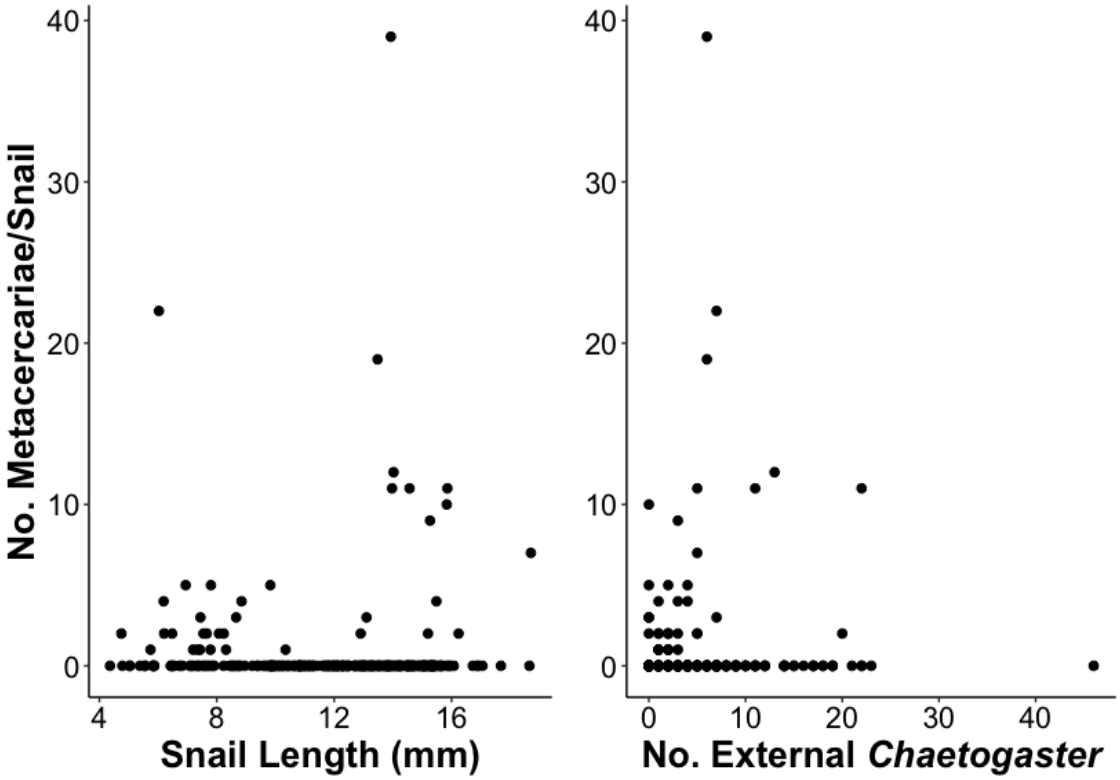

Figure 2-9. Metacercariae counts from 241 Lafarge snails in 2022. The left panel shows the number of metacercariae/snail relative to snail size (mm), while the right displays the number of metacercariae/snail relative to the number of external *Chaetogaster*/snail. See Table 2-2 for a summary of collection dates.

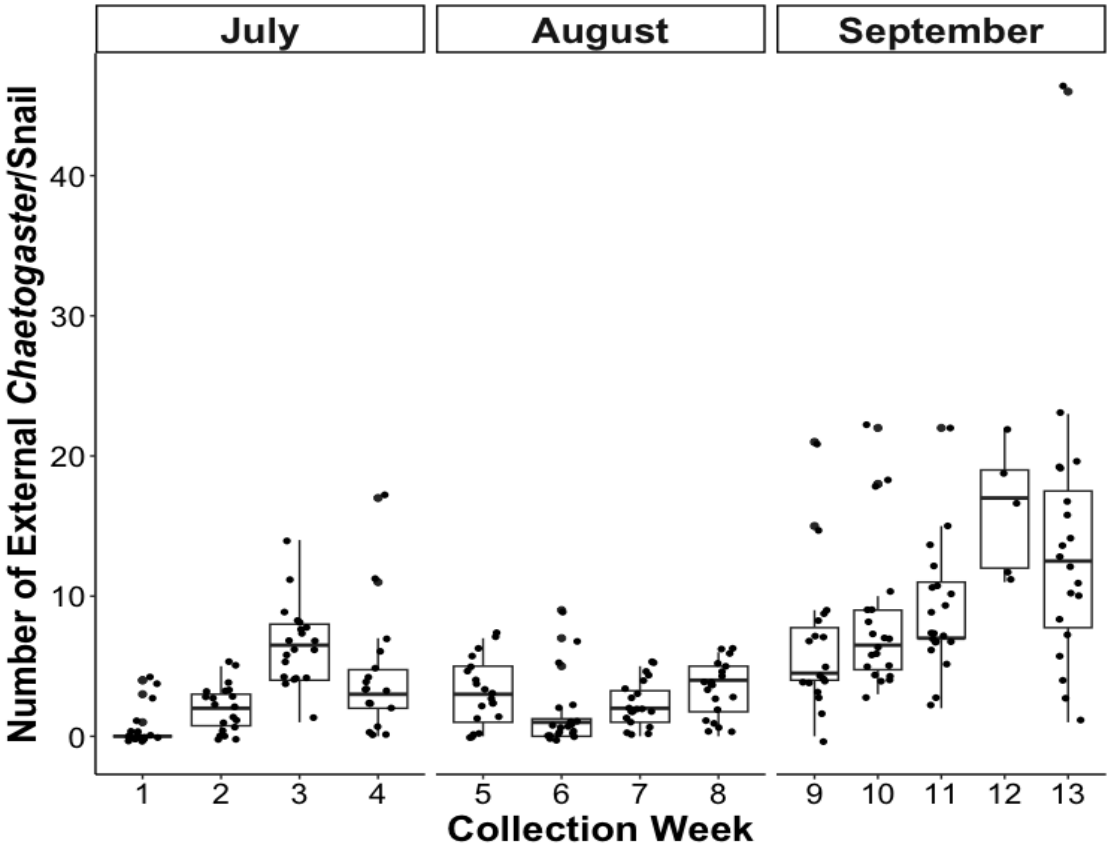

Figure 2-10. Number of external *Chaetogaster*/snail in Lafarge per month in 2022. A total of 241 snails collected over 13 weeks were considered in this dataset, see Table 2-2 for a summary of collection dates. Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values. Data points have been jittered for clarity.

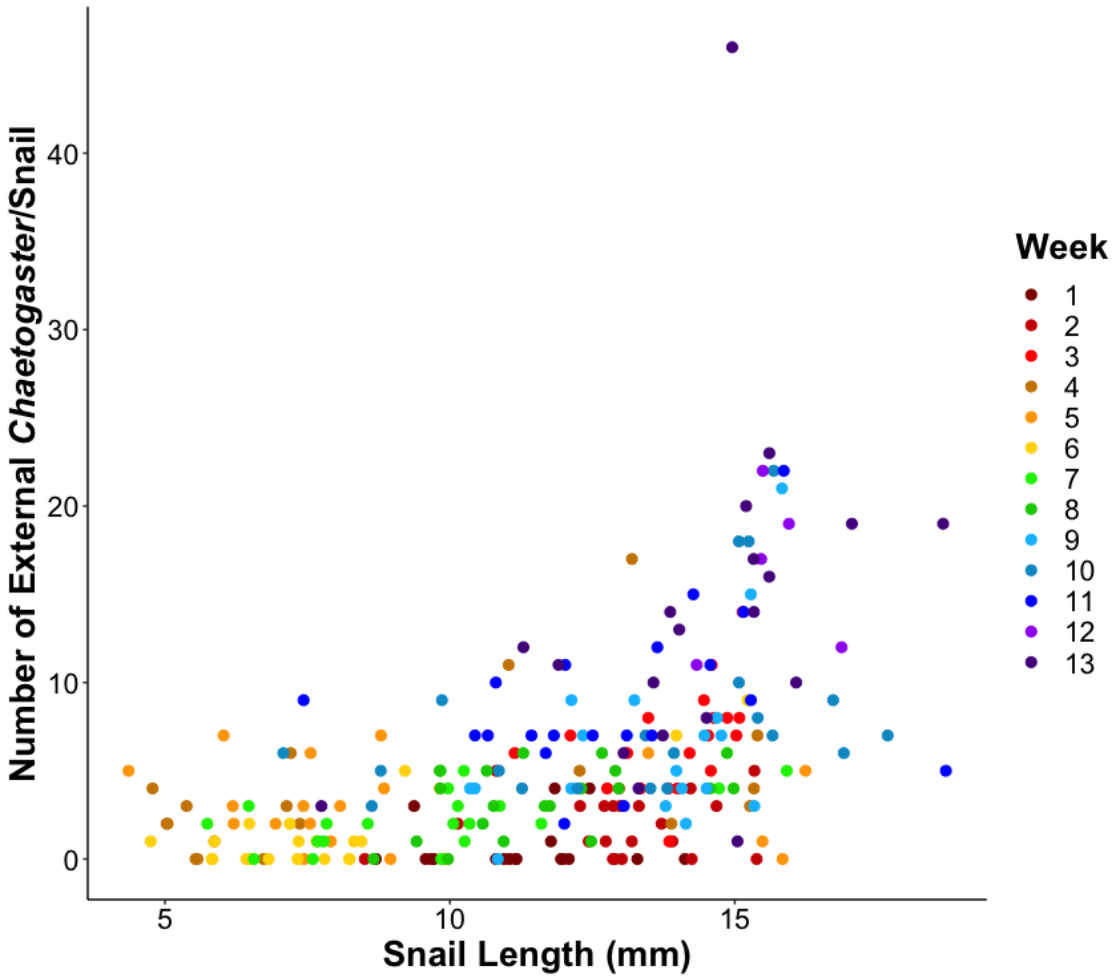

Figure 2-11. *Chaetogaster*/snail numbers from 241 Lafarge snails over summer 2022. See Table 2-2 for a summary of collection dates.

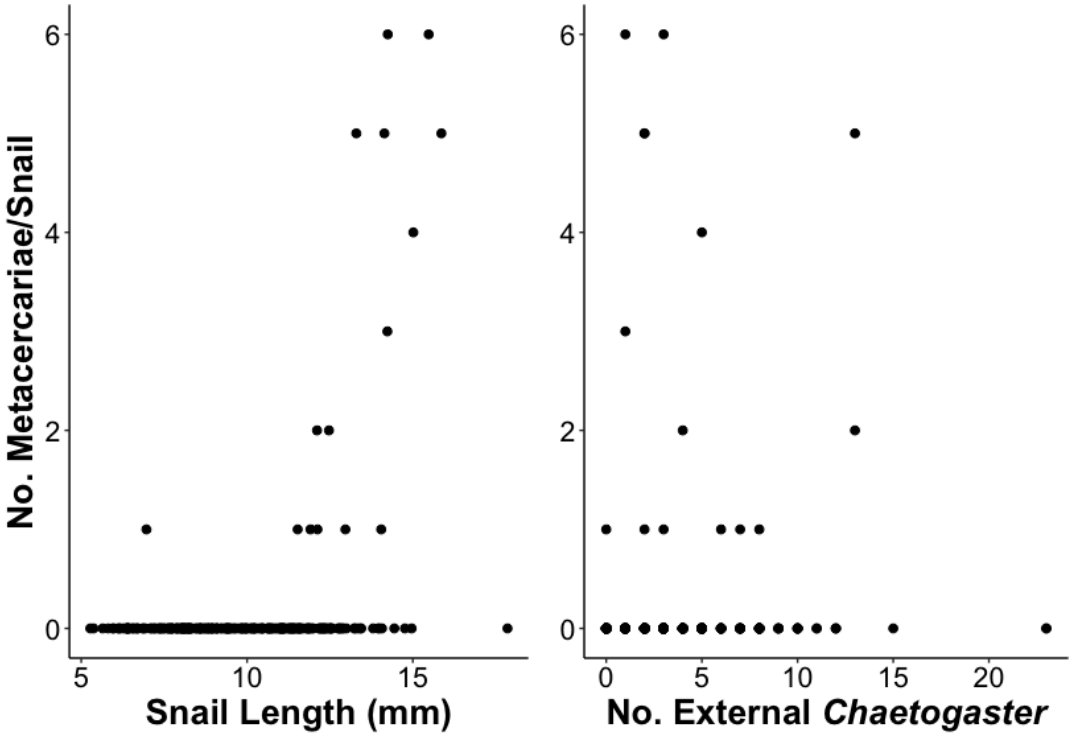

Figure 2-12. Metacercariae counts from 243 Morinville snails in 2022. The left panel shows the number of metacercariae/snail relative to snail size (mm), while the right displays the number of metacercariae/snail relative to the number of external *Chaetogaster*/snail. See Table 2-2 for a summary of collection dates.

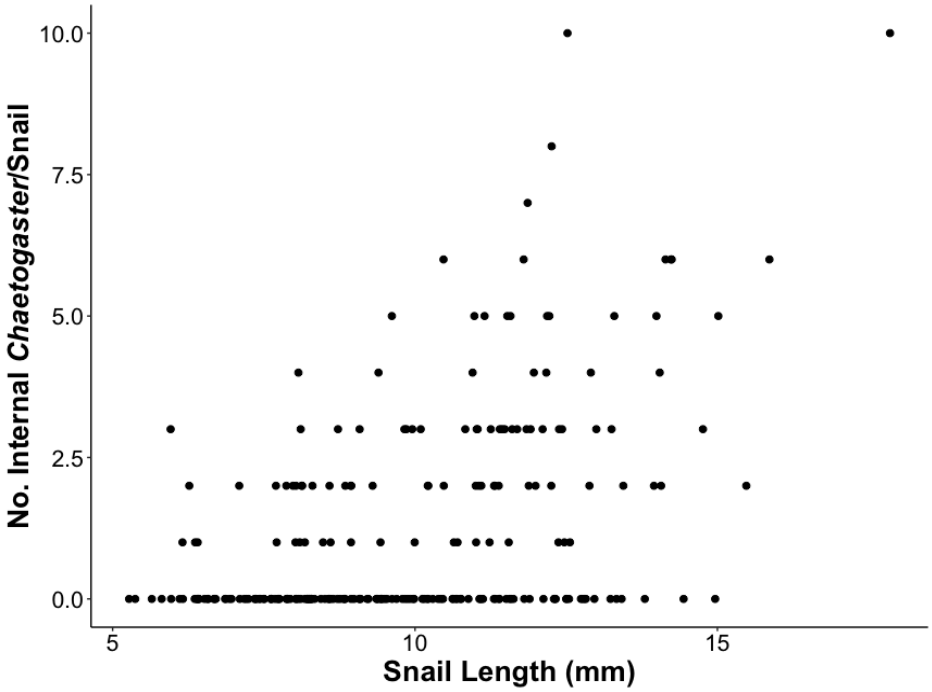

Figure 2-13. The number of internal *Chaetogaster* by snail size for Morinville 2022. A total of 243 snails were considered in this dataset.

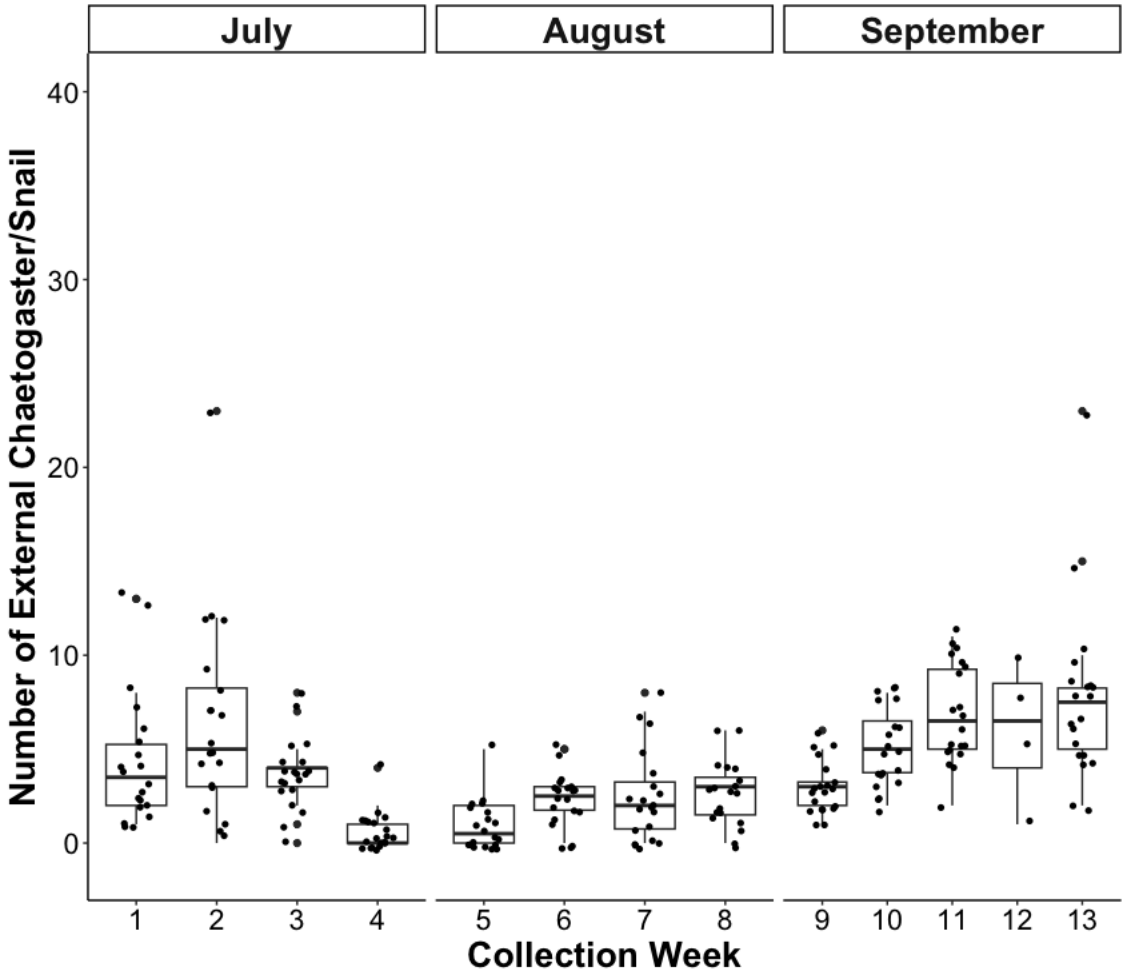

Figure 2-14. Number of external *Chaetogaster*/snail in Morinville over summer 2022. Twenty snails were examined for external *Chaetogaster* each week for 13 weeks (See Table 2-2 for dates and exceptions), the data has been partitioned by month. Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values. Data points have been jittered for clarity.

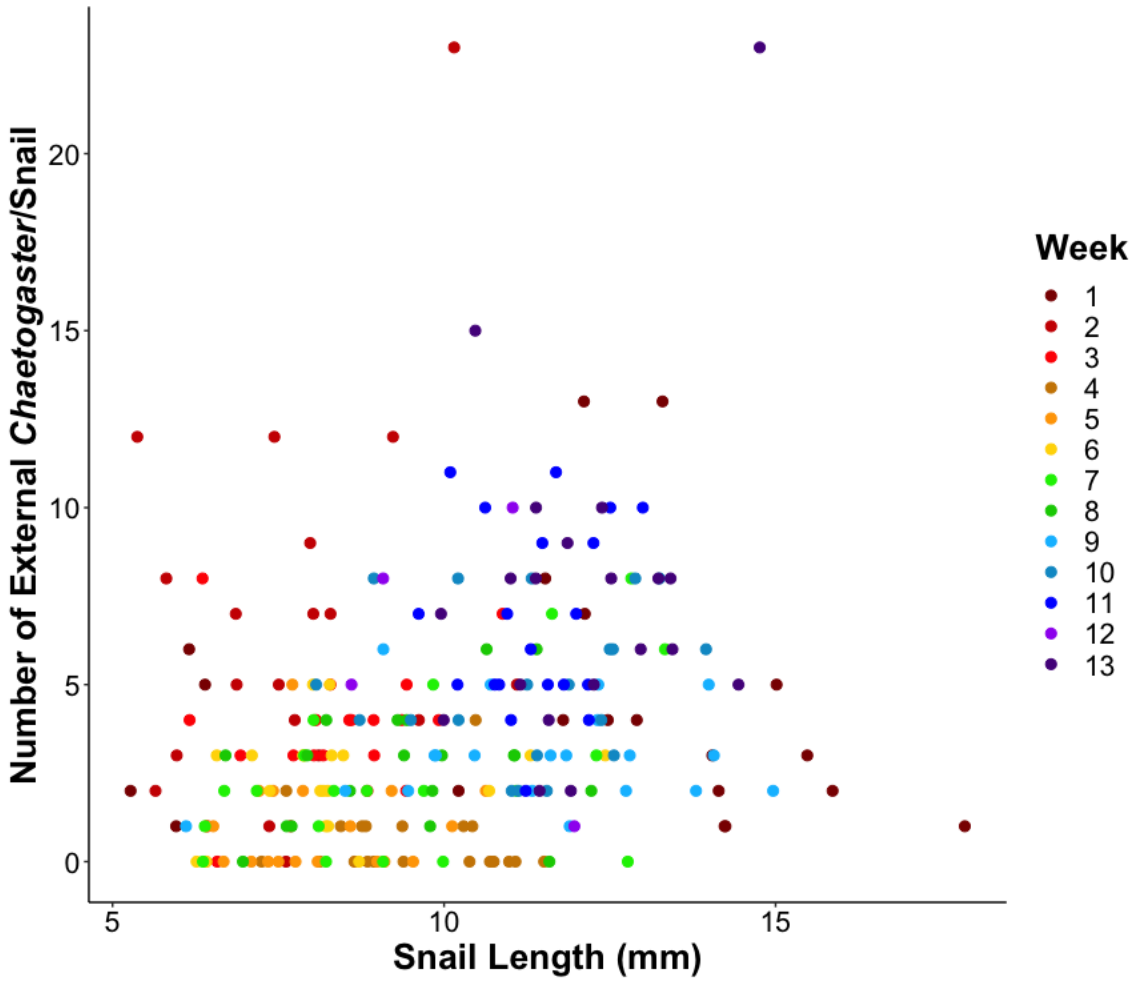

Figure 2-15. *Chaetogaster* abundance by snail size in Morinville over summer 2022. Twenty snails were examined for external *Chaetogaster* each week for 13 weeks (See Table 2-2 for dates and exceptions).

Figure 2-16. Snail cages disturbed by local wildlife in 2021. A cluster of dislodged cages is indicated by the arrow.

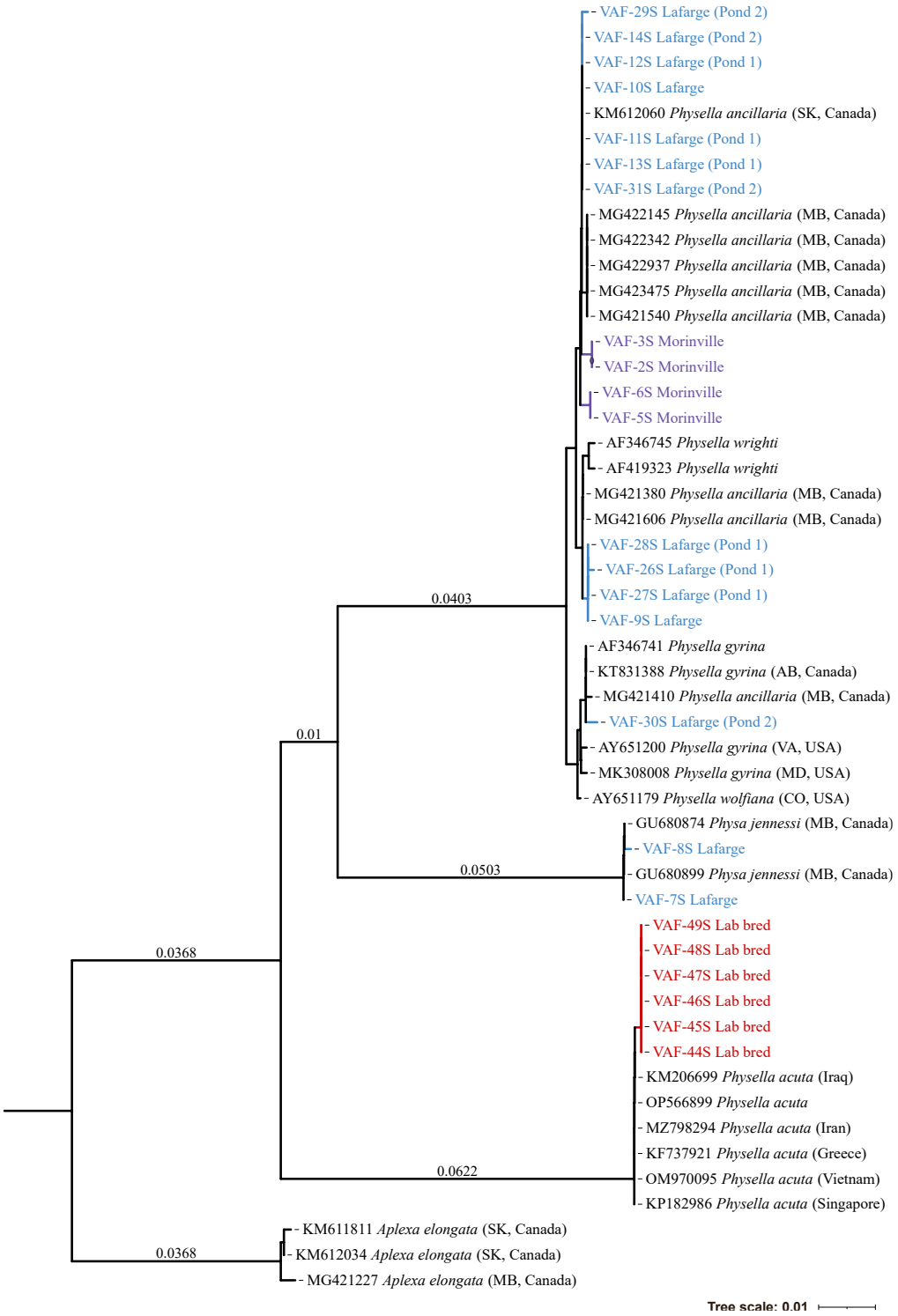

Figure 2-17. Physid neighbour-joining tree with p-distances. Created with COI 'barcode' sequences from 24 physid specimens collected from Lafarge and Morinville in 2021 and 2022 (Table 2-3) as well as 27 sequences pulled from GenBank (see Table 2-4 for list Appendix 2-2 for attribution). Numbers above branches are branch lengths calculated using p-distances, only branch lengths above 0.01 were included. My samples are colour coded according to origin.

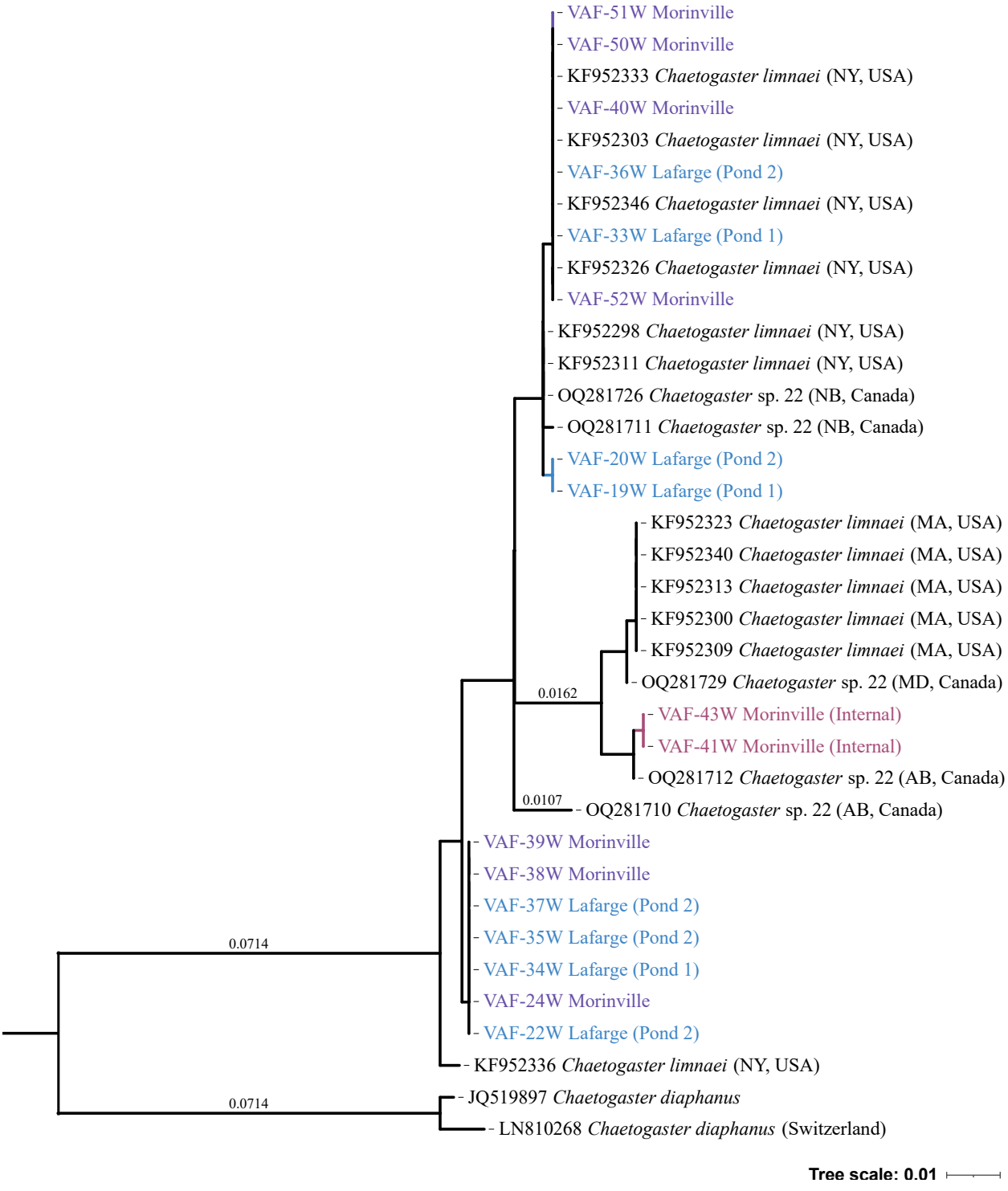

Figure 2-18. *Chaetogaster* Neighbour Joining tree with p-distances. Created with COI 'barcode' sequences from 17 specimens collected from physid snail hosts from Lafarge and Morinville in 2021 and 2022 (Table 2-3) as well as 19 sequences pulled from GenBank (see Table 2-5 for list and Appendix 2-4 for attribution). Numbers above branches are branch lengths calculated using p-distances, only branch lengths above 0.01 were included. My samples are colour coded according to origin.

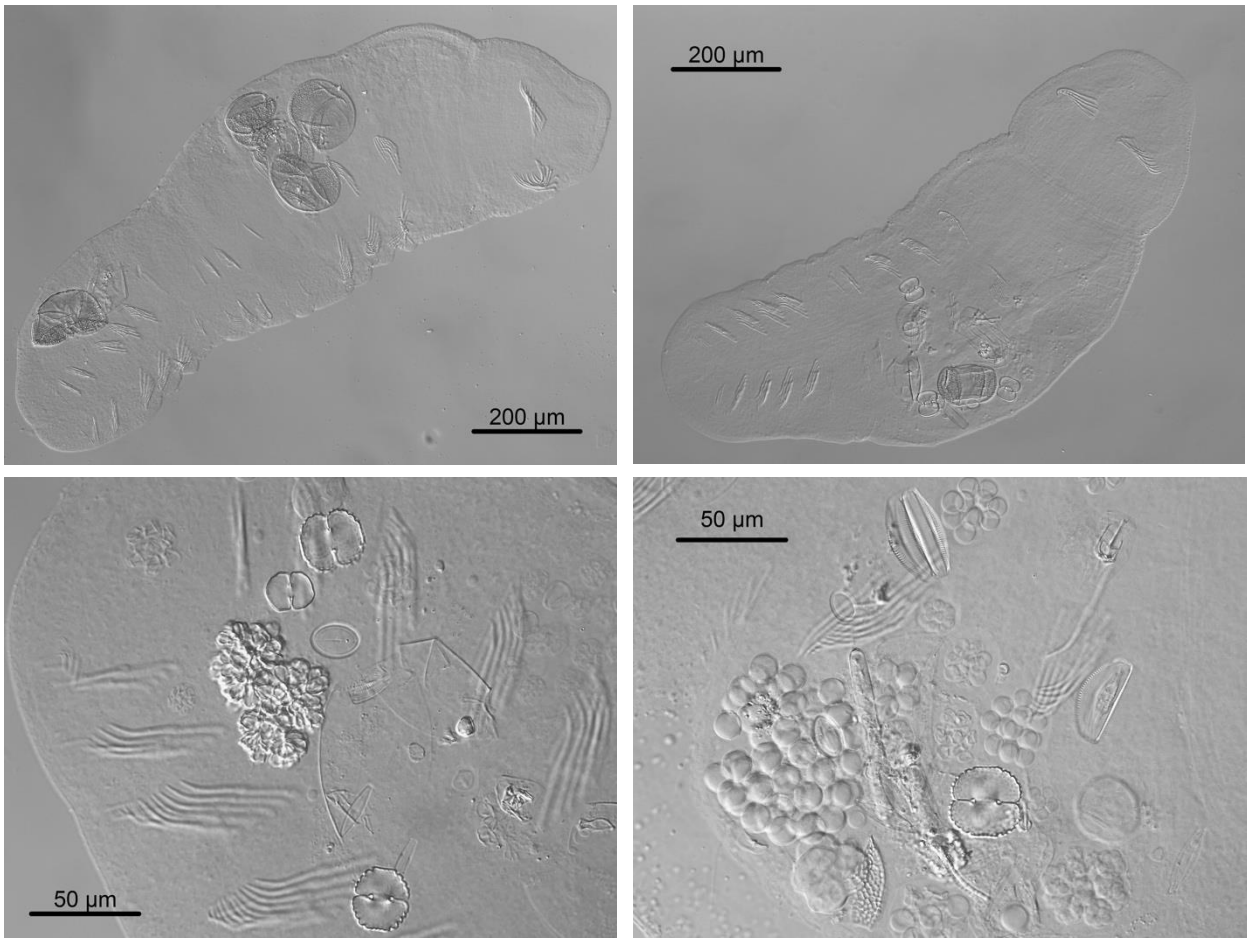

Figure 2-19. Images of slide mounted external *Chaetogaster* from field-collected physids. Top images are of *Chaetogaster* at 100x magnification with examples of pollen in their gut contents. Bottom images are *Chaetogaster* gut contents at 400x magnification, including examples of diatoms and other algae.

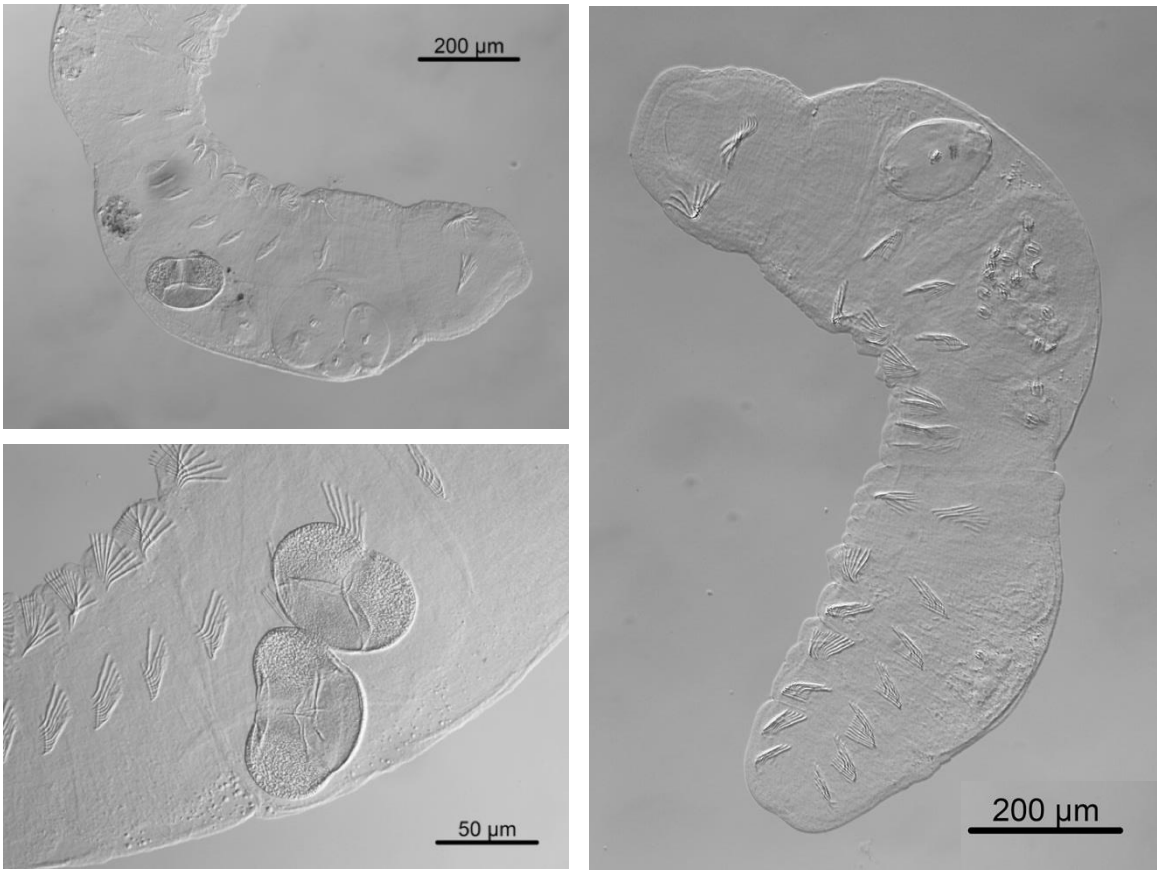

Figure 2-20. Images of slide mounted *Chaetogaster* from lab-bred snails. Top left and right images are both at 100x magnification and show pollen and rotifers in *Chaetogaster* gut contents. Bottom left image is at 400x magnification and shows pollen in a *Chaetogaster* gut contents.

Chapter 3. Fitness Experiments

Introduction

In Chapter 2, I investigated the association between *Chaetogaster* and host snails in the field. Here in Chapter 3, I examine the possible effects of *Chaetogaster* on host fitness in the lab. Previous lab experiments suggest that *Chaetogaster* may have negative effects on host fitness by affecting their reproduction as well as foraging and resting behaviour, especially when snails are heavily infected (Stoll et al. 2013; Stoll et al. 2017).

Two growth experiments were conducted by Stoll et al. (2013) with *Physella acuta* (Draparnaud) (Physidae) host snails, the first conducted over the span of one week and the other over five weeks. In the short-term experiment, 10-day old snails were given 0, 1, 5, 10 or 20 *Chaetogaster*. After one week, snails given 20 worms had grown the least, those given 0 worms the most with those given 10 worms falling in the middle. These results suggest that heavy *Chaetogaster* colonization may decrease snail growth. In the long-term growth experiment, 10-day old snails were placed into two treatments (*Chaetogaster*((+/-)) and monitored for growth and reproduction over five weeks (Stoll et al. 2013). Here it was again found that snails with *Chaetogaster* had lower growth and reproductive success. *Chaetogaster*(+) snails laid fewer eggs with a lower hatch rate than those in the *Chaetogaster*(-) treatment.

More recently, a survey of snails in seven streams in Germany found that population-level reproductive success appeared to be inversely related to *Chaetogaster* abundance regardless of snail species (Stoll et al. 2017). In this study, 'reproductive success' was estimated by proportion of juveniles in the population. Since *Chaetogaster* worms colonize some snail species but not others and may vary in colonization intensity between species, these symbionts may play a role in structuring freshwater snail communities.

Here I present two laboratory experiments similar to the long-term growth and reproduction experiment conducted by Stoll et al. (2013). I hypothesized that *Chaetogaster* presence would negatively impact host fitness in relation to host reproduction and host growth and predicted I would see a similar pattern as in Stoll et al. (2013), with *Chaetogaster*(+) snails having lower growth and reproductive rates. I use lab-bred snails that are very likely also *P. acuta*, (see Chapter 2 for more details), so the Stoll et al. (2013) experiment is useful comparison to my own.

Methods

Here I describe the first fitness experiment in detail followed by a short description of the changes made to the methods for the second experiment.

Fitness Experiment 1

Sixty lab-bred *Physella acuta* were randomly divided into two equal groups and placed into two separate containers. One group was assigned to be the *Chaetogaster*(+) group and was given *Chaetogaster* from field-collected snails while the other did not receive any *Chaetogaster* but was otherwise treated the same. Worms (exact number not recorded although likely around ~70) for the *Chaetogaster*(+) treatment were collected from physid snails from Heritage Lake in Morinville (see Chapter 2) and pipetted gently into the *Chaetogaster*(+) container, where they were left to colonize the snails and reproduce for ~3 weeks.

On May 27th, 2022, Fitness Experiment 1 began. At this time, each snail was taken from their respective container (either *Chaetogaster*(-) or *Chaetogaster*(+) as described above)and individually photographed on a piece of graph paper so that it could be measured later using ImageJ (see below for details of photography). Snails in the *Chaetogaster*(+) group were also checked to ensure the presence of worms on each snail used for this treatment. Each snail was then put in a small clear plastic container by itself with ~80 ml of artificial spring water (ASW; see Appendix 2-1 for recipe) and a small piece of organic lettuce. Generally red-leafed lettuce was used, however other lettuce varieties were also occasionally used. All the containers had screw top lids that were left unscrewed and slightly tipped open to allow air circulation in the container. The containers were left near a north-facing window so that they were exposed to a

natural day/night cycle (~16:37 to 15:31 hrs:mins of daylight) from 27 May 2022 to 8 July 2022 (Figure 3-1).

Containers were checked three times a week for snail deaths and to replenish lettuce that had been eaten. Once a week the snails were also given a water change in which a pipette was used to remove ~20 mL of water and any feces at the bottom of cup. New artificial spring water was added top up the water level to ~80 mL. At the same time as the water change, each snail was removed and photographed on graph paper again so that I could track the growth of the snails over time. Additionally, any egg masses found in the containers were removed and the eggs counted before being placed in a well-plate with some ASW. Egg masses were monitored for 20 days after collection and the number of hatched snails was counted at the end of this time.

Although the original intent was to run Experiment 1 until all snails had died, after four weeks I observed that several snails in the *Chaetogaster*(+) treatment had lost *Chaetogaster,* so the experiment was ended at six weeks. At the end of the experiment all snails were photographed one last time and snails in the *Chaetogaster*(+) treatment were dissected so that a final *Chaetogaster* count could be made.

Fitness Experiment 2

For my second Fitness Experiment, I collected 70 lab-bred *P. acuta* and randomly assigned 30 to the *Chaetogaste*r(-) treatment and 40 to *Chaetogaster*(+). On 8 August 2022, each snail was photographed on graph paper and then placed in the same clear plastic containers as described above. Snails in the *Chaetogaster*(+) treatment were each given five *Chaetogaster* collected from physid snails from Heritage Lake in Morinville. The *Chaetogaster* were gently pipetted into each snail's container and then left for 24 h to colonize the snail. After 24 h each

snail in the *Chaetogaster*(+) treatment was checked under a dissecting microscope for *Chaetogaster* colonization. Colonization was a success in all snails, and the experiment ran for 6 weeks from the 9th of August to the 20th of September 2022.

In this iteration of the Fitness Experiment, each container was checked daily from Monday-Friday for snail mortality and lettuce was replenished as necessary. The position of the snail in the container (below or above the water line) was also noted. Once a week, snails were given a water change, at which time snails in the *Chaetogaster*(+) treatment were checked under the dissecting microscope for continued presence of *Chaetogaster*. Snail eggs were removed and counted from each container during these checks; however, they were not saved to assess hatching rates. Snails were not photographed weekly in this experiment, only at the start and end of the experiment (or if the snail died before the end of the experiment). All other aspects of this experiment were the same as in the first Fitness Experiment

Statistics

At the start of Fitness Experiment 1 my plan was to use snail growth, egg production and egg hatching rate as proxies for snail fitness. Snail growth was calculated by comparing the size of snails at the start and end of the experiment; however, growth was minimal and measurement error was high relative to the snails' body size range (4-6 mm), so apparent changes in size over the period were not reliable. Also, ImageJ-based estimates of snail body size based on photographs showed imperfect repeatability, with snails photographed only four days apart showing an average of 0.832 mm difference in length (Appendix 3-1). Because of this, I did not do any statistical analyses on changes in size for either Fitness Experiment 1 or 2 (see Appendix

3-2 for raw data). Instead, for both experiments egg production was used as the primary metric to determine the effect of *Chaetogaster* presence on host fitness.

In Experiment 1, egg hatch rate was also measured; however, it became evident that the wells in the 24-well plates were not large enough for the eggs as the water became murky close to the 20-day mark, suggesting bacterial growth. At this point it was not feasible to put the collected eggs in larger containers, so this part of Experiment 1 was not duplicated in experiment 2. A summary of the hatch rate of the eggs in Experiment 1 is presented in the Results but not formally analyzed.

In Experiment 1, I noted that many snails chose to the leave the water and climb up the side of the container by a couple of centimeters, and most of these were in the *Chaetogaster*(+) treatment. Therefore, in Experiment 2, I changed the protocol so that the snails were checked every day and I noted when snails were not in the water. In Experiment 2 there were very few instances of snails leaving the water so while the results are reported below, they were not formally analyzed.

In both Experiment 1 and 2, about half of the snails in the *Chaetogaster*(+) treatment lost their symbionts during the experimental period. I decided that the *Chaetogaster*(+) group would be split in two for analysis: snails that kept *Chaetogaster* (= KC) and those that lost *Chaetogaster* (= LC). The rate of egg production analysis considered the number of eggs/week. Snails that died before the 3-week mark were not included, since they did not live long enough to produce meaningful data.

The egg production analysis was conducted in R 4.2.2 (R Core Team 2022), primarily using the package glmmTMB (Brooks et al. 2017) to create Generalized Linear Mixed Models (GLMMs). Due to the type of data being analyzed (count data with overdispersion) a negative

binomial distribution was used. The initial model included these variables: *Chaetogaster* status at the end of the experiment, initial snail size and the interaction between the two. Subsequent models were created with the removal of the interaction term. *Chaetogaster* status at the end of the experiment and snail size were not removed from the model, as exclusion would diminish my ability to answer the original question. An additional random effect was added to each model, which included snail ID nested within week to account for the counts of eggs from the same snails each week. In Experiment 2, an additional zero inflation parameter was added to all models, which improved the fit of the model residuals. Once all models had been created they were compared using AIC. The model with the lowest AIC was selected as the best. If two models fell within 2 AIC of each other, then an ANOVA comparison was completed between the two (assuming both met model assumptions) and the simplest model was chosen if the two were not significantly different. The selected model was then examined and interpreted.

Results

Fitness Experiment 1

Eleven of the 60 snails died in this experiment, but only the 6 that did not survive past the third week of the experiment were excluded from analyses (Table 3-1 and Appendix 3-3 for raw data). Of the 28 snails in the *Chaetogaster*(+) treatment, 11 kept their symbionts through to dissection at the end, while the rest lost their worms. The average number of eggs produced each week by the 8 snails that kept *Chaetogaster* was 4 ± 9.5 (SD), and the 15 that had lost *Chaetogaster* produced an average of 1.8 ± 4.8 (SD) (Table 3-2). Two of the KC snails and 8 of the LC snails produced no eggs. Snails in the *Chaetogaster*(-) treatment produced on average 5 ± 8.5 (SD) eggs per week, and five *Chaetogaster*(-) snails produced no eggs. The total egg production over the entire experiment was also examined graphically (Figures 3-3 to 3-5). Overall, more snails in the *Chaetogaster*(-) treatment produced eggs while the LC group had the greatest number of eggless snails (Figure 3-2 and Figure 3-3). Interestingly, the number of snails producing eggs increased over time in the *Chaetogaster*(-) group, while remaining mostly the same for the other two group (Figure 3-4). Hatch rate of eggs from KC snails was 18.3%, from LC snails 30% and from *Chaetogaster*(-) snails 31.8% (Table 3-3).

During Fitness Experiment 1 I noted that snails of smaller sizes tended to fall into the 'lost *Chaetogaster*' group while larger ones kept their symbionts (Figure 3-6). A comparison of the initial start sizes indicated that, despite the random sorting of snails into treatment groups, the spread in sizes for the *Chaetogaster*(+) treatment was slightly larger, with there being more small-bodied snails than in the *Chaetogaster*(-) treatment (Figure 3-6).

Two Generalized Linear Mixed Models with negative binomial regression considering snail-symbiont status (*Chaetogaster*(-), LC, KC), snail length and their interaction were created

to test if the number of eggs produced/snail was affected by these variables (Table 3-4). Since this experiment included repeated measures over time, a random effect of snail nested within week was included in each model. A comparison of AIC values indicated that models with and without the interaction term explained the data equally well. Since both models met the necessary assumptions, they were compared with an ANOVA test. Since the inclusion of the interaction term did not significantly improve the model ($X^2 = 3.582$, $p = 0.167$), the simplest model (Model 2) was chosen.

Of the two parameters, only *Chaetogaster* status at the end of the experiment had a significant effect on weekly egg production, and that effect itself was slight ($X^2 = 6.539$, $p = 0.0380$, Table 3-5). A follow up Tukey comparison did not find a difference among the three means and so while there might be a slight difference among the groups it is likely not biologically meaningful. There was no apparent effect of snail size on egg production.

Fitness Experiment 2

In this experiment 70 snails were checked a total of 32 times each over 6 weeks for their position in the container (in or out of the water). Five of these snails died before the end of the experiment, all of which were from the *Chaetogaster*(+) groups. One snail from the *Chaetogaster*(-) treatment was accidentally lost in the third week when the lettuce was being replaced (Table 3-6). This left a total of 2152 instances of a snail being checked for its position in the container. Of this total, only eight snails came above the water line to create 11 instances of a snail being found out of the water, 10 of which were from snails in the *Chaetogaster*(+) treatment.

Although five snails died in this experiment, none were excluded from the analysis, since they all survived past the third week of the experiment (Table 3-6); however, the *Chaetogaster*(-) snail that was lost during a water change was excluded. Of the 40 snails in the *Chaetogaster*(+) treatment, 20 kept their symbionts through the entire experiment. The average number of eggs produced each week by snails that kept *Chaetogaster* was 10.7 ± 18.8 (SD), and those that lost *Chaetogaster* was 8.9 ± 18.0 (SD) (Table 3-7). Snails in the *Chaetogaster*(-) treatment produced on average 17.3 ± 22.7 (SD) eggs per week. Generally, snails that produced eggs were consistent in producing them most weeks while the rest produced none throughout the experiment. The number of snails that produced no eggs at all was 11 for the *Chaetogaster*(-) treatment, and 9 and 10 for snails that kept and lost *Chaetogaster* respectively. Generally, the *Chaetogaster*(-) group produced the most eggs over the course of the whole experiment (Figure 3-7 and Figure 3-8). As in Fitness Experiment 1, the snails that lost their symbionts generally produced the fewest eggs. The number of snails producing eggs each week was largely consistent in each group, although there was a small increase over time in the 'lost *Chaetogaster'* group (Figure 3-9).

Again, there was a general trend of the snails chosen for the *Chaetogaster*(+) treatment including a disproportionate number of individuals of small initial size, however it is less pronounced in this experiment than in Fitness Experiment 1 (Figure 3-10 and Figure 3-11). Interestingly, body size of snails that lost *Chaetogaster* in Fitness Experiment 2 ranged quite widely, with this group including the largest snail in this experiment.

Four models were created to analyze these data, examining the effect of *Chaetogaster* status at the end of the experiment, snail size and their interaction (Table 3-8). Two models also included a term for zero inflation, since it was observed that the dataset had a high number of zeros, which could be due to the individual snails more than the different treatments. As in the

first experiment, an additional random effect of snail nested in week was added to all models to account for the repeated measures.

Model selection using AIC comparison determined that model 3 fit the data best (Table 3-8). This model was checked for model assumptions and found not to be in violation. This model indicates that all variables, including the interaction, had a mildly significant effect on the egg production of snails over time (Table 3-9). Since there is a potential for snail size to affect the probability of symbiont loss, the results should be interpreted with caution. A Tukey comparison found the *Chaetogaste*r(-) treatment to be different from the lost *Chaetogaster* group (t ratio = 3.649, p = 0.001) and the kept *Chaetogaster* group (t ratio = 2.858, p = 0.0125). The two *Chaetogaster* groups were not different from each other (t ratio = 0.410, p =0.911).

Discussion

The general goal of my thesis research was to explore the relationship between physid snails and their symbiont *Chaetogaster* within the context of the mutualism-to-parasitism continuum. Chapter 3 in particular investigates the direct effects of *Chaetogaster* colonization on snail hosts, by measuring the fitness of snails with and without symbionts. Based on the experimental results of Stoll et al. (2013), I hypothesized that *Chaetogaster* presence would negatively impact host fitness in relation to host reproduction and host growth.

My examination into the effect on reproduction was relatively successful, with the data showing relatively consistent trends, although at a very low significance. Over both experiments I found a mild significant relationship between the status of a snail (i.e., *Chaetogaster*(-), KC, or LC before the end of the experiment) on egg production. In particular, I found that *Chaetogaster*(-) snails generally produced more eggs than snails in either of the *Chaetogaster*(+) categories (Table 3-2 and Table 3-7). In both of my fitness experiments, the group that lost worms produced the fewest number of eggs, while the *Chaetogaster*(+) snails that kept *Chaetogaster* to the end fell somewhere in the middle. This doesn't match what one would predict if worm colonization were directly negative to reproduction, as in that case, snails that kept *Chaetogaster* to the end of the experiment should produce the fewest eggs, and snails that lost *Chaetogaster* during the experiment should produce an intermediate number of eggs. This suggests that there might be other factors at play here. One of which may be the heterogeneous nature of the 'Lost *Chaetogaster*' group. Since I was unable to pinpoint the exact time when snails lost their symbionts over the course of the experiment, this group of snails within the data should be interpreted with caution. It is possible that some snails may have lost their *Chaetogaster* much more quickly than others leading to incongruous patterns within this group.

Another caveat to consider is the slight size discrepancy between the treatment groups, with the snails in the *Chaetogaster*(-) treatment generally being larger than the other groups (Figure 3-6 and Figure 3-11). In the second experiment, I found the interaction term between snail group and size to be significant, suggesting that size has an impact on if snails kept or lost their *Chaetogaster*. That being said, a direct visual comparison of the *Chaetogaster*(-) group with KC does suggest that *Chaetogaster*(-) snails overall produced the most eggs, so there is no evidence that symbiont colonization benefitted snail fitness.

Stoll et al. (2013) found that snails with *Chaetogaster limnaei* had significantly reduced reproductive output. They examined not only direct egg counts but also egg size and hatch rate, with both metrics lower in snails carrying *Chaetogaster*. I didn't measure egg size in my experiment, but an attempt was made to quantify hatch rate for Fitness Experiment 1. Numbers are only presented but not formally analyzed (Table 3-3) in this thesis due to the rotting associated with anoxia/bacteria growth in the small wells holding the egg clusters.

Unlike my investigation into snail reproduction, I was not able to make a reliable comparison of growth in snails between the treatment groups, due to a combination of the slow growth of snails and the measurement protocol I used. Snails were measured by taking images on my phone of each snail on a piece of grid paper and measuring the snail shell length (proxy for total size) using the measurement tool in ImageJ. Some variation was likely introduced due to snails holding their shell at different angles in different images. Additionally, for the image to be captured clearly on my phone, the camera needed to be 7 cm away, which resulted in the snails being quite small in the images. Enlarging these images for measurement in ImageJ introduced pixilation-associated error into the final measurements. See Methods for a test of measurement repeatability (also Appendix 3-1).

Very likely due to measurement error in both the initial and final snail size, I found that several snails had "shrunk", over the course of the experiment (Appendix 3-2). While it is not wholly impossible for snails to lose body mass or shell thickness due to starvation (Smith 1991; Porcel et al. 1996), declining in shell length was a rather unlikely scenario. I think it is more likely that the measurement error was generally the same or larger than the amount that the snails actually grew, resulting in an unreliable dataset.

In the similar experiment by Stoll et al. (2013), the authors were able to quantify growth in *Physella acuta* with and without *Chaetogaster*. As my snails were found to be closely related (and in fact likely members of that species) to *Physella acuta* samples from Genbank (See Chapter 2 for more details), the Stoll et al. (2013) experiment is useful comparison to my own. However, this experiment was done with much younger snails (e.g., 10 or 14 day old snails) and with a high quality camera attached to a stereomicroscope. This likely gave the authors the ability to properly measure the change in size of snail over time. Stoll et al. (2013) also noted that most snails levelled off in growth at around 28 days at around 5-6 mm in length. Since my experiment started with snails that were older than this and that had already reached a steady size, it is not surprising that it was difficult for me to detect differences in growth over the course of these two experiments.

Another caveat to consider with the results of these experiments is the history of the snail hosts. The lab-bred snails I used were from a colony of the snails that have lived either in captivity or in pipes of the local aquaculture facility. In both cases, these snails potentially had not been exposed to *Chaetogaster* or other symbionts/parasites (besides rotifers) for many generations. It is possible that this lack of exposure negatively affected the population's ability to cope with such relationship. While this may be a general concern, it could also be argued that

since the experiments conducted in Stoll et al. (2013) used snails collected from the wild (and that had been exposed to *Chaetogaster* in recent generations) found similar results to my own, that this is only a mild concern.

The results of both experiments in Chapter 3 support my initial hypothesis with some reservation. Overall, I found *Chaetogaster* colonization to have a slight negative effect on snail fitness, as measured by egg production, although considering the caveats discussed above this result should be interpreted with caution. This suggests that this genotype of *Chaetogaster* may lean towards parasitism on the mutualism-to-parasitism continuum when in symbiosis with this lineage of physid snails.

Tables

Table 3-1. Snails used for and excluded from analysis in Fitness Experiment 1. 'Dead snails' includes all snails that died before the end of the experiment, while 'excluded snails' are the subset of dead snails that didn't survive past the third week of the experiment.

Group	Dead snails	Excluded snails	Total snails in analysis
***Chaetogaster*(-)**	2	1	29
Lost *Chaetogaster* (LC)	4	2	15
Kept *Chaetogaster* (KC)	5	3	8

Table 3-2. Average number of eggs produced/week by snails in Fitness Experiment 1. Data is arranged by category, only snails included in the analysis are included here (see Table 3-1).

Group	No. of included snails	Mean No. eggs/week	SD	No. snails with no eggs
***Chaetogaster*(-)**	29	5	8.5	5
Lost *Chaetogaster* (LC)	15	1.8	4.8	8
Kept *Chaetogaster* (KC)	8	4	9.5	2

Table 3-3. Percent of snail eggs that hatched, Fitness Experiment 1.

Group	No. of snails laying eggs	Total eggs produced by group	No. eggs hatched	Percent hatched
***Chaetogaster*(-)**	24	799	254	31.8%
Lost *Chaetogaster* (LC)	6	130	39	30%
Kept *Chaetogaster* (KC)	6	120	22	18.3%

Table 3-4. Model selection for Fitness Experiment 1. The chosen model is indicated by an asterisk '*'. The response variable is the number of eggs laid/snail over the course of the experiment.

Model	Parameters	Interaction terms	Random effect	AIC
1	*Chaetogaster* status at end of experiment, snail length	Between both variables	Snail ID nested in Week	969.61
2*	*Chaetogaster* status at end of experiment, snail length	NA	Snail ID nested in Week	969.19

Table 3-5. Statistical results for Fitness Experiment 1. Significant results are indicated with an asterisk '*'. The response variable is the number of eggs laid/snail over the course of the experiment.

Parameter	X^2 value	DF	P value
Status at end of experiment	6.5393	2	0.03802*
Snail Length	2.4515	1	0.11742

Table 3-6. Snails used for analysis for Fitness Experiment 2. 'Dead snails' includes all snails that died before the end of the experiment, while 'excluded snails' are the subset of dead snails that didn't survive past the third week of the experiment.

Group	Dead snails	Excluded snails	Total snails in analysis
***Chaetogaster*(-)**	0	1	29
Lost *Chaetogaster*	5	0	20
Kept *Chaetogaster*	0	0	20

Table 3-7. Average number of eggs produced/week by snails in Fitness Experiment 2. The data are arranged by category.

Group	**No. of included snails**	**Avg No. eggs/week**	**SD**	**No. snails with no eggs**
Control	29	17.3	22.7	11
Lost *Chaetogaster*	20	8.9	18.0	10
Kept *Chaetogaster*	20	10.7	18.8	9

Table 3-8. Model selection for Fitness Experiment 2. The chosen model is indicated with an asterisk '*'. The response variable is the number of eggs laid/snail over the course of the experiment.

Model	**Parameters**	**Interaction terms**	**Random effect**	**Zero-inflation parameter**	**AIC**
1	*Chaetogaster* status at end of experiment, snail length	Between both variables	Snail ID nested in Week	No	2134.949
2	*Chaetogaster* status at end of experiment, snail length	NA	Snail ID nested in Week	No	2132.204
3*	*Chaetogaster* status at end of experiment, snail length	Between both variables	Snail ID nested in Week	Yes	1959.282
4	*Chaetogaster* status at end of experiment, snail length	NA	Snail ID nested in Week	Yes	1963.196

Table 3-9. Model summary for Fitness Experiment 2. Significant results are indicated with an asterisk '*'. The response variable is the number of eggs laid/snail over the course of the experiment.

Parameter	X^2 **value**	**DF**	**P value**
Status at end of experiment	9.1782	2	0.010*
Snail Length	7.2887	1	0.007*
Status: snail length	12.749	2	0.002*

Figures

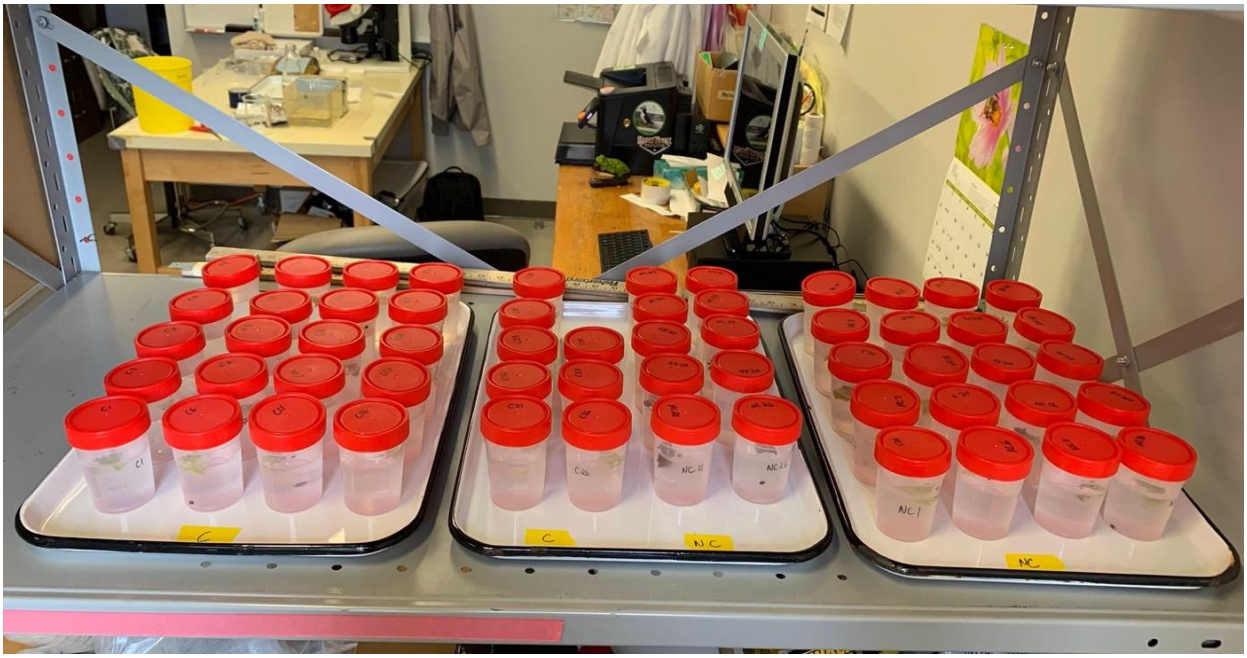

Figure 3-1. Image of Fitness Experiment set up. ‘C’ label refers to *Chaetogaste*r(+) treatment and ‘NC’ to *Chaetogaster*(-) treatment.

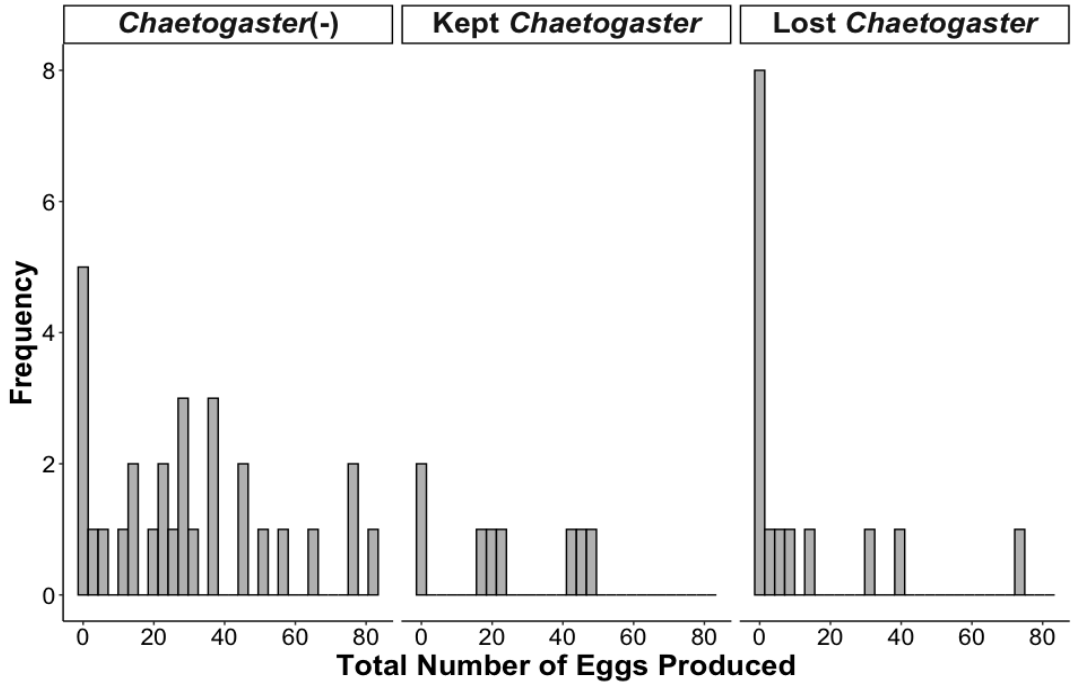

Figure 3-2. Frequency of total egg production/snail in each group in Fitness Experiment 1. Groups include: *Chaetogaster*(-) (n=29), kept *Chaetogaster* (n =8), lost *Chaetogaster* (n=15).

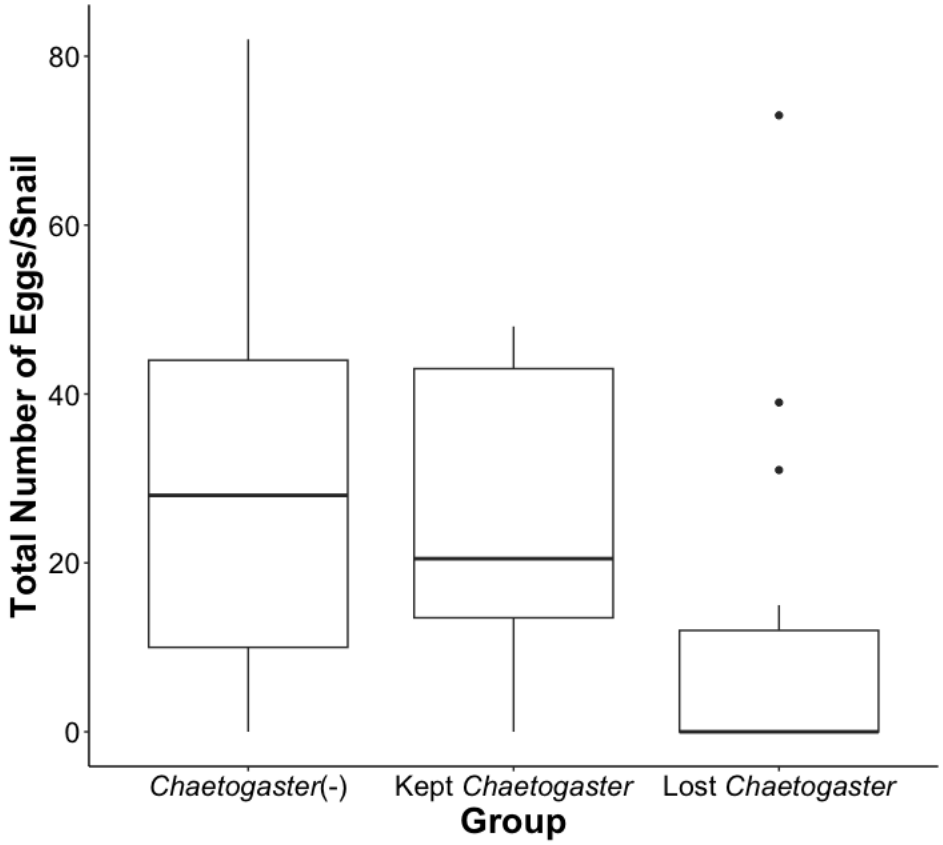

Figure 3-3. Total number eggs produced/snail in Fitness Experiment 1 averaged per group. Groups include: *Chaetogaster*(-) (n=29), kept *Chaetogaster* (n =8), lost *Chaetogaster* (n=15). Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values.

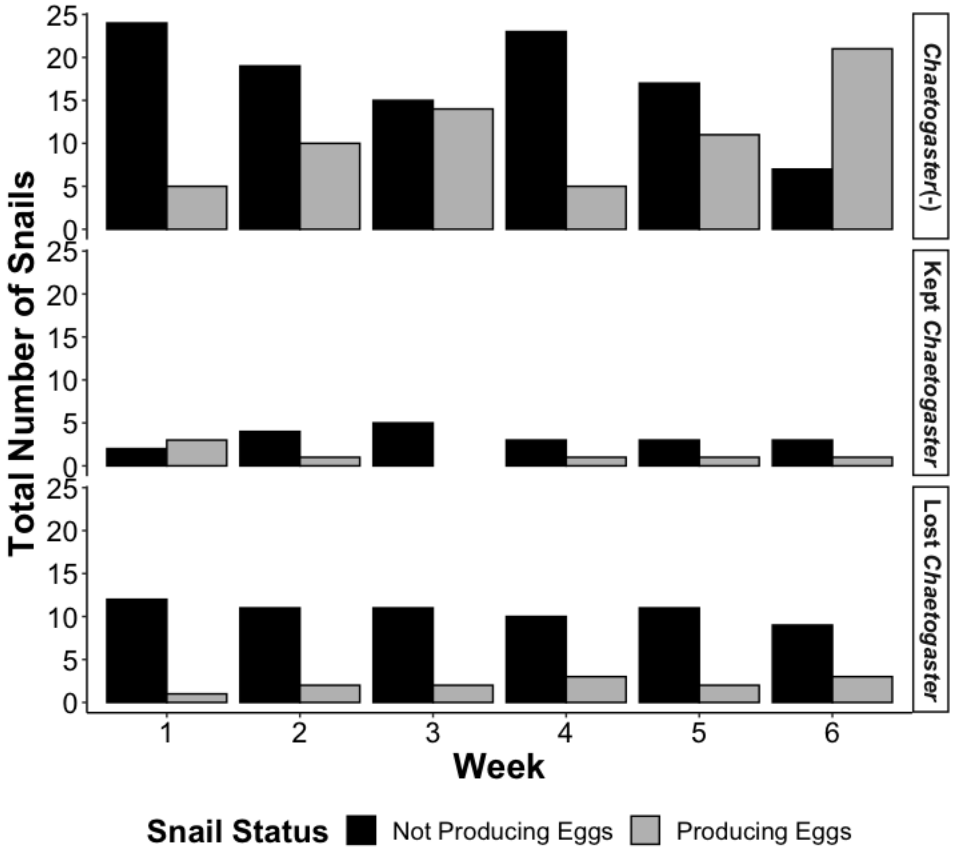

Figure 3-4. Egg production over the 6-week period of Fitness Experiment 1, by group. Groups include: *Chaetogaster*(-) (n=29), kept *Chaetogaster* (n =8), lost *Chaetogaster* (n=15).

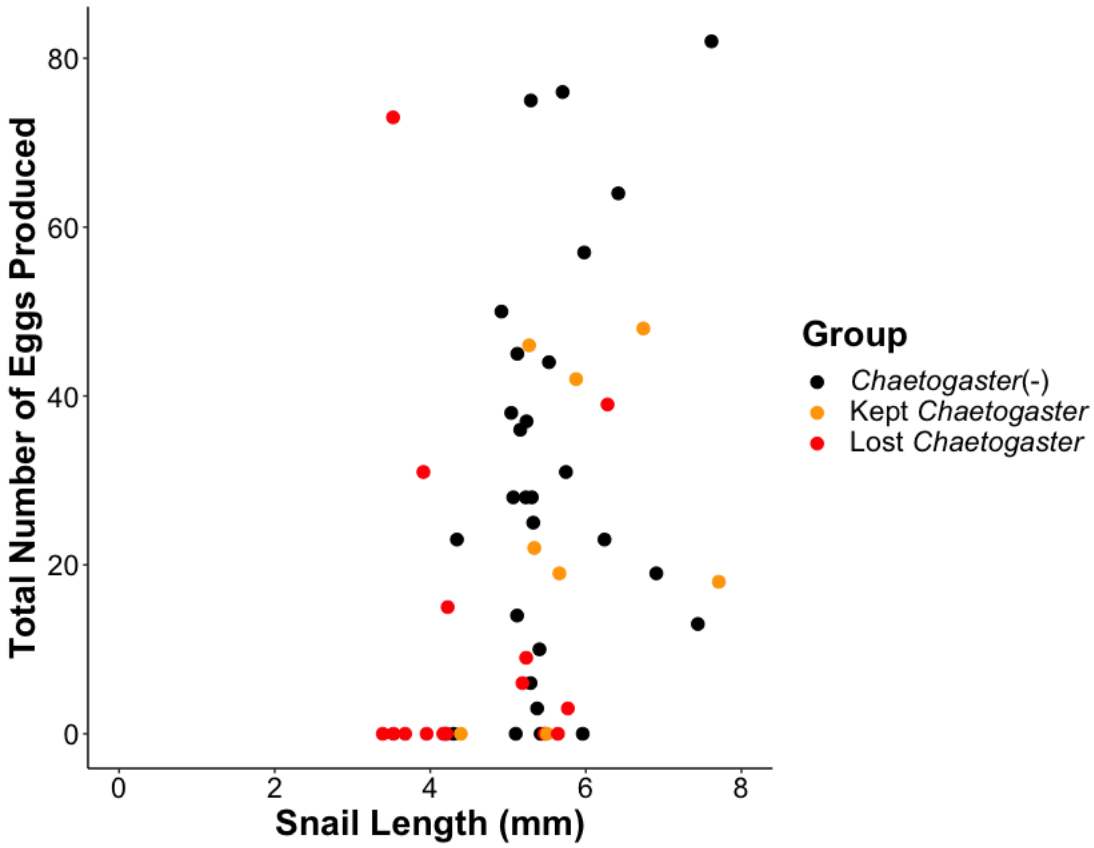

Figure 3-5. The total number of eggs/snail in Fitness Experiment 1 arranged by initial length. Snail length is based on snail size at the start of the Fitness Experiment. Groups include *Chaetogaster*(-) (n=29), kept *Chaetogaster* (n =8), lost *Chaetogaster* (n=15).

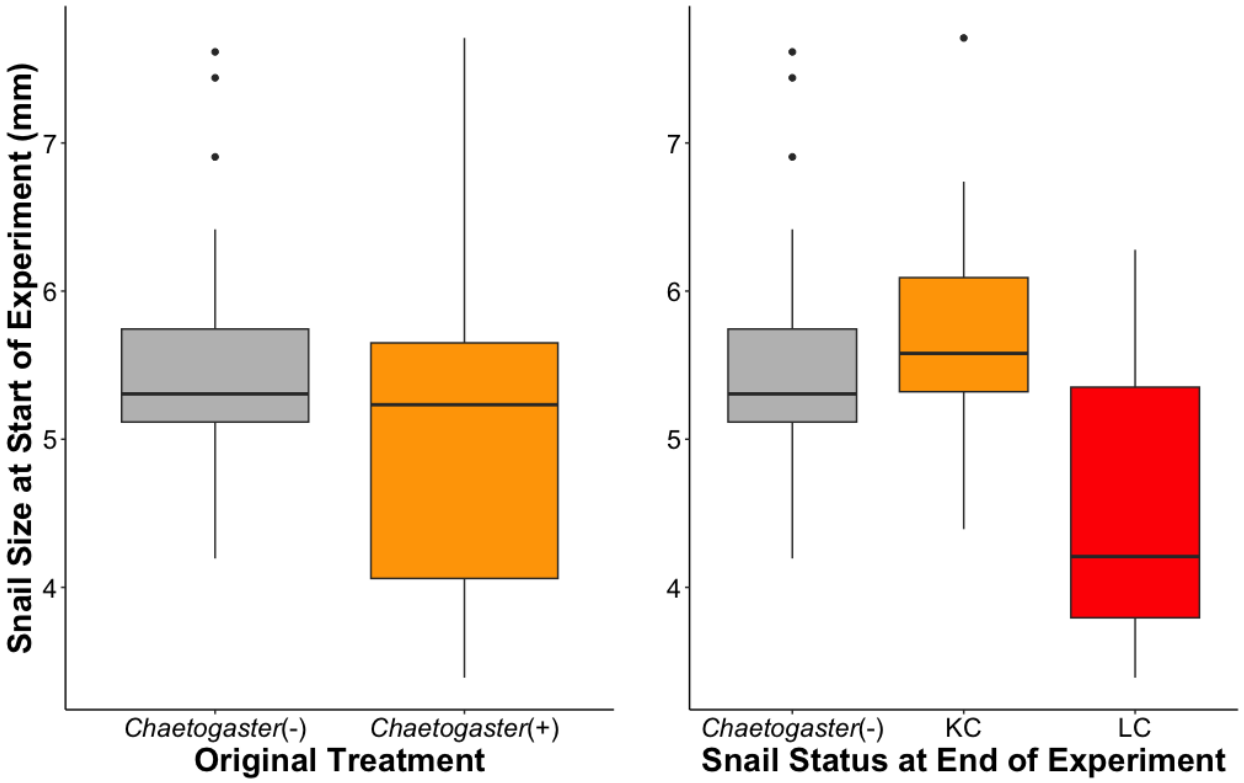

Figure 3-6. Size comparison between treatment considering initial treatment and end groups. Snail length is based on snail size at the start of Fitness Experiment 1. Groups at the end of Fitness Experiment 1 include *Chaetogaster*(-) (n=29), kept *Chaetogaster* (KC: n =8), lost *Chaetogaster* (LC: n=15). Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values.

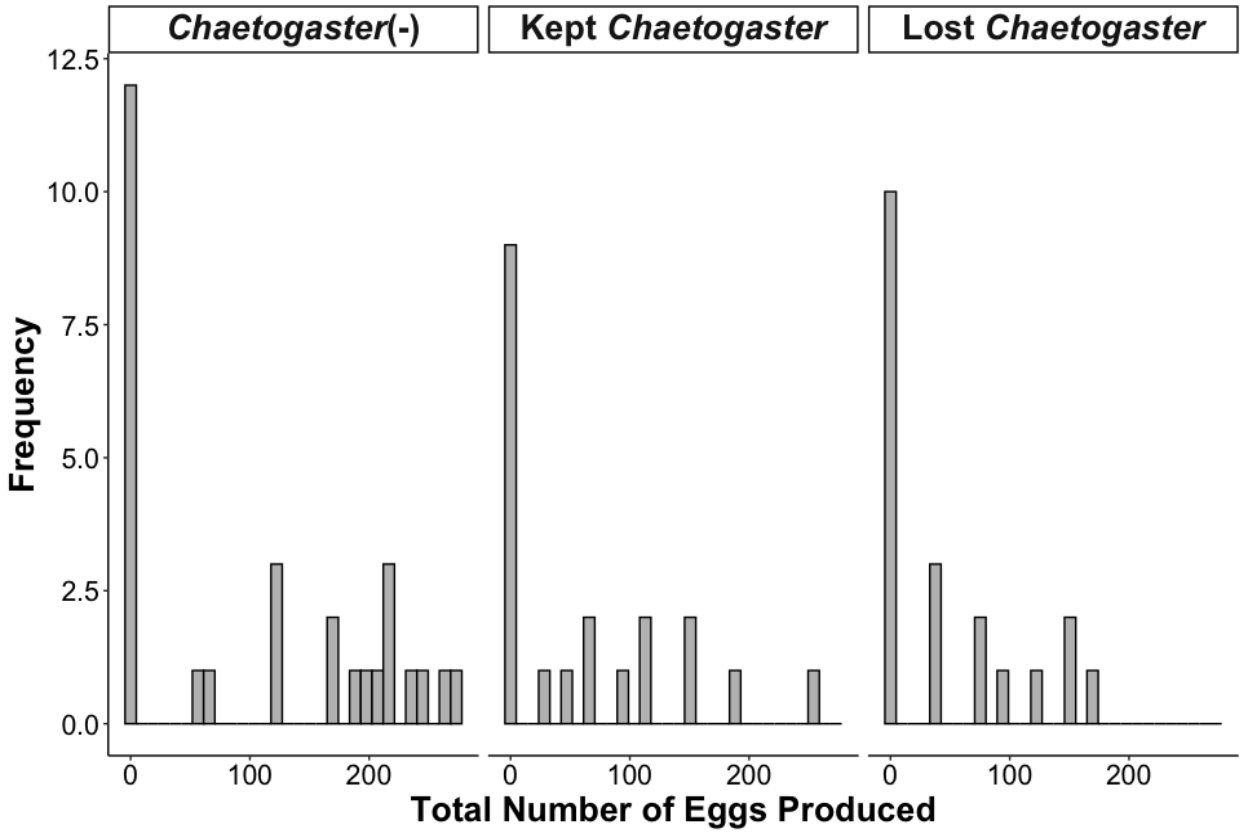

Figure 3-7. Frequency of total egg production/snail in each group in Fitness Experiment 2. Groups include: *Chaetogaster*(-) (n=29), lost *Chaetogaster* (n=20) and kept *Chaetogaster* (n=20).

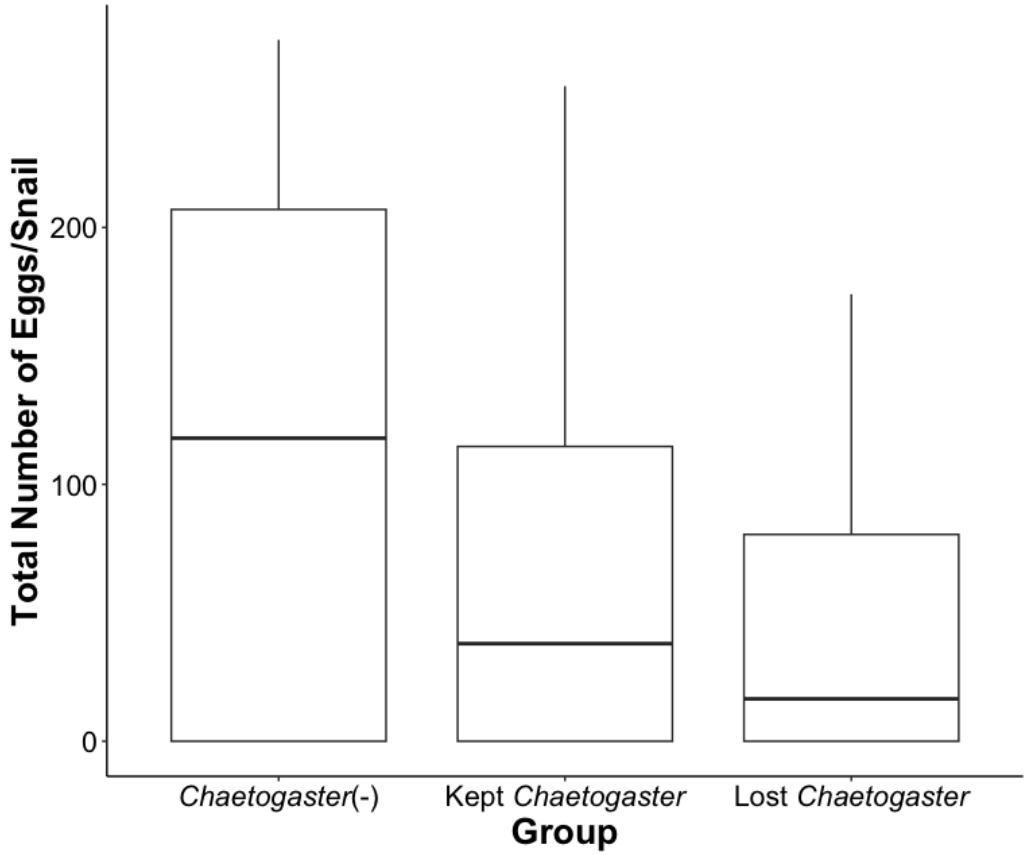

Figure 3-8. Total number of eggs produced/snail in Fitness Experiment 2 averaged per group. Groups include: *Chaetogaster*(-) (n=29), lost *Chaetogaster* (n=20) and kept *Chaetogaster* (n=20). Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values.

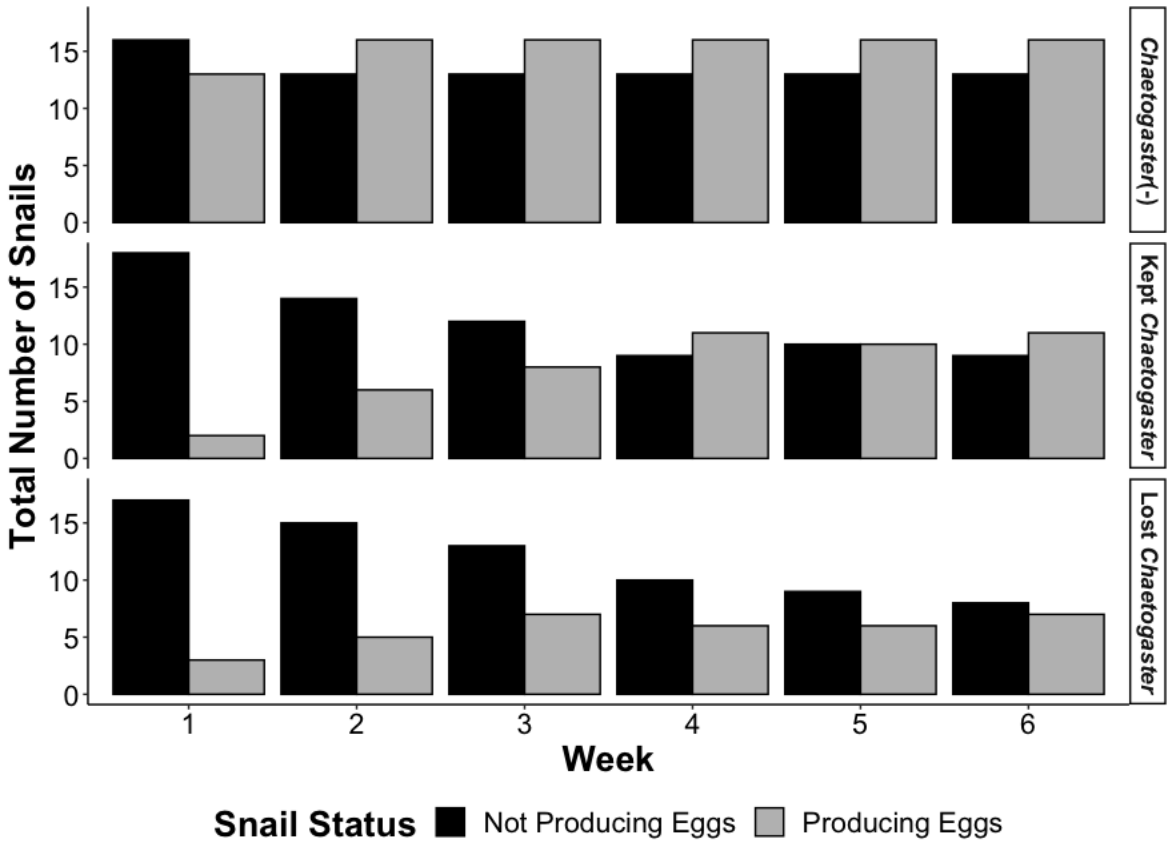

Figure 3-9. Snail status over time by group in Fitness Experiment 2.Groups include: *Chaetogaster*(-)(n=29), lost *Chaetogaster* (n=20) and kept *Chaetogaster* (n=20).

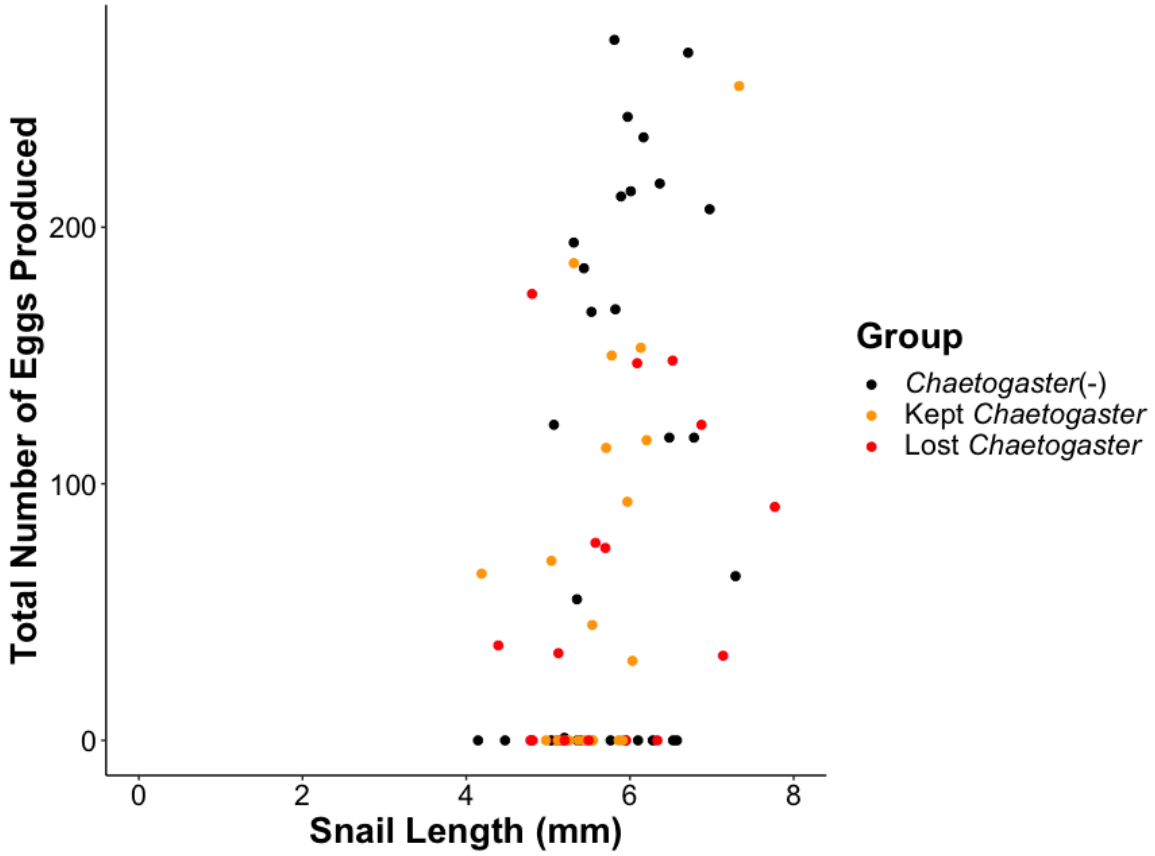

Figure 3-10. Total number of eggs/snail in Fitness Experiment 2 arranged by initial length. Groups include: *Chaetogaster*(-) (n=29), lost *Chaetogaster* (n=20) and kept *Chaetogaster* (n=20).

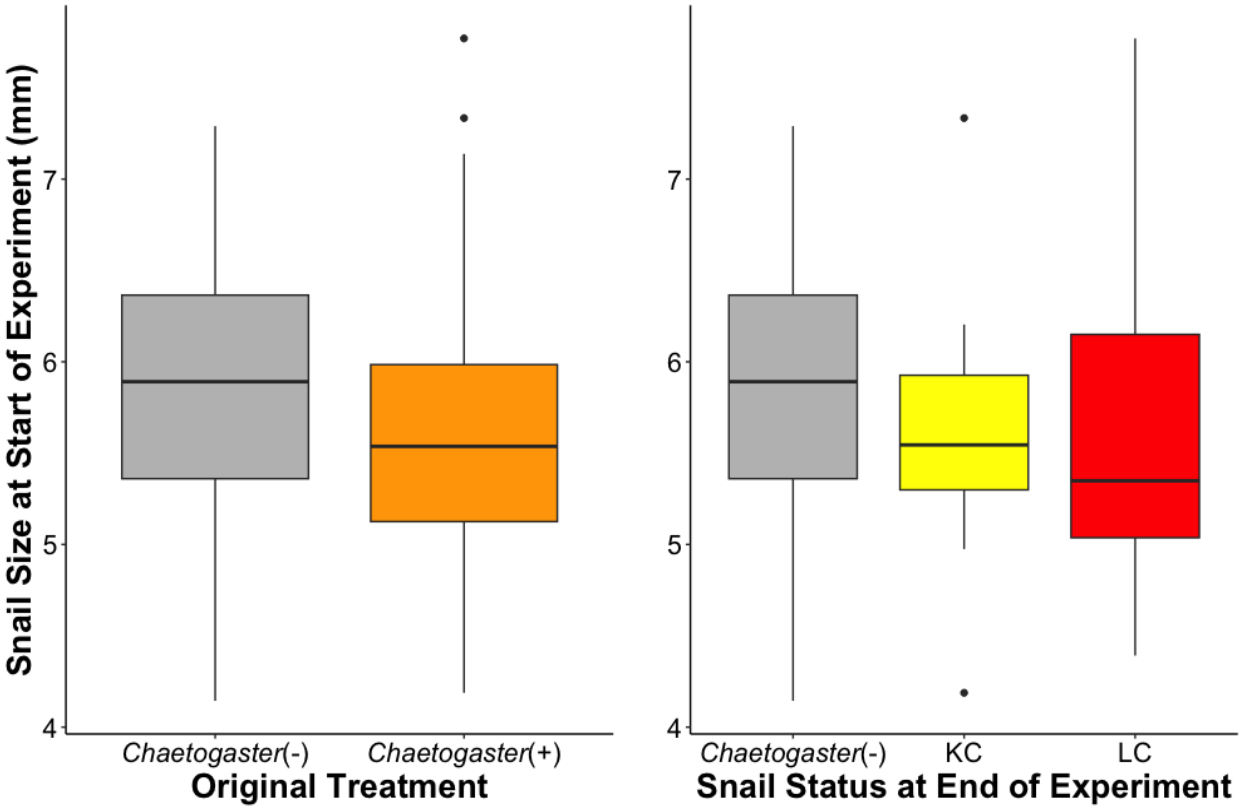

Figure 3-11. Size comparison between treatments and end groups. Snail length is based on size at the start of Fitness Experiment 2. Groups at the end of Fitness Experiment 2 include *Chaetogaster*(-) (n = 29), kept *Chaetogaster* (KC: n = 20), lost *Chaetogaster* (LC: n = 20). Upper and lower box edges represent the first and third quartiles, while the middle line indicates the median. Points beyond the whiskers may be considered extreme values.

Chapter 4. Behaviour Experiments

Introduction

Host Manipulation

One of the most fascinating aspects of symbiosis is the idea of host manipulation by parasites. Commonly cited examples of this phenomenon include fungi creating 'zombie ants' (Hughes et al. 2011; de Bekker et al. 2015; Andriolli et al. 2019) and the protist *Toxoplasma gondii* (Nicolle & Manceaux) causing a 'fatal feline attraction' in rodents (Berdoy et al. 2000; Vyas et al. 2007; Worth et al. 2013). In the first example, worker ants infected by *Ophiocordyceps* (Ophiocordycipitaceae) fungi will leave the nest and bite into a leaf before dying (Hughes et al. 2011). This death-grip behaviour ensures that the ant stays in an elevated position after death, allowing the infecting fungus to grow a reproductive stalk and release spores from that location. Studies indicates that in some cases the selection of death-grip location will be so specific that a 'zombie ant graveyard' is created (Hughes et al. 2011; Andriolli et al. 2019). The second example is an apicomplexan parasite that has felines as the definitive host (where sexual reproduction occurs) while intermediate hosts can be a wide range of mammals including rodents, sheep, sea otters, cattle, pigs, horses, and humans (Dubey 2008; McConkey et al. 2013). Some studies have found rodents infected with *T. gondii*, unlike uninfected conspecifics, do not avoid cat odour cues, suggesting that the parasite may be manipulating the intermediate host to increase chances of trophic transmission to a definitive host (Berdoy et al. 2000; Vyas et al. 2007; McConkey et al. 2013). However, others question this link, citing lack of convincing evidence (Worth et al. 2013). Beyond these, are many other examples of parasites manipulating host behaviour in spectacular or more subtle ways (Poulin 2010). Generally, host manipulation is

any change in host behaviour caused by the symbiont that increases symbiont fitness (usually in terms of transmission for completion of the life cycle) (Poulin 2010). Despite having such a simple and all-encompassing definition, identifying instances of host manipulation as adaptive as opposed to coincidental can be tricky, and there is much discussion on what criteria must be met for a behaviour to be considered adaptive manipulation (Poulin 1995; Thomas et al. 2005; Poulin 2010; Herbison et al. 2018).

In the realm of snails and their symbionts, there are examples of parasites altering host physiology and chemical cues (Bates et al. 2011; Friesen and Detwiler 2021; Friesen et al. 2022a; Friesen et al. 2022b). *Zeacumantus subcarinatus* (Sowerby) intertidal snails infected by *Maritrema* spp. (Microphallidae) or *Philophthalmus* spp. (Philophthalmidae) trematodes had different thermal tolerances depending on the infecting species (Bates et al. 2011), and host thermal tolerance aligned with the thermal range of infecting parasite. The authors suggested that the decreased thermal tolerance of snails infected by *Philophthalmus* spp. was likely a pathological side effect. Conversely, they considered the increase in heat tolerance with *Maritrema* spp. infection a potential adaptive manipulation since parasite productivity increased in warmer temperatures.

Chemical cues (e.g., alarm cues, sex pheromones, etc.) are an important mode of communication in aquatic communities, providing information to individuals that may induce changes in their behaviour or physiology (Turner et al. 2000; Jacobsen and Stabell 2004; Gerald and Spezzano 2005; Friesen and Detwiler 2021; Friesen et al. 2022a; Friesen et al. 2022b). Both symbionts and hosts participate in the production and reception of these chemical cues, and this has impacts on the transmission of symbionts; however, despite the ubiquity of symbiotic relationships this is a relatively unexplored field (Friesen and Detwiler 2021; Friesen et al.

2022a; Friesen et al. 2022b). It is assumed that chemical cues are a mediating factor in host manipulation by parasites; however, direct evidence in this area is lacking (Friesen and Detwiler 2021). Recent research on the topic of chemicals called oxylipins aims to untangle these complex interactions (Friesen et al. 2022a; Friesen et al. 2022b). Findings to date suggest that snails with trematode infections produce different amounts and types of oxylipins, which may be an example of host manipulation if it increases the probability of transmission success by making a parasitized snail more readily detected or more attractive to the next host species in the trematode's life cycle (Friesen et al. 2022a; Friesen et al. 2022b).

Although snail-associated *Chaetogaster* cannot be confidently labelled as parasitic, mutualistic, or commensal due to lack of consistent evidence (possibly because published studies have examined different snail species and *Chaetogaster* lineages), it is reasonable to hypothesize that these worms might influence host behaviour and/or chemical cue production and reception. This chapter discusses past forays into *Chaetogaster*-snail behavioural studies and presents two new experiments that further investigate the potential for this phenomenon.

Changes in Host Activity

A behaviour experiment conducted by Stoll et al. (2013) investigated the effect of *Chaetogaster* colonization on *Physella acuta* (Draparnaud) (Physidae) snails. *S*nails were given 0, 10 or 20 *Chaetogaster*. Then two observation periods of 7.5 h each were conducted over the course of two days, where each snail was observed at 30 min intervals. The authors found that snails with 10 or 20 worms spent less time crawling/feeding and more time resting than their uncolonized conspecifics, although there was no significant difference between snails with 10 or 20 symbionts. Two hypotheses were proposed to explain these results: (1) that *Chaetogaster*

bristles may irritate snail epithelium, which requires rest to heal; (2) that increased mucus production due to *Chaetogaster* colonization may limit oxygen uptake in colonized snails and so reduce activity levels. Although unlikely to be 'host manipulation' as defined above, this result is an interesting example of how *Chaetogaster* worms may affect the behaviour of their hosts.

I conducted observational assays investigating the effect of *Chaetogaster* presence on snail movement behaviour. I took snails collected from the field with varying numbers of worms and analyzed their movements over a period of an hour. I expected to see similar results as with the behaviour experiment in Stoll et al. (2013), with increased *Chaetogaster* abundance increasing host resting behaviour and decreasing crawling/feeding.

Chaetogaster Dispersal

Several studies have investigated the dispersal of *Chaetogaster* from snail to snail. These studies generally focus on the ability of *Chaetogaster* individuals to find snail hosts (e.g., via mucus trails) or in terms of what may prompt a *Chaetogaster* to leave their host (Gruffydd 1965b; Shaw 1992; Hopkins et al. 2015). Generally, laboratory experiments investigating *Chaetogaster* dispersal suggest that worms will only leave a live host for a different one if there is physical contact between the two, although there is some evidence of dispersal without host-to-host contact (Gruffydd 1965b; Hopkins et al. 2015). Dispersal dynamics in the field are still not well understood, but here again it is suggested that snail-to-snail contact increases *Chaetogaster* dispersal (Gruffydd 1965b).

None of the above experiments investigated the dispersal of *Chaetogaster* from the snail's perspective (e.g., are snails that lack *Chaetogaster* attracted to or repelled from snails that have *Chaetogaster*?). Since it is suggested that freshwater snails can alter their behaviour

depending on symbiont-affected chemical cues detectable in the environment (Turner et al. 2000; Gerald and Spezzano 2005; Henry et al. 2006; Friesen et al. 2018; Friesen and Detwiler 2021; Friesen et al. 2022a; Friesen et al. 2022b), it's possible that snails with *Chaetogaster* differ in their attractiveness to other snails and/or in their tendency to approach other snails. For my second behavioural experiment I tested whether *Chaetogaster* colonization changes the behaviour of nearby conspecific snails, hypothesizing that *Chaetogaster* presence would change the preference of snails in associating with each other. I placed a snail with or without *Chaetogaster* in an arena with options to move towards or away from conspecific snails with and without *Chaetogaster*. If tested snails spend more time near conspecifics with *Chaetogaster* then the worms are likely attractive to host snails, though it would not be possible to differentiate between snails choosing to aggregate near snails with *Chaetogaster* for their own benefit (i.e., *Chaetogaster* colonization is actually beneficial to the host) or if the *Chaetogaster* were manipulating hosts to be more attractive to other snails (Table 4-1). If the opposite preference is seen, then this may suggest that *Chaetogaster* are harmful to host snails causing snails to actively avoid snails with symbionts-associated chemical cues.

Methods

Movement Assays

I conducted two Movement Assays in the Summer of 2021, which are described here. These experiments were based on two trial assays conducted earlier in the summer (data and methods not included here). Partial data from all four assays can be found in Chapter 2 when I discuss the number of worms per snail in 2021. Both Assays 1 and 2 followed the same protocol except that Movement Assay 1 was completed using snails collected from the Lafarge properties on August 31st, 2021, while Movement Assay 2 used snails collected from Heritage Lake, Morinville, on September 27th, 2021.

Preparation for Assays

Physid snails were collected from the field the day before each assay was to be completed. Snails were collected with a net and individually placed in small containers with some pond water. About thirty snails for each assay were collected and brought back to the lab. The snails in their containers were placed in an incubator overnight (18ºC) with their lids cracked to allow gas exchange.

Ethovision Trials

The Movement Assay began at around 8 am on the morning after the snails were collected. To start, six snails were placed individually into 3.5 cm diameter wells in a six-well-plate with some water from the collection site and allowed to acclimate to the new environment for an hour at room temperature. After acclimation the well-plate was carefully placed into a DanioVision chamber (https://www.noldus.com/daniovision) with the chamber light on, and the

assay commenced. While the DanioVision video recorded the movements of the snails for 1 hr, the next set of six snails was placed into another well-plate to acclimate and the cycle continued until all 30 snails had been recorded in the DanioVision chamber. After the video recording period, the length of each snail's shell was measured with electronic calipers (Fisher Scientific digital calipers, Model: 14-648-17) and the snail was dissected. While dissecting each snail I counted the number of external and internal *Chaetogaster*, the number of metacercarial cysts and noted the presence/absence of trematode infection (rediae/sporocysts). Once all videos had been recorded the videos were analyzed in EthoVisionXT behavioural analysis software (https://www.noldus.com/ethovision-xt) to assess total distance traveled by each snail.

Statistics

Total distance travelled was calculated in centimeters and analyzed using linear models (stats package: R Core Team 2022) or Generalized Linear Models (MASS package: Venables & Ripley 2022) in R version 4.2.2 (R Core Team 2022). Models included these variables: number of external *Chaetogaster*, number of internal *Chaetogaster*, snail shell length, number of encysted metacercariae, presence/absence of other trematode life stages. Symbionts other than external *Chaetogaster* were only included in potential models if they were present in more than 10% of the population. Once predictors were chosen, all possible models were created before model selection via AICc. To simplify model creation, selection, and interpretation, only one interaction was included per model and interactions were only considered between two predictors at a time (i.e., higher level interactions of three or more variables were not included). The chosen model was then examined for model assumptions and output. Data analysis for Movement Assay 2 required some adjustments due to multiple extra predictors that could be

included in the analysis and extreme data points; those are described in the results section for that experiment.

Preference Experiment

Arena Set Up

For this experiment a narrow rectangular container 39.5 cm long, 3.5 cm wide and 3.5 cm high with no top, made from black acrylic, was used as an arena (Figure 4-1). The arena had been created for previous experiments completed by another student and required some adjustments for my experiment. First the arena was partitioned in half by a rectangular piece of mesh (1 mm openings), which allowed water and dissolved chemical cues, but not worms or snails, to move between both sides. I used only one half of the container for my experimental arena, and that half was divided into smaller sections. Small pieces of mesh (1 mm openings) were added to each end of the arena to create small spaces on each end for separating bait snails from the tested snails. The middle, main part of the arena, was visually delineated into three sections with pieces of string attached to the rim of the container. This set up allowed the tested snail to move about the main arena freely, while remaining separated from the bait snails along the sides. The main arena was 18 cm long, while the bait snail enclosures were 1-2 cm in length. The pieces of string acted as indicators for me, for how close each tested snail was to either of the bait snail enclosures. The set-up arena and all plastic parts were soaked overnight in water taken from the lab snail colonies to remove any chemical irritants in or on the equipment.

Experimental Trials

I removed 78 *Chaetogaster* from six physid snails I had collected from Heritage Lake in Morinville in 2021 (Likely in early fall, however the exact date is not available) and transferred them to a container of lab-bred *Physella acuta* on February 8th, 2022. Another container of lab-bred snails was not given *Chaetogaster*. These containers were left undisturbed (other than water and food changes) until March 12th, 2021, which was the first day of this experiment.

At the start of each trial, the experimental container (Figure 4-1) was filled with ~150 mL of pre-conditioned ASW (i.e., water from the tanks that had housed the snails prior to the experiment) to ~1.25 cm in depth and three snails were put in each of the separated 'bait snail' areas. One group of the bait snails was from the *Chaetogaster*(+) chamber and the other was from the *Chaetogaster*(-) container; (+) and (-) sides were randomly assigned via a coin toss. Once the arena was set up, a snail with or without *Chaetogaster* was placed in Area 2 of the main arena, this was the 'tested snail'. Small pieces of plastic were briefly inserted along the delineated lines to prevent the snail from moving to a different area during acclimation. This tested snail was given 5 minutes to acclimate, and then the trial started. At the start of the trial, the plastic barriers used during acclimation were removed and the snail was allowed to move freely about all three areas of the main arena. I checked the tested snail every minute for 20 minutes and recorded what area in the arena it was occupying: near *Chaetogaster*(+) bait snail, near *Chaetogaster*(-) bait snail, or no choice (Area 2: in the middle). Snails that did not leave Area 2 within 5 minutes after acclimation were removed from the experiment and the trial stopped at this point. After 20 minutes the trial ended and I removed the tested snail, measured its shell using calipers (Fisher Scientific Model: 14-648-17) and dissected it. During dissection the number of external *Chaetogaster* were counted.

The water from in the arena was removed and replaced at the end of each trial, and the arena was wiped down with paper towels to remove mucus trails. The entire experiment was conducted over the course of 5 days, with 5 trials conducted each day. The same six bait snails (3 with and 3 without *Chaetogaster*) were used each day (i.e., used in each trial on that day) and replaced with new ones the next day. This measure was taken to reduce the influence of individual bait snails, while also minimizing the number of extra snails needed to conduct the experiment. Bait snails were dissected at the end of the day to confirm their *Chaetogaster* status.

Arena Testing

In order to test if the arena used was an appropriate size for the Preference Experiment, I set up an arena test using the same snail set up as described above (i.e., three bait snails in each side arena and one 'tested' snail in the main arena) and some food colouring. There was some concern that whatever chemical cue that the bait snails/*Chaetogaster* were producing (assuming that they were in fact doing so) would spread too quickly through the arena, confusing the tested snail on the direction of origin since the arena did not have any water flow. To address this concern, I did mock trials in which I put food colouring into one of the bait snail enclosures and timed the movement of the colour across the arena. I found food colouring was heavier than water and generally sunk to the lower portion of the arena and even with snail movement in the arena it barely made it to the middle of the main arena in 25 minutes (i.e., the time of the acclimation period and trial together). This suggests that any chemical cue as heavy or heavier than food colouring was likely restricted to the side of the arena in which it was produced over most or all of the testing period. A lighter chemical cue may have travelled further across the arena in the allotted time period. However, in this arena of standing water I think it is likely that

a chemical cue gradient was created even with lighter molecules due to the short length of the trial.

Statistics

Data from this experiment was separated into several different metrics that were analyzed separately, so that different aspects of snail behaviour could be examined. The different datasets were as follows (note: 'choice' is used for the sake of brevity rather than to indicate that a conscious choice was made by the snails):

i. First Choice: Considers which group of bait snails the tested snail investigated first. This was counted by examining which area of the arena the tested snail moved into first after leaving Area 2 at the start of the experiment. This metric was broken into two aspects, the amount of time a snail took to make their first choice (i.e., to leave Area 2), and bait snail group that was first chosen.

ii. First Arrestment: An arrestment was defined as any period that the tested snail spent two or more consecutive time checks (i.e., at least two minutes) in the same area of the arena (excluding the middle 'no choice' section). This metric was broken into two aspects, the amount of time before the tested snail's first arrestment and bait snails chosen for the first arrestment.

iii. Longest Arrestment: the tested snail's longest period of arrestment. This metric was broken into two sections, the amount of time before the tested snail's longest arrestment and the bait snails chosen for the longest arrestment.

iv. Time Spent Near Bait Snails: This considers the total number of time checks that each snail spent in the two 'choice' sections, Area 1 and Area 3.

v. Movement: The number of times a tested snail moved from section to section. This was used as a proxy for total movement during the experiment. For example: all tested snails started in the middle section, if a snail then moved to the Area 1 by the first time-check, then that was one switch. If by the second time check, it had moved all the way to Area 3 then that would be an additional 2 switches (since they had passed through the middle section).

All data were first examined graphically for general trends and distribution. Afterwards, data were analyzed in R 4.2.2 (R Core Team 2022) using Generalized Linear Mixed Models (glmmTMB package: Brooks et al. 2017), with a logistic regression or a negative binomial regression. Each model was chosen by first creating all possible models and then eliminating variables (primarily interaction terms) through comparison of model AICc. To simplify model creation, selection, and interpretation, interactions were only considered between two predictors at a time (i.e., higher level interactions of three or more variables were not included). If models were less than 2 AICc then an ANOVA comparison was done to select the best one. All models included the variables of treatment and length of the tested snail, as well as an additional random effect of trial date to account for the same group of snails being used as bait snails on each day. Additional parameters were added as necessary and described in the Results section. Model results were checked using the plot() function in DHARMa (negative binomial regression) or by comparing logit values to the continuous predictor (logistic regression).

Results

Movement Assays

Assay 1 (Lafarge)

Snails collected for this experiment on Aug. 31st, 2021 (see Appendix 4-1 for raw data), from Lafarge properties had 0 to 10 external *Chaetogaster*. Snails ranged from 4.3 mm to 10.2 mm in length with an average shell length of 6.7 mm. On average, snails moved 58 ± 9.5 cm in the allotted hour (Table 4-2). Those with fewer *Chaetogaster* (e.g., less than 4) generally travelled farther than those with more (e.g., 10 worms). Of all snails tested, the minimum distance travelled was ~18 cm while the maximum was ~281 cm (Figure 4-2).

Six inverse Gaussian models were created to determine if number of external *Chaetogaster* and snail length affected distance the snail moved. Trematode infection (rediae/sporocysts) was also considered as a potential variable as more than 10% of the snails in this assay were infected. In contrast, internal *Chaetogaster* were extremely rare and were not considered. Snail length and external worms were included in all potential models, but trematode infection was not (Table 4-3). The best model for the data was Model 6, which had the lowest AICc and met necessary assumptions (Table 4-3). Model 6 only included external *Chaetogaster* numbers, and snail length, no interaction or trematode data was included. Overall, the model indicated that neither the number of external *Chaetogaster* (X^2 = 1.340, p = 0.247) nor snail size (X^2 = 2,813, p = 0.094) had an effect on the distance travelled by each snail (Table 4-4).

Assay 2 (Morinville)

Snails were collected for this experiment on Sept 27th, 2021, from Heritage Lake in Morinville (see Appendix 4-2 for original data). All snails in this experiment were found to have

external *Chaetogaster*, the number of which ranged from 5 to 21 per snail (Table 4-5 and Figure 4-3). This dataset had two extreme values of distance travelled (1067.8 cm and 5269.9 cm), both values would have required unrealistic speeds for snail subjects. It was assumed that these values were due to errors in the tracking software rather than true measurements and were therefore removed. As a precaution, the chosen model was run with both datasets (complete and subset) and the significance of the results did not change. All calculations and results from here forward will only consider the dataset excluding the two 'long-distance' snails.

Of the 28 included snails, the minimum distance travelled was 67.7 cm while the greatest was 499.9 cm. The average distance travelled was 314.7 ± 18.8 cm (SE) in the allotted time frame (Table 4-5). Snails with fewer worms (e.g., 5-9 worms/snail) generally moved more than snails with more worms (e.g., 20-24 worms/snail). Snails ranged in size from 8.15 mm to 15.45 mm with an average shell length of 13.64 mm. This dataset only included 3 snails infected with trematodes; however, all snails contained at least one metacercarial cyst (with a maximum of 51 cysts/snail). All but two snails also carried internal *Chaetogaster*, with density ranging from 1 to 11 per snail.

The predictors investigated in this set of models was external *Chaetogaster*, snail length and internal *Chaetogaster*. Trematode infection (rediae/sporocysts) was not included in model selection as more than 10% of the population did not carry an infection. With internal *Chaetogaster* and metacercarial cysts both affecting most if not all of the sampled snails, both variables could have been included in model selection; however, including both (for a total of 4 main variables and all resulting interactions) would have resulted in an unwieldy model creation and selection process, so I chose to only include one of the two variables. I chose internal *Chaetogaster* rather than metacercariae because *Chaetogaster* are the main focus of this thesis.

Snail length and external *Chaetogaster* were included in all potential models, but internal *Chaetogaster* were not.

Six linear models were created for this analysis after the variables of interest in this dataset were all determined to be approximately normal. The top two models, both within 2 AICc of each other were Models 1 and 4 which both included internal *Chaetogaster* as a predictor (Table 4-6Table 4-3). An examination of the models indicated both met the necessary assumptions and therefore an ANOVA comparison was conducted to choose the best model. The ANOVA indicated that model fit did not significantly increase with the addition of an interaction term, so the simplest model (Model 4) was chosen. Overall, the model indicated none of the three main predictors had an effect of the distance travelled by each snail (Table 4-7).

Snail Preference Experiment

First Choice

A total of 60 snails were tested in this preference experiment conducted over 5 days (see Appendix 4-3 original data). In total, 15 tested *Chaetogaste*r(-) snails chose bait snails with *Chaetogaster* first while the remaining 15 chose bait snails without *Chaetogaster* (Table 4-8 and Figure 4-4). In the *Chaetogaster*(+) treatment, 18 snails chose bait snails with *Chaetogaster* first, while 12 chose bait snails without *Chaetogaster* first. In both treatments, snails whose first choice was bait snails without *Chaetogaster* chose approximately twice as fast as those that chose bait snails with *Chaetogaster* (Table 4-8 and Figure 4-5).

Two models using logistic regression (glmmTMB package: Brooks et al. 2017)) were created to assess whether treatment, snail length and their interaction affected the first choice made by the tested snails (Table 4-9). A random effect of trial date was also included to account

for bait snails being used repeatedly on the same day. Models were within 2 AICc, so a model comparison using ANOVA was completed and model assumptions were checked for both. The ANOVA determined that the addition of the interaction term did not significantly improve the model ($X^2 = 0.525$, p = 0.469). Additionally, Model 2 fit the assumptions best (a linear relationship between logit values and continuous predictor values (snail length)). Therefore, Model 2, including only treatment and snail length, was chosen as the best model (Table 4-9). Overall, the treatment of the tested snail (presence or absence of *Chaetogaster*) did not have a significant effect on the first choice made by that snail ($X^2 = 2.039$, p = 0.153), nor did the length of the tested snail ($X^2= 3.060$, p =0.080, Table 4-10).

Two models were created to determine if members of one treatment group made their choice significantly faster or slower than the other (Table 4-11). The models used a negative binomial regression but were otherwise the same as those above. Comparison by AICc found both models to explain the data equally well, and so an ANOVA comparison was used to determine if including the interaction term significantly improved the model. Since the ANOVA was not significant ($X^2 = 0.495$, p = 0.482) and both models met the necessary assumptions, the simpler model was chosen (Model 2).

Overall, neither treatment of the tested snail (presence or absence of *Chaetogaster*) ($X^2 = 2.068$, p = 0.150) nor snail size ($X^2 = 1.110$, p = 0.292), had an effect on the number of time checks before a snail made its first choice (Table 4-12).

First Arrestment

Here, first arrestment is defined as the first choice (i.e., section of arena) that a tested snail stayed in for the duration of 2+ consecutive time checks. In the *Chaetogaster*(-) group of tested snails, 12 chose bait snails with *Chaetogaster* as their first location for arrestment, while

the remaining 18 chose bait snails without *Chaetogaster* (Table 4-13 and Figure 4-6). In the *Chaetogaster*(+) treatment, exactly half of the tested snails chose bait snails with *Chaetogaster* and the other half bait snails without *Chaetogaster.* In both treatments, tested snails that chose bait snails with *Chaetogaster* made their first arrestment faster on average than those that chose bait snails without *Chaetogaster* (Table 4-13 and Figure 4-7).

Two models using logistic regression (glmmTMB package: Brooks 2017) were created to analyse if treatment, snail length and their interaction affected the first choice made by the tested snails (Table 4-14). A random effect of trial date was also included so account for bait snails being used repeatedly on the same day. Model 2, including only treatment and snail length, was chosen as the best model as it had the lowest AICc (Table 4-14). A comparison of logit values to snail length (i.e., the continuous predictor) determined that the model met necessary assumptions. Overall, the treatment of the tested snail (presence or absence of *Chaetogaster*) did not have a significant effect on the first choice made by that snail ($X^2 = 2.039$, $p = 0.153$), nor did the length (i.e., size) of the snail ($X^2 = 3.060$, $p = 0.080$, Table 4-15).

Additional models were created to test if the time to first arrestment was different. Two negative regression models were created, using the same parameters as above, again with the inclusion of a random effect for trial date (Table 4-16). Comparison through AICc indicated that both models fit the data equally well. An ANOVA comparison ($X^2 = 2.044$, $p = 0.153$) and examination of the assumptions, determined that the simplest model (Model 2) should be chosen.

Overall, neither the treatment of the tested snail (presence or absence of *Chaetogaster*) ($X^2 = 1.976$, $p = 0.160$) nor snail size ($X^2 = 1.675$, $p = 0.196$) had an effect on the time taken for a snail to make its first arrestment (Table 4-17).

Longest Arrestment

One control snail was removed from the analysis in this section, because it had two equally long arrestments in both choice areas, and therefore could not be properly classified into either choice. In the control treatment, 17 out of 29 snails chose the area closest to the *Chaetogaster*(+) bait snails, while the rest chose the opposite (Table 4-18 and Figure 4-8). Of the *Chaetogaster*(+) treatment exactly half of the test snails chose *Chaetogaster*(+) and the other half *Chaetogaster*(-). Length of longest arrestments were generally similar across groups, ranging from 2 to 20 time checks (Table 4-18 and Figure 4-9). On average, snails in the *Chaetogaster*(+) treatment had slightly longer average arrestments than those in the control.

Two models using logistical regression (glmmTMB) were created to assess whether treatment, snail length) or their interaction affected the area in which tested snails completed their longest arrestment (Table 4-19). An additional random effect of trial date was added to both models. The simplest model, Model 2, including only treatment and snail length as predictor values was selected as the best model (Table 4-19). Overall, the treatment of the tested snail (presence or absence of *Chaetogaster*) did not have a significant effect on the location of the longest arrestment made by that snail ($X^2 = 0.798$, $p = 0.372$), nor did the length (i.e., size) of the snail ($X^2 = 0.526$, $p = 0.468$, Table 4-20).

Two additional models were created to determine if the duration of longest arrestment was different between groups. Models were created using a negative binomial regression, with the variables treatment, snail length and their interaction as well as trial date for a random effect (Table 4-21). Model 2 was selected by comparison of AICc as the best model. Overall, neither treatment of the tested snail (presence or absence of *Chaetogaster*) ($X^2 = 2.388$, $p = 0.122$) nor

snail length (X^2 = 0.017, p = 0.895) significantly affected the duration of longest arrestments for individual snails (Table 4-22).

Time Spent

On average, snails in both treatments spent a similar number of time checks near both groups of bait snails, but less time in the middle section (Table 4-23, Figure 4-10 and Figure 4-11).

Four Generalized Linear Models using the negative binomial regression (due to over dispersed count data) were created to assess whether treatment, snail length, area or their interaction affected the amount of time that tested snails spent in each section of the arena (Table 4-24). An additional random effect was added to account for trial date. The simplest model, Model 4, excluding all interaction terms, was selected as the best. Overall, none of the variables examined had a significant effect on where the snails spent their time (Table 4-25). However, there was a visible trend of tested snails preferring to spend time in the 'choice' sections near conspecifics rather in the middle.

Movement between Sections

Here, the number of times a tested snails switched sections in the arena was considered. 'Total number of switches' is defined as the number of times a tested snail moved from one section of the arena to another. On average, *Chaetogaster*(-) snails made 1.5 more switches than those in the *Chaetogaster*(+) Treatment (Table 4-26 and Figure 4-12). The maximum number of switches in each group was 16 and 11, for the *Chaetogaster*(-) and *Chaetogaster*(+) treatment respectively.

To investigate the movement of snails in each treatment, two models were created (glmmTMB) using negative binomial regression with treatment, snail length and their interaction as predictors (Table 4-27). A random effect of trial date was also included. A comparison of AICc indicated that Model 2, the simplest model, fit the data best. An examination of the model also concluded that it met the necessary assumptions. Overall, treatment had a weakly significant effect ($X^2 = 4.252$, p = 0.039) with *Chaetogaster*(-) snails moving more during the trial than those with *Chaetogaster*. Snail length did not have a significant effect on number of switches ($X^2 = 0.610$, p = 0.435, Table 4-28).

Discussion

Movement Assays

The two experiments included in this chapter investigated two different, but complementary aspects of the *Chaetogaster*-physid relationship. In particular, these experiments aimed to determine if *Chaetogaster* presence affected host behaviour.

In my movement assays I hypothesized that *Chaetogaster* presence changes the activity (movement vs rest) of host snails. In particular, I expected that colonized snails would spend more time resting in accordance with results found by Stoll et al. (2013). However, I did not find *Chaetogaster* to have a significant effect on snail movement in either of my assays (Figure 4-2 and Figure 4-3). The lack of pattern may be a result of the small range in number of *Chaetogaster*/snail, differences in snail and worm lineages/species as well as time frame (my experiment only involved 1 h of observation while Stoll et al. (2013) used two 7.5-h observation periods) could also have caused the difference. Further discussion on these caveats can be found below. Overall, the results of my Movement experiments did not support my hypothesis that *Chaetogaster* colonization changes the activity (movement vs rest) of host snails.

As explained previously, my Movement Assays emulated experiments by Stoll et al. (2013). Stoll and colleagues experimentally manipulated worm abundance on their physid snails and then determined the time budget (i.e., time spent feeding, resting, etc.) of each snails over two 7.5 h intervals. Initially, I had wanted to complete my experiment in a similar way; however, certain details were not feasible to replicate. In particular, scheduling and supplies did not allow for such long trials, so the assays were shortened to just one hour in length. I also chose to use the EthoVision program rather than manually track snail movement. This allowed me to gain data quickly with a relatively high level of precision; however, it did mean that I could not

collect certain types of data. Stoll et al. (2013) watched their experimental organisms in a glass-bottomed container that allowed them to make estimates on the feeding behaviour of the individual snails. With my chosen experimental setup, I was not able to see my snails from below and therefore was not able to determine if a snail was resting or feeding while staying in one place.

Also, wild snail populations without worms were not readily available so I could not collect wild snails and experimentally colonize them as was done in Stoll et al. (2013). Using worm-free lab-bred snails was a possible option, but one that was ultimately decided against due to limited numbers in the lab colonies. A concern with using lab-bred snails is that they might react more negatively to sudden presence of worms after presumably not encountering *Chaetogaster* in many generations. With these options unavailable, my next idea was to use wild snails that had natural variation in worm abundance. While this option had its potential flaws, it also had merits, particularly in that wild snails with *Chaetogaster* were abundant, allowing me to repeat this experiment as needed. This choice actually became quite an advantage for me as I repeated the experiment a total of four times (only the last two attempts are discussed in this chapter) in order to perfect the experimental design.

Most of the snails collected for the Movement Assays were already colonized by *Chaetogaster*, so there was no way to control the numbers of worms per snail for this experiment, since actively removing worms would have likely distressed the hosts and altered their subsequent movement patterns. Adding worms to a snail would have been possible, but since it is not feasible to exactly count the number of worms/snail on live snail adding worms would have been a gamble for the total. It was assumed that snails in wild populations with *Chaetogaster* experience colonization on and off throughout their life cycle according to changes

in *Chaetogaster* abundances (see Chapter 2). By general observation, I found that populations with *Chaetogaster* tended to have a narrow range in the number of symbionts/snail (with occasional outliers) and so it was a concern that worm numbers would not be different enough to result in varied host behaviour. Snails in Assay 1 had a range of 0-10 external worms, while those in Assay 2 ranged from 5-21 worms/snail. With the minimal number of snails completely without *Chaetogaster*, it is difficult to make robust observations on how symbiont presence affects snail behaviour. In comparison to other collections of this species and others at the same location, these (0-10 or 0-21 *Chaetogaster*) are relatively conservative ranges of symbionts/snail (pers. obs. VAF and Figure 2-3). Other surveys have also found much higher numbers of *Chaetogaster*, including an extreme value of 90 worms on one *Lymnaea pereger* (Gruffydd 1965b) or 85 worms on an individual *Bithynia leachi* (Sheppard) (Buse 1974).

Another concern with using wild snails is that I could not control the exact species used. As discussed in Chapter 2, I found multiple species/groups of snails at my collection sites that I was not able to differentiate by eye. It is possible that multiple snail species were included in my movement assays, which could have contributed to the lack of conclusive patterns in the data.

Preference Experiments

The second behavioural experiment included in this chapter aimed to investigate whether snails actively avoid (or seek out) other snails with or without *Chaetogaster*, and if this depended on whether the focal snails themselves were already bearing *Chaetogaster*. Since this experiment required snails without worms, lab-bred snails were used for this investigation despite the concerns outlined previously.

In this experiment, a snail (*Chaetogaster*(+/-)) was given the choice of spending time near *Chaetogaster*(+) or *Chaetogaster*(-) conspecifics and the proportion of time spent near each was examined. The prediction here was that if *Chaetogaster* emit some sort of attractive chemical cue, either as an honest signal of 'utility' to snails (if they are beneficial mutualists) or as a type of manipulation (if they are detrimental), snails would spend more time near conspecifics with worms (Table 4-1). If *Chaetogaster* presence was detrimental to host fitness and they do not manipulate snails using chemicals, then one might expect to see the opposite pattern, with snails avoiding conspecifics with worms. Analysis of this experiment did not find evidence of snails spending significantly more or less time with conspecifics with *Chaetogaster* colonization (Figure 4-11). Thus, there was no evidence of attractive or repellent chemicals associated with *Chaetogaster*. There was a slight pattern of tested snails preferring the sections of the arena near conspecifics over the middle section, which suggests that the bioassay was successful in allowing test snails to chemically detect bait snails.

Henry et al. (2006) performed a similar experiment to determine if *Physella acuta* altered their behaviour in the presence of a gradient chemical cues. In this experiment, snails were put in a small Y-maze in which each arm of the maze was ~5cm long and 2.5 cm wide with a central circular chamber ~5cm in diameter. Pure water was put in one arm of the maze, while conditioned water of conspecifics and heterospecifics was placed in the other two. The tested snail was placed in the center of the maze and allowed to move freely. The results of this experiment determined that the tested snails did not move randomly but did alter their behaviour depending on the chemical cues emitted from each arm. This experiment is of particular interest to me for several reasons. The first is that this experiment indicates that *Physella acuta* can change its behaviour depending on the chemical cues of conspecific and heterospecific snails.

The second is that the snails could detect chemical gradients in a similarly sized arena to my own, suggesting that a larger or smaller arena was not necessary. Additionally, Henry et al. (2006) argued that only the first choice made by the snail was a reliable metric of preference, since snails are well known to follow mucus trails (Ng et al. 2013; Bergey et al. 2023), and so future 'choices' may be altered by trails left by the snail itself previously. This argument suggests that the lack of significant preference for *Chaetogaster*(+) or (-) in my examination of the snails 'first choice' is the strongest evidence that the tested snails do not, in fact, have a preference. One last interesting observation from the experiment of Henry et al. (2006) is that their snails preferred water conditioned by heterospecifics over that conditioned by conspecifics, which is a curious phenomenon. One would wonder if my results would have been different if I had tested my snails with heterospecific snails with and without *Chaetogaster*.

The first arrestment and longest arrestment (definitions in Methods of Chapter 4) were also investigated, and no patterns were found. These results may imply that worms are not producing any particular snail attracting or repelling chemical cues or that effect was negated in some way by the experiment set-up (e.g., bait snails emitted distress cues from being moved around repeatedly and this overwhelmed any other chemical cues present) or the use of this particular snail-*Chaetogaster* species combination (e.g., *Chaetogaster* chemical cues may be specific to a different host physid species). Without further testing, the results cannot be disentangled beyond speculation.

There was one interesting pattern found in the data from the preference experiment, and that was concerning the movement of tested snails within the arena. Although the previously discussed movement experiment, in which I used field-caught physids, did not find any effect of colonization on snail movement, in this experiment there was a difference between snails with

and without worms. In fact, snails with *Chaetogaster* moved between areas of the arena significantly less than those without worms. This result is in line with that found by Stoll et al. (2013). Since based on genetic analysis presented in Chapter 2, my lab-bred snails are the same species used by Stoll et al. (2013), this might suggest that host behaviour in reaction to colonization is species dependent. It could also suggest that snails that have hosted *Chaetogaster* for most of their lifespan are relatively unaffected, but adult snails colonized for a short amount of time (e.g., 1 day (Stoll et al. 2013) or ~1 month (this experiment)) show signs of behavioural changes.

Overall, my Preference Experiment did not support my hypothesis that *Chaetogaster* worms actively attract other snails to their host to facilitate snail-to-snail transfer. However, these results are potentially due to the experimental methodology, and I would generally encourage future investigations into this topic. An examination of the chemical cues produced by snails with and without *Chaetogaster* would be of particular interest to move this line of questioning forwards. The surprising results of an effect of *Chaetogaster* on snail movement in in this preference experiment in the movement experiments also suggests new lines of research, especially in terms of investigating species-specific and duration-specific reactions to *Chaetogaster* colonization.

Tables

Table 4-1. Some implications of potential outcomes of the Preference Experiment. The following is not considered an exhaustive list.

		Status of the Tested Snail	
		Chaetogaster(-)	*Chaetogaster*(+)
Category of Bait Snails selected by Tested Snail	*Chaetogaster*(-)	Implication: Uncolonized snails have adapted to avoid conspecifics with *Chaetogaster*, which would imply a negative effect of *Chaetogaster*	Implication: *Chaetogaster* manipulate their hosts to seek out uncolonized conspecifics for increased dispersal.
	Chaetogaster(+)	Implication: Colonized snails emit a chemical cue that attracts other snails, likely for improved *Chaetogaster* dispersal	Implication: Colonized snails emit a chemical cue that attracts other snails, likely for improved *Chaetogaster* dispersal
	No Preference	Implication: Either no special chemical cue is emitted by host snails, or uncolonized snails are indifferent to such a cue.	Implication: Either no special chemical cue is emitted by host snails, or colonized snails are indifferent to such a cue.

Table 4-2. Average distance travelled Movement Assay 1.Assay was conducted over a span of one hour and measured the distance travelled by 36 field-collected physid snails with differing numbers of *Chaetogaster*. The tested snails were also broken into groups of snails with 0-4, 5-9, and 10-14 worms, for a more refined examination of the data.

Group	**No. of snails**	**Avg. distance (cm)**	**SE distance (cm)**
All sampled snails	36	58.7	9.5
0-4 worms/snail	28	65.3	11.70
5-9 worms/snail	7	37.9	10.42
10-14 worms/snail	1	NA (individual = 20.70)	NA

Table 4-3. Model selection and creation for Movement Assay 1'No. *Chaetogaster*' = Number of external *Chaetogaster*/snail, and 'trematode infection' = presence of rediae/sporocysts in snail. The chosen model is indicated with an asterisk '*'.

Model	Parameters	Interaction terms	AICc
1	No. *Chaetogaster*, Snail length, trematode infection	No. *Chaetogaster*: Snail length	350.82
2	No. *Chaetogaster*, Snail length, trematode infection	Snail length: trematode infection	351.36
3	No. *Chaetogaster*, Snail length, trematode infection	No. *Chaetogaster*, trematode infection	349.65
4	No. *Chaetogaster*, Snail length, trematode infection	No	348.47
5	No. *Chaetogaster*, Snail length	Between both variables	348.57
6*	No. *Chaetogaster*, Snail length	No	346.28

Table 4-4. Analysis of Deviance table (Type II tests) for Model 6 (Assay 1). There were no significant results.

Parameter	X^2 value	DF	p Value
No. *Chaetogaster*	1.340	1	0.247
Snail Length	2.813	1	0.094

Table 4-5. Average distance travelled Movement Assay 2. Assay was conducted over a span of one hour and measured the distance travelled by 28 field-collected physid snails with differing numbers of *Chaetogaster*. The tested snails were also broken into groups of snails with 0-4, 5-9, 10-14, 15-19, and 20-24 worms, for a more refined examination of the data.

Group	**No. of snails**	**Avg. distance (cm)**	**SE distance (cm)**
All snails	28	314.7	18.8
0-4 worms/snail	0	NA	NA
5-9 worms/snail	7	330.5	54.8
10-14 worms/snail	8	313.7	36.0
15-19/worms/snail	10	310.4	29.7
20-24 worms/snail	3	294.9	51.4

Table 4-6. Model selection for Movement Experiment (Assay 2). The chosen model is indicated with an asterisk '*'.

Model	**Parameters**	**Interaction terms**	**AICc**
1	No. *Chaetogaster*, Snail length, Internal *Chaetogaster*	No. *Chaetogaster*: Snail length	339.74
2	No. *Chaetogaster*, Snail length, Internal *Chaetogaster*	Snail length: Internal *Chaetogaster*	340.97
3	No. *Chaetogaster*, Snail length, Internal *Chaetogaster*	No. *Chaetogaster*, Internal *Chaetogaster*	341.37
4*	No. *Chaetogaster*, Snail length, Internal *Chaetogaster*	No	338.58
5	No. *Chaetogaster*, Snail length	Between all variables	348.88
6	No. *Chaetogaster*, Snail length	No	347.87

Table 4-7. ANOVA table (Type II tests) for model 4 (Assay 2).There were no significant results.

Parameter	Sum of Squares	DF	F Value	p Value
No. *Chaetogaster*	10171	1	0.853	0.365
Snail Length	5791	1	0.486	0.493
Internal *Chaetogaster*	5663	1	0.475	0.498
Residuals	274201	23		

Table 4-8. Summary of snail first choice by treatment. A total of 30 snails in each treatment group (*Chaetogaster*(+/-)) were tested for a preference in being near bait snails with or without *Chaetogaster*.

Tested snail	First Choice	Total No. of snails	Mean time to first choice (min)
***Chaetogaster*(-)**	Bait snails with *Chaetogaster*	15	1.4
	Bait snails without *Chaetogaster*	15	0.6
***Chaetogaster*(+)**	Bait snails with *Chaetogaster*	18	1.6
	Bait snails without *Chaetogaster*	12	0.75

Table 4-9. Model selection for first choice in Preference Experiment. The chosen model is indicated using an asterisk '*'.

Model	Parameters	Interaction terms	Random effect	AICc
1	Treatment, Snail length	Yes	Trial date	88.79
2*	Treatment, Snail length	NA	Trial date	86.93

Table 4-10. Summary statistics table for Model 2 (First choice). There were no significant results.

Parameter	X^2 value	DF	p Value
Treatment (Tested snail +/- *Chaetogaster*)	2.039	1	0.153
Snail Length	3.060	1	0.080

Table 4-11. Model selection for time to first choice (Preference Experiment). The chosen model is indicated using an asterisk '*'

Model	Parameters	Interaction terms	Random effect	AICc
1	Treatment, Snail length	Yes	Trial date	185.47
2*	Treatment, Snail length	NA	Trial date	183.49

Table 4-12. Summary statistics table for Model 2 (time to first choice). There were no significant results.

Parameter	X^2 value	DF	p Value
Treatment (Tested snail +/- *Chaetogaster*)	2.068	1	0.150
Snail Length	1.110	1	0.292

Table 4-13. Summary table for first arrestment choice (Preference Experiment). A total of 30 snails in each treatment group (*Chaetogaster*(+/-)) were tested for a preference in being near bait snails with or without *Chaetogaster*.

Treatment	**First arrestment choice**	**Total No. of snails**	**Mean time to first arrestment (min)**
***Chaetogaster*(-)**	Bait snails with *Chaetogaster*	12	2
	Bait snails without *Chaetogaster*	18	1.6
***Chaetogaster*(+)**	Bait snails with *Chaetogaster*	15	2.7
	Bait snails without *Chaetogaster*	15	1.6

Table 4-14. Model selection for first arrestment choice (Preference Experiment). The chosen model is indicated using an asterisk '*'.

Model	**Parameters**	**Interaction Terms**	**Random Effect**	**AICc**
1	Treatment, Snail length	Between treatment and snail length	Trial date	90.15
2*	Treatment, Snail length	NA	Trial date	87.81

Table 4-15. Summary Statistics for Model 3 (First arrestment choice). There were no significant values.

Parameter	X^2 **value**	**DF**	**p Value**
Treatment (Tested snail +/-*Chaetogaster*)	1.755	1	0.185
Snail Length	2.629	1	0.105

Table 4-16. Model selection for time to first arrestment (Preference Experiment). The chosen model is indicated using an asterisk '*'.

Model	Parameters	Interaction Terms	Random Effect	AICc
1	Treatment, Snail length	Between treatment and snail length	Trial date	233.24
2*	Treatment, Snail length	NA	Trial date	232.81

Table 4-17. Summary Statistics for Model 3 (time to first arrestment). There were no significant values.

Parameter	X^2 value	DF	p Value
Treatment (Tested snail +/-*Chaetogaster*)	1.976	1	0.160
Snail Length	1.675	1	0.196

Table 4-18. Summary table of longest arrestment data. A total of 30 snails in each treatment group (*Chaetogaster*(+/-)) were tested for a preference in being near bait snails with or without *Chaetogaster*.

Treatment	Longest arrestment choice	Total No. of snails	Min length of longest arrestment (min)	Mean time for longest arrestment (min)	Max length of longest arrestment (min)
***Chaetogaster*(-)**	Bait snails with *Chaetogaster*	17	5	10.5	20
	Bait snails without *Chaetogaster*	12	3	8.1	20
***Chaetogaster*(+)**	Bait snails with *Chaetogaster*	15	2	10.6	19
	Bait snails without *Chaetogaster*	15	6	12.7	20

Table 4-19. Model selection for longest arrestment choice (Preference Experiment). The chosen model is indicated using an asterisk '*'.

Model	Parameters	Interaction Terms	Random Effect	AICc
1	Treatment, Snail length	Between both variables	Trial date	91.16
2*	Treatment, Snail length	NA	Trial date	89.09

Table 4-20. Summary statistics of Model 3 (longest arrestment choice). There were no significant values.

Parameter	X^2 Value	DF	p Value
Treatment (Tested snail +/- *Chaetogaster*)	0.798	1	0.372
Snail Length	0.526	1	0.468

Table 4-21. Model selection for duration of longest arrestment. The chosen model is indicated using an asterisk'*'.

Model	Parameters	Interaction Terms	Random Effect	AICc
1	Treatment, Snail length	Between both variables	Trial date	360.75
2*	Treatment, Snail length	NA	Trial date	358.62

Table 4-22. Analysis of variance summary table (duration of longest arrestment). There were no significant values.

Parameters	X^2 Value	DF	p Value
Treatment (Tested snail +/- *Chaetogaster*)	2.388	1	**0.122**
Snail Length	0.017	1	**0.895**

Table 4-23. Mean number of time checks spent in each area of arena. Snails are split according to treatment *Chaetogaste*r(+) (n=30) and *Chaetogaster*(-) (n=30).

Treatment	***Chaetogaster*(+) bait snails**	**Middle (No choice)**	***Chaetogaster*(-) bait snails**
***Chaetogaster*(-)**	7	4.3	8.7
***Chaetogaster*(+)**	7.8	4.8	7.4

Table 4-24. Model selection for time spent in each area. The chosen model is indicated using an asterisk '*'.

Model	**Parameters**	**Interaction terms**	**Random Effect**	**AICc**
1	Area, Treatment, Snail length	Area: Treatment	Trial date	1068.54
2	Area, Treatment, Snail length	Treatment: Snail length	Trial date	1068.00
3	Area, Treatment, Snail length	Area: Snail length	Trial date	1070.06
4*	Area, Treatment, Snail length	NA	Trial date	1065.81

Table 4-25. Model 4 analysis of deviance table (time spent in each area). There were no significant values.

Parameter	**X^2 value**	**DF**	**p Value**
Area	5.403	2	0.067
Treatment (Tested snail +/- *Chaetogaster*)	1.0939	1	0.296
Snail Length	0.039	1	0.843

Table 4-26. Summary table for the movement of tested snails (Preference Experiment). Data are split into *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30) treatments. Switches are counted as the tested snail moving from one section of the arena to an adjacent section.

Treatment	**Min No. of switches**	**Mean No. of switches**	**Max No. of switches**
***Chaetogaster*(-)**	1	6.5	16
***Chaetogaster*(+)**	1	4.5	11

Table 4-27. Model selection for movement analysis (Preference experiment). The selected model is indicated with an asterisk '*'.

Model	**Parameters**	**Interaction terms**	**Random Effect**	**AICc**
1	Treatment, Snail length	Yes	Trial date	306.96
2*	Treatment, Snail length	NA	Trial date	304.88

Table 4-28. Model 2 analysis of deviance table (movement analysis). Significant results are indicated with an '*'.

Parameter	**X^2 value**	**DF**	**p Value**
Treatment (Tested snail +/- *Chaetogaster*)	4.252	1	**0.039***
Snail length	0.610	1	0.435

Figures

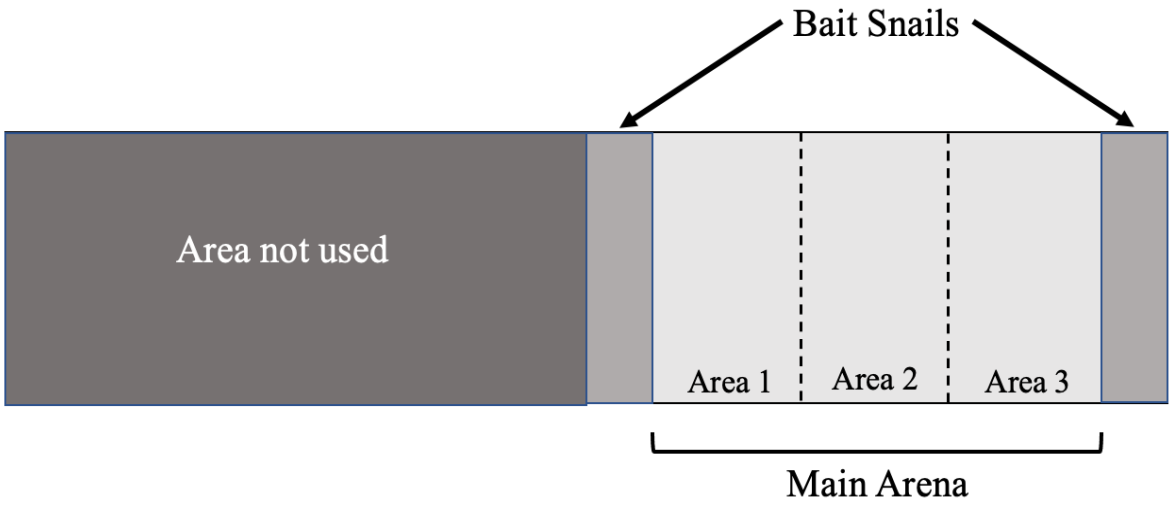

Figure 4-1. Diagram of Preference Experiment arena. The used area of the container was split into different sections (two bait snail enclosures and a main arena). Dotted lines in the main arena do not indicate barriers, but rather visually delimited areas for data recording purposes. The main arena was 18 cm in length while the bait snail enclosures were 1-2 cm wide.

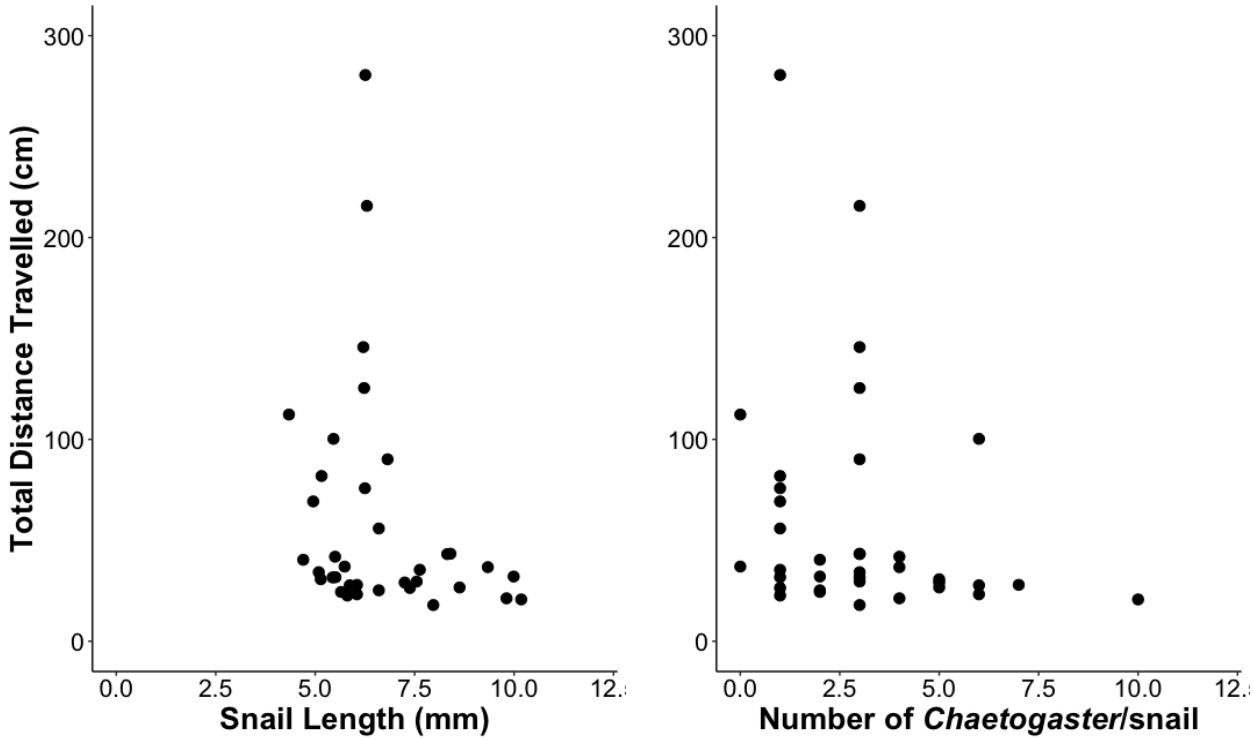

Figure 4-2. Total distance travelled by physid snails in Movement Assay 1. Data are arranged according to snail size (left) and number of external *Chaetogaster* (right). Sample size is 36 snails from Lafarge.

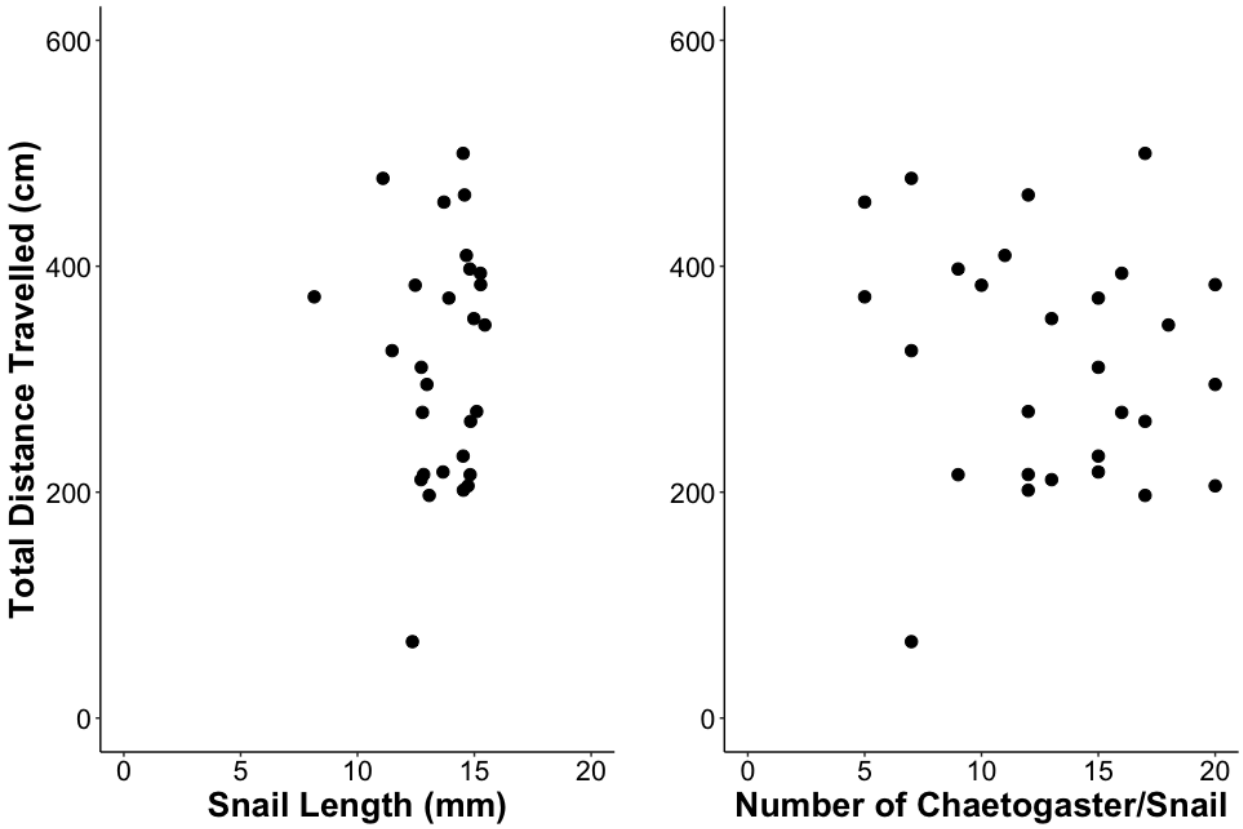

Figure 4-3. Total distance travelled by physid snails in Movement Assay 2. Data are arranged according to snail size (left) and number of external *Chaetogaster* (right). Sample size is 28 snails from Morinville.

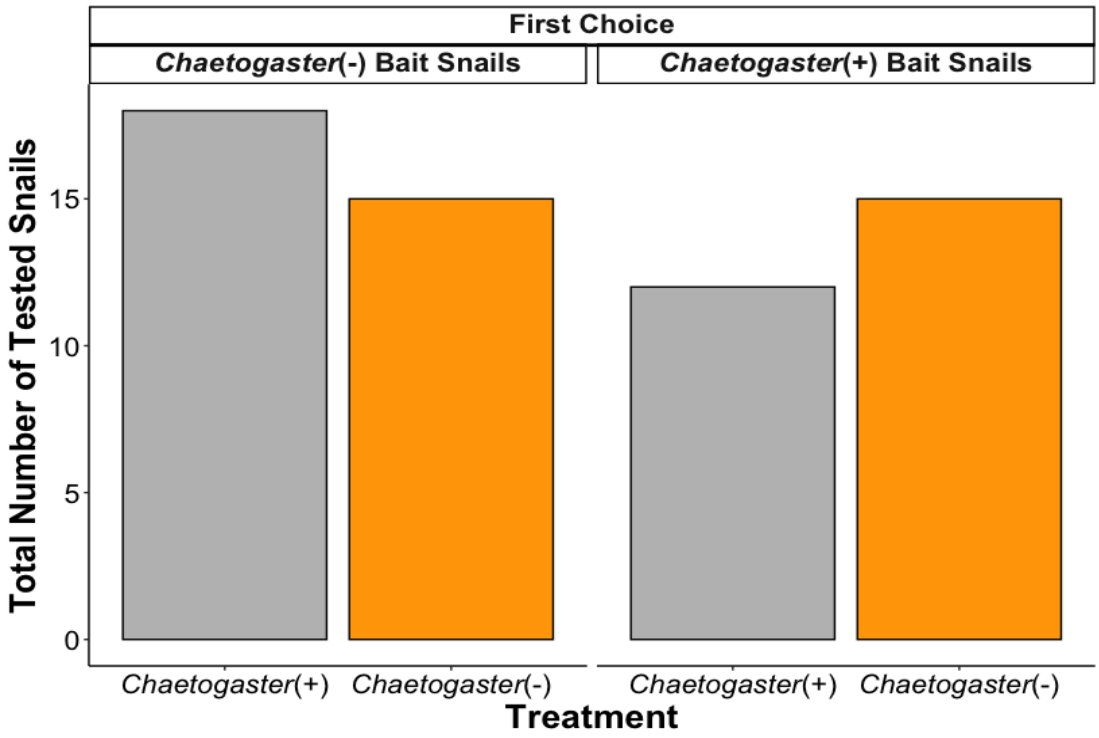

Figure 4-4. First choice made by tested snails. Snails are split according to treatment of Test Snails: *Chaetogaste*r(+) (n=30) and *Chaetogaster*(-) (n=30).

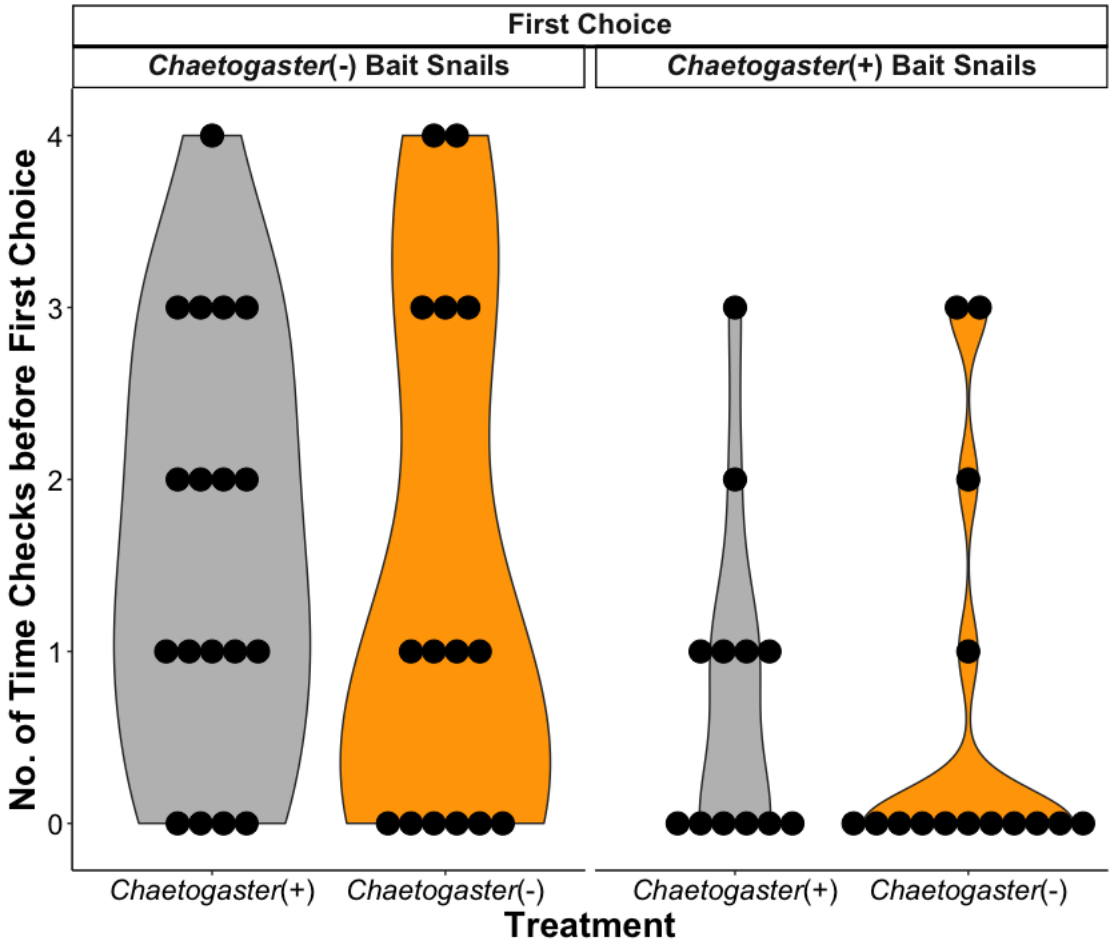

Figure 4-5. Time to first choice made by tested snails. The maximum number of time checks here is 4, since snails that reached the fifth time check without making a choice were removed from the experiment. Here, a value of zero indicates that the snail made a choice before the first time check. Snails are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30).

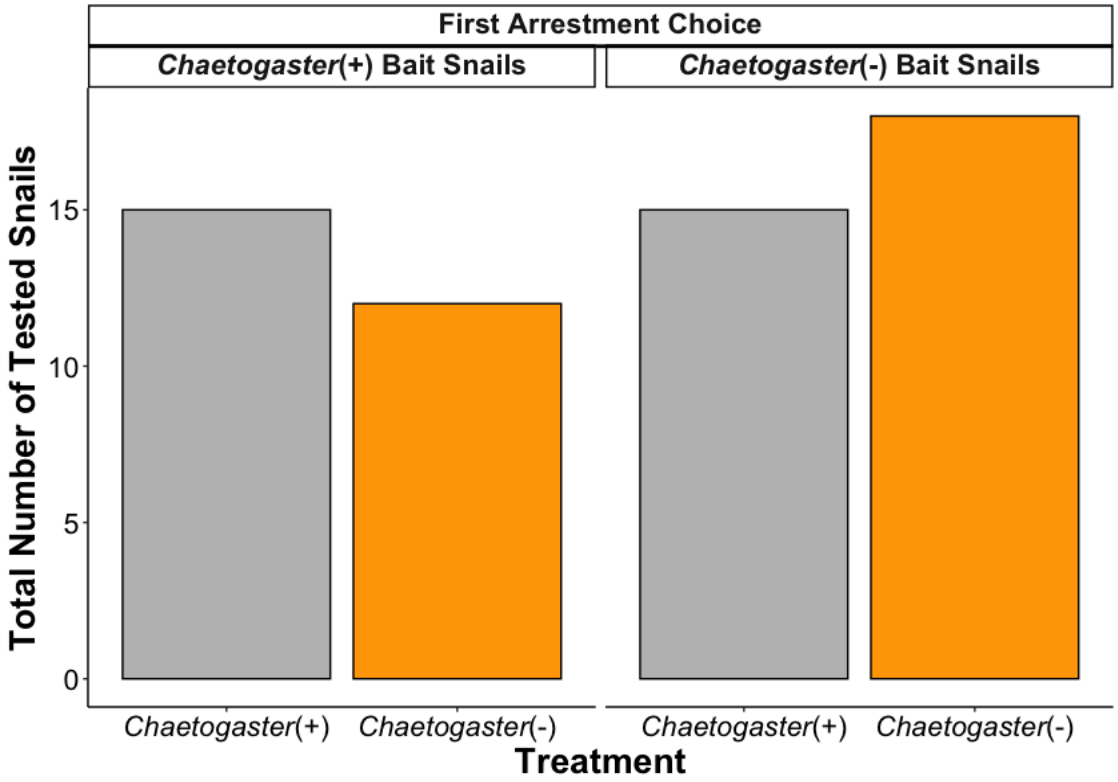

Figure 4-6. First arrestment made by tested snails. Snails are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30).

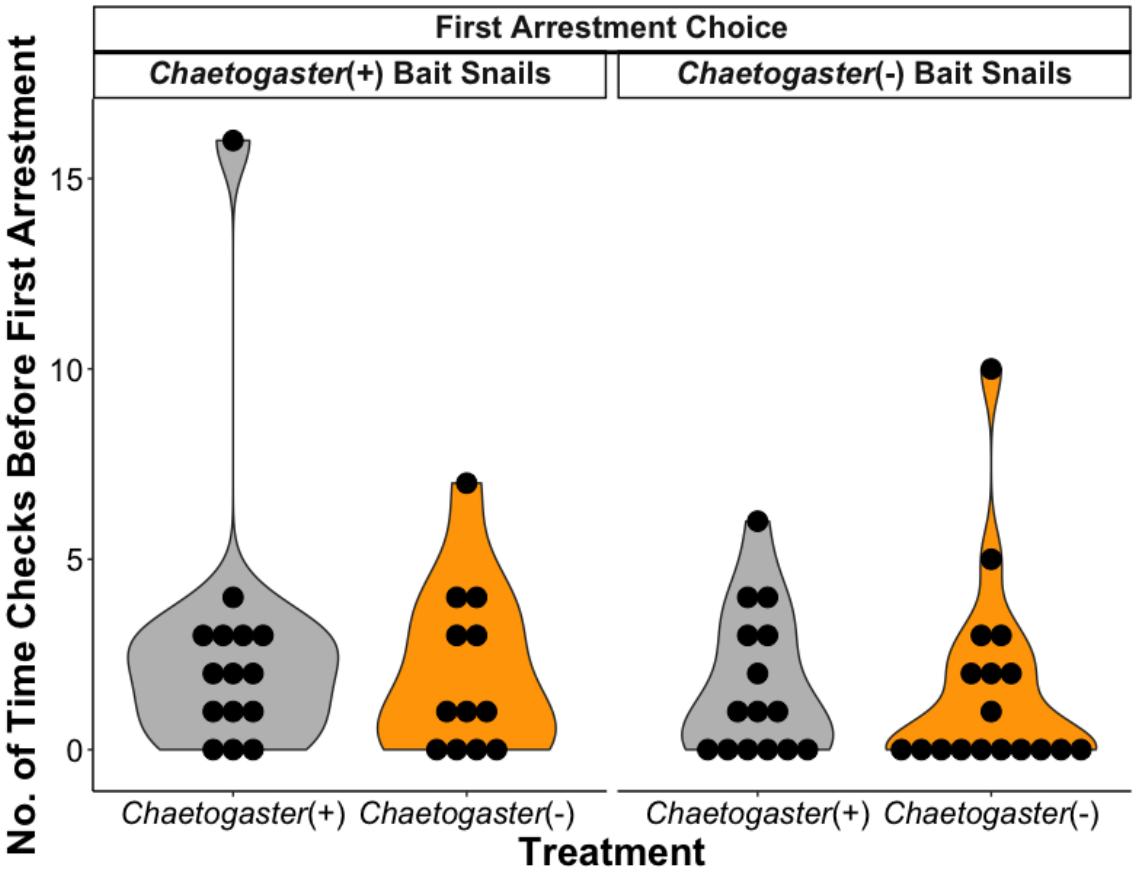

Figure 4-7. Tested snails from each treatment according to area of first arrestment. Snails are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30). Here, a value of zero indicates that the snail started its first arrestment before the first time check.

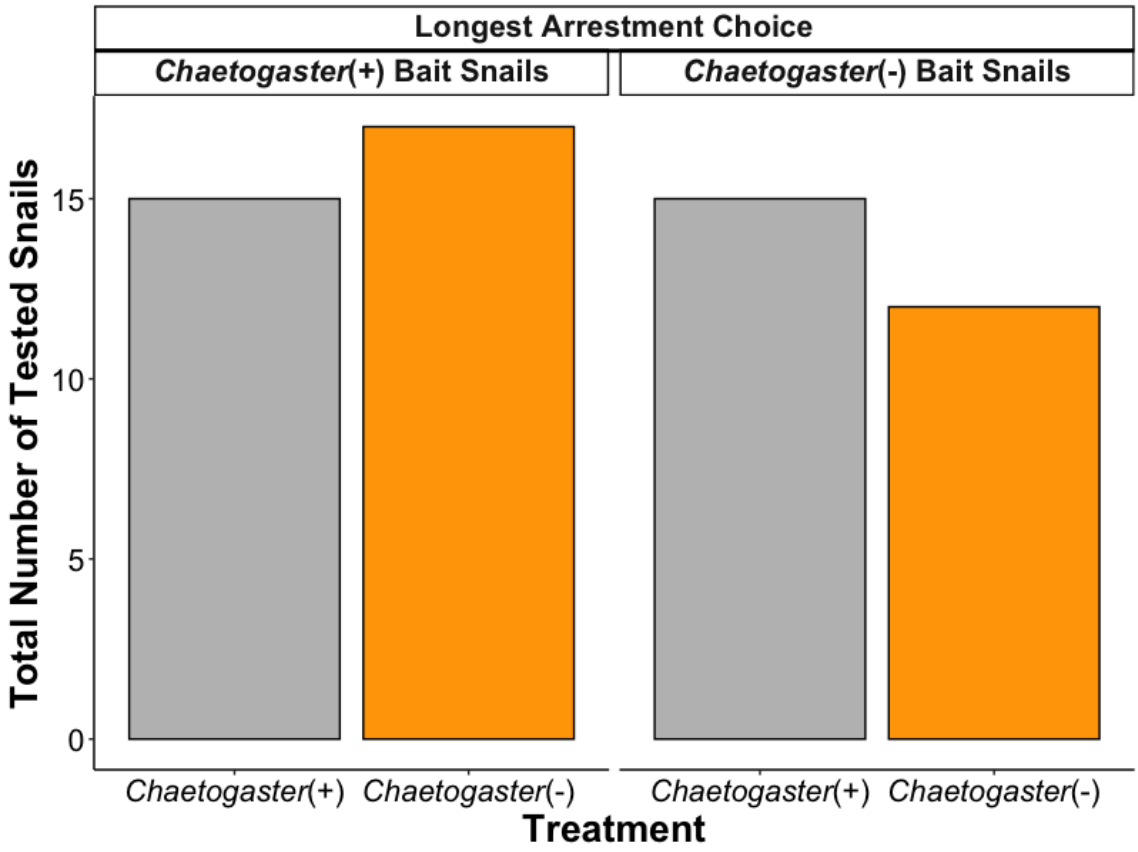

Figure 4-8. Longest arrestment choice made by tested snails. Groups are split by choice of area and treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=29).

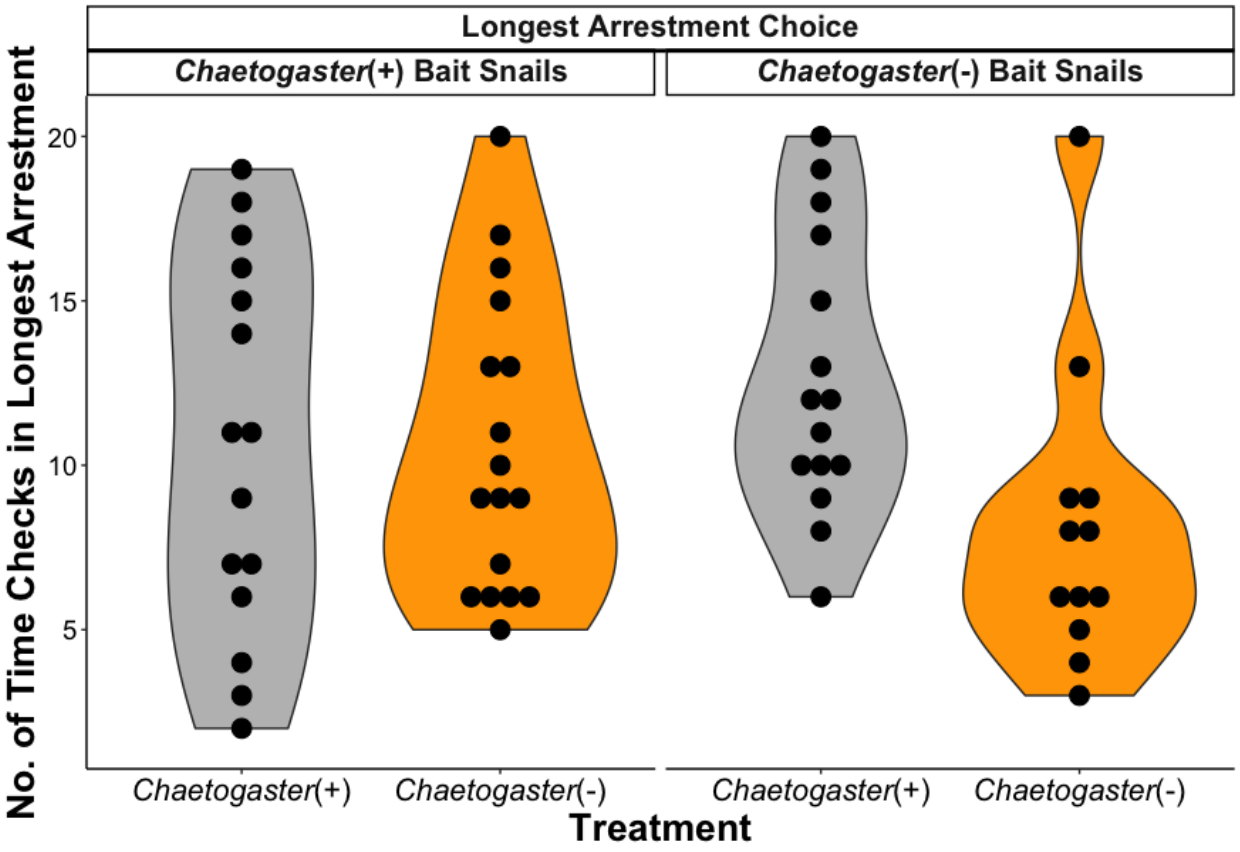

Figure 4-9. Time spent in longest arrestment. Groups are split by choice of area of longest arrestment and treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=29).

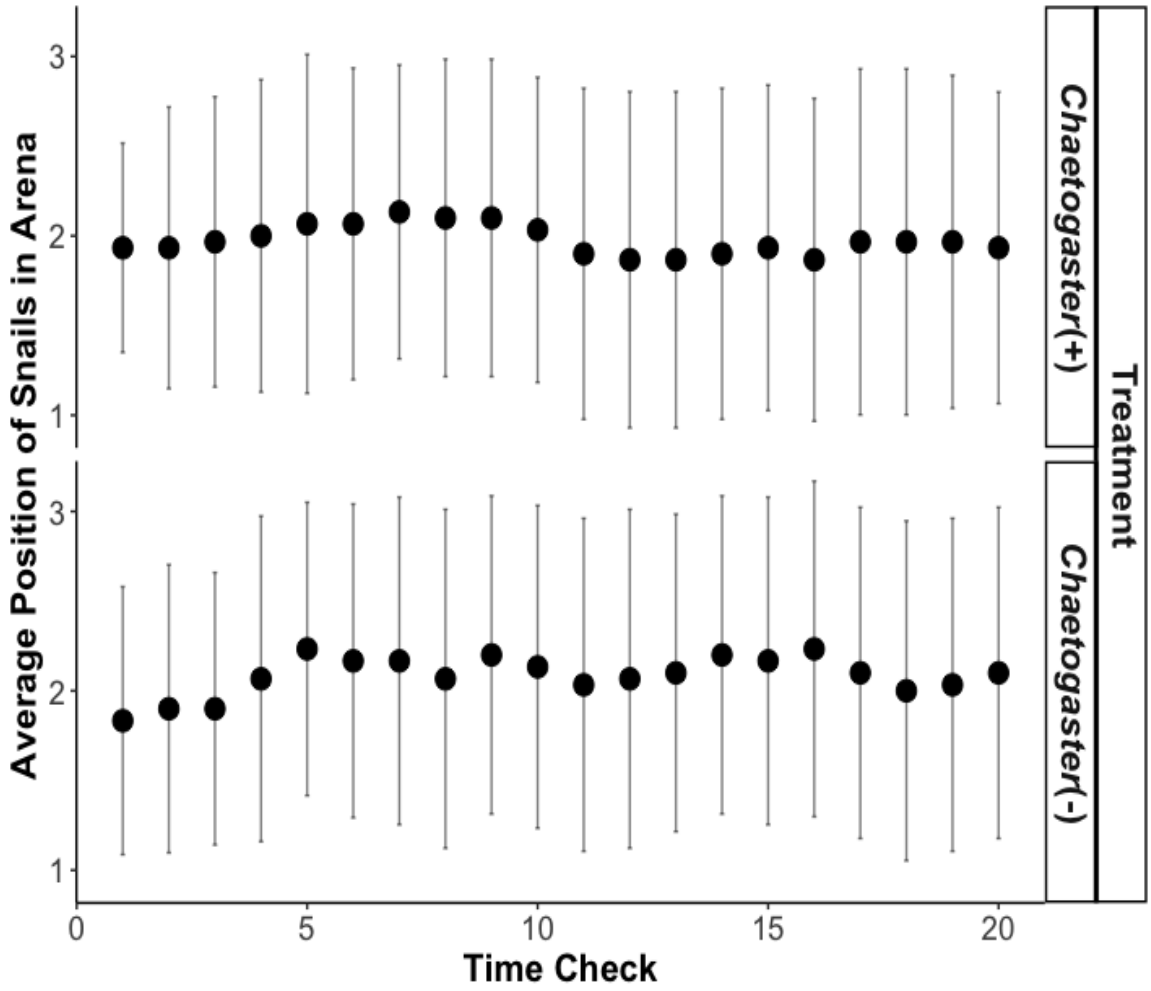

Figure 4-10. Average snail position in arena at each time check. Position 1 = Bait Snails with no *Chaetogaster*, position 2 = middle (no choice), position 3 = Bait Snails with *Chaetogaster*. Data are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30).

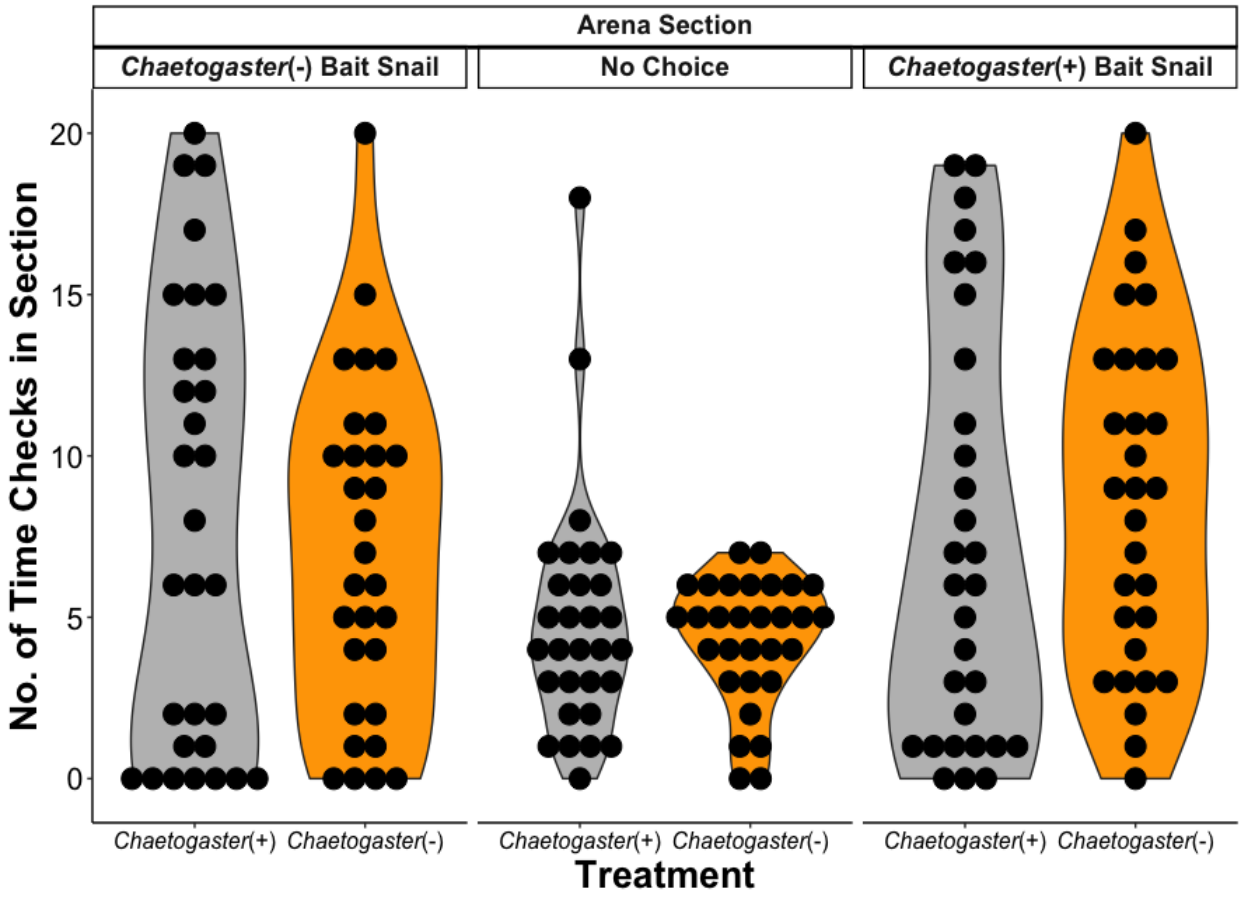

Figure 4-11. Number of time checks tested snails spent in each section of arena. Data are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30).

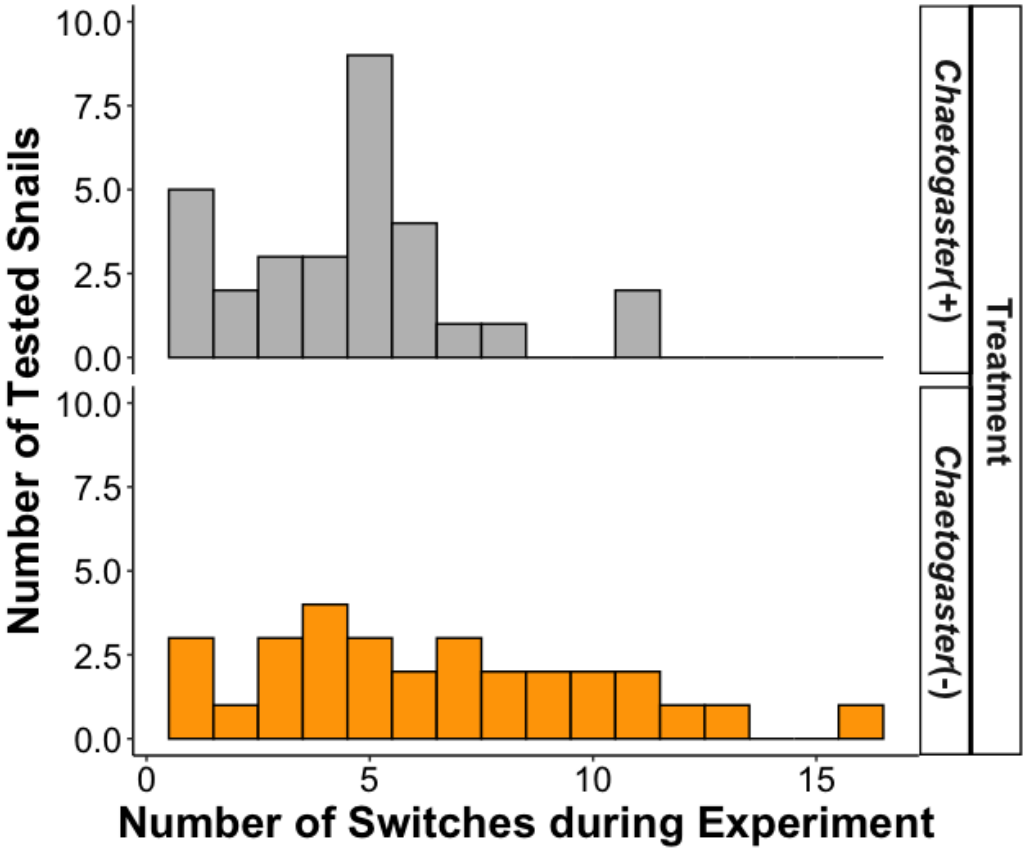

Figure 4-12. Number of switches by tested snails between sections of arena. Data are split according to treatment of Test Snails: *Chaetogaster*(+) (n=30) and *Chaetogaster*(-) (n=30).

Chapter 5. Synthesis

Summary and Conclusions

Over the course of six studies including both manipulative experiments and field surveys, I have attempted to disentangle the mysterious relationship between physid snails and *Chaetogaster* worms. Being a patchwork of success and lack thereof, my experiments highlight our current knowledge in this area as well as areas where more research is needed.

Chapter 2 details my *Chaetogaster*-snail survey and manipulative experiments completed in the field. My survey data did support my predictions in Chapter 1 that *Chaetogaster* abundance is affected by season as well as snail size. These results support previous research on *Chaetogaster* fluctuations over time (Gruffydd 1965b; Buse 1974; Young 1974; Sankurathri and Holmes 1976; Fernandez et al. 1991; Ibrahim 2007; Stoll et al. 2017). My results also indicate that the ponds surveyed (Lafarge and Morinville) had two or more species of physid snail present, along with one common species of *Chaetogaster*. I was not able to visually distinguish between the physid species based on shell characteristics, but it is possible that differences in soft tissues (e.g., degree of dentation of mantle edges) might differ between them. This could be tested by photographing live snails prior to preservation and barcoding, and then examine whether the barcode results correlate with particular morphological features.

Despite successfully addressing the problem of wildlife destroying the lines of cages by using a chicken-wire fence to exclude them, my cage experiment(s) overall did not result in useable data, and therefore my original hypothesis that *Chaetogaster* presence minimizes host infection by trematode parasites could not be formally tested. Future field experiments would benefit from use of snails sourced from the water body in which the experiment is run rather than using snails from a lab population that had likely adapted to different water quality conditions

over many generations. It would also be wise to check the experimental containers more frequently, perhaps daily, to address problems as they arise.

In Chapter 3, I describe two lab experiments aimed to determine the effect of *Chaetogaster* colonization on physid fitness, and I hypothesized that *Chaetogaster* colonization negatively affects host fitness. Using egg production as a proxy for host fitness, I found that snails without *Chaetogaster* produced more eggs on average than those given *Chaetogaster*, which does suggest that worm presence decreases host fitness. While I am confident in the interpretation of this experiment for the lab population of *Physella acuta* involved, future experiments like this one could be improved in a number of ways. Primarily the testing of multiple species, including snail populations that had been in recent generations in contact with the lineage of *Chaetogaster* used to test fitness effects. I also think a potential solution to my problems with examining hatch rate of eggs would be to move each snail from its container into a new one every few days (leaving the eggs behind). This would leave eggs undisturbed in their original container (i.e., no possibility of harming the eggs by moving them with tweezers) which would presumably be large enough to prevent bacterial growth in the water. I also think a fitness experiment that attempts to follow a larger portion (or in fact, the entirety) of the snail life span would solve the problem of lack of measurable growth) that I encountered when doing this experiment.

Chapter 4 presents two experiments regarding the behaviour of snails with and without *Chaetogaster*. In the first, I observed the movement of field-caught snails with varying numbers of worms and found no difference in the behaviour of snails with a higher number of worms. This did not support my hypothesis that *Chaetogaster* presence changes the activity (movement vs rest) of host snails. However, these experiments used snails collected in the field and therefore

the number of worms/snail was not controlled. It is possible that controlling the colonization of the snails tested (and perhaps expanding the range of *Chaetogaster* abundance) would allow a pattern to emerge. I also think that investigating snail behaviour after short and long intervals of colonization may garner interesting results, if perhaps snails are able to cope with colonization in the short term but not over long periods of time (or vice-versa).

In the preference experiment, I exposed snails to conspecifics with and without *Chaetogaster* to determine if *Chaetogaster* presence attracts or repels conspecific snails, and whether that depended on the *Chaetogaster*(+) or (-) status of the focal snail. Although I found little evidence of any influence of *Chaetogaster* presence on this aspect of snail behaviour, I suspect that an improved experimental design may result in a pattern of 'preference'. In particular, I believe that this experiment would be best repeated with snails that are either field collected or had been reared from snails that had been recently collected from the field. Using field snails, that have had the opportunity to have encountered snails in the past few generations might show population specific adaptations to *Chaetogaster* colonization.

Future Directions

Despite being a crucial aspect of snail-associated *Chaetogaster* ecology, very few studies have rigorously investigated the distribution and seasonal dynamics of *Chaetogaster*. As of yet, none of these studies genetically characterized both host and worm. I believe that a thorough investigation into local (or beyond local if you are feeling adventurous!) populations of *Chaetogaster* symbionts to determine the effect of season on population dynamics would be beneficial to our understanding of these worms and their ecological niche. Combining this research with environmental tolerance assays on *Chaetogaster* would be particularly useful. It is likely that different species of worm have different temperature tolerances, which may have been a factor in the peak-and-valley seasonal pattern that I observed in my survey of *Chaetogaster* 'sp. 22'. This combined line of inquiry will be most effective for determining which species of *Chaetogaster* may be affected by changing temperatures in future years, and hence which populations of snails may lose these symbionts.

While I was not able to successfully use the cage experiment to determine if *Chaetogaster* colonization affected the rate of trematode infection in snails, I used survey data to investigate if there was a correlation between *Chaetogaster* and trematode infections. I did not find a significant pattern between worm colonization and trematode infection; however, this is still an intriguing line of inquiry that deserves further investigation. Relating to my point above, it would be particularly important to consider the species involved in such interactions, as *Chaetogaster* may prevent parasitic infection more effectively against specific species of trematode (e.g., depending on parasite transmission mode – active or passive [Hobart et al. 2022]). Additionally, trematode species may vary in their host preferences, so the role of

Chaetogaster as a protective symbiont (if it indeed is one) may be more obvious for some snail/trematode species pairs than others.

In Chapter 3, my results suggest that hosting *Chaetogaster* sp. 22 negatively affects egg production in *Physella acuta*; while this result is corroborated by some previous experiments (Stoll et al. 2013), there are still aspects that need further investigation. I was unable to measure realized fecundity of my snails due to poor water quality causing eggs to deteriorate prior to hatching. This could be remedied in future experiments in which eggs are kept in larger containers, and possible also aerated. Also, I am particularly interested in the long-term effects of *Chaetogaster* worms in this captive snail population. Would the negative effect of colonization continue from generation to generation? Or would later snails adapt to this symbiont, causing the dynamic to change? Expanding research from *Physella acuta* to other snail species (including non-physid species) that host *Chaetogaster* worms, and to other species of snail-associated *Chaetogaster*, would determine if this negative effect of fitness is universal or specific to host and worm species-pairs. I additionally suggest testing the effect of *Chaetogaster* on host snails when another stressor is in play (e.g., poor water quality, extreme temperatures, etc.) since the effect of worm presence may be exacerbated in these situations. This would again, be informative for how snail and *Chaetogaster* may react to the changing environment caused by human activities.

My behavioral studies in Chapter 4 did not find evidence that *Chaetogaster* colonization affects snail behaviour with regard to distance traveled or attraction to other snails; however, there were small hints that the situation may be more complex than it seems. In particular, continued research into the activity of *Chaetogaster*-bearing snails would be of interest, especially if combined with assays of relative fitness. Beyond that, an in-depth examination of

the chemical cues produced by host and symbiont when together and separate might determine if snail-worm combinations do produce differing chemical cues than uncolonized snails. If a difference is found, then another attempt at determining if snails are attracted to or repelled from colonized snails would be paramount.

Overall, with the dearth of research on *Chaetogaster*-snail symbiosis there are many avenues of inquiry still to explore on this topic. As *Chaetogaster* worms (of varying species) are found almost ubiquitously in freshwater habitats around the globe, I strongly encourage future research to focus on these exciting organisms.

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Appendices

Chapter 1. Appendices

Appendix 1-1. A list of scientific literature focused on snail-associated *Chaetogaster limnaei*. Citations are organized by year. Information provided includes the *Chaetogaster* group of interest (as identified by the authors), host family, geographical origin of hosts, and whether or not the paper investigated the ecological relationship between host and symbiont. This list is not exhaustive and only includes literature that was in English and was readily found online by searching Google Scholar for "*Chaetogaster*".

Citation	***Chaetogaster* Group**	**Host Families**	**Host origin**	**Investigated ecological relationship between host and symbiont?**
Khalil 1961	*Chaetogaster limnaei* (external)	Lymnaeidae	Africa	No
Wajdi 1964	*Chaetogaster limnaei*	Not given	NA	Yes: Host defence from trematodes
Michelson 1964	*Chaetogaster limnaei* (external and internal)	Physidae, Planorbidae	Snails from Puerto Rico	Yes: Host defence from trematodes
Gruffydd 1965a	*Chaetogaster limnaei limnaei* and *Chaetogaster limnaei vaghini*	Lymnaeidae	UK	No
Gruffydd 1965b	*Chaetogaster limnaei limnaei* and *Chaetogaster limnaei vaghini*	Lymnaeidae	UK	No
Buse 1972	*Chaetogaster limnaei limnaei* and *Chaetogaster limnaei vaghini*	Lymnaeidae	UK	No

Buse 1974	*Chaetogaster limnaei limnaei* and *Chaetogaster limnaei vaghini*	Physidae, Planorbidae, Lymnaeidae, Tateidae, Bithyniidae, Acroloxidae, Succineinae, Valvatidae	UK	No
Young 1974	*Chaetogaster limnaei limnaei*	Physidae, Lymnaeidae, Bithyniidae	UK	Yes: Host defence from trematodes
Gamble and Fried 1976	*Chaetogaster limnaei limnaei*	Physidae	USA	No
Fernandez et al. 1991	*Chaetogaster limnaei limnaei*	Planorbidae	USA	Yes: Host defence from trematodes
Shaw 1992	*Chaetogaster limnaei limnaei*	Physidae	Canada	No
Conn et al. 1996	*Chaetogaster limnaei*	Dreissenidae	USA and Canada	No
Rodgers et al. 2005	*Chaetogaster limnaei limnaei*	Planorbidae	USA	Yes: Host defence from trematodes
Ibrahim 2007	*Chaetogaster limnaei*	Physidae, Lymnaeidae, Planorbidae	Egypt	Yes: Host defence from trematodes
Fried et al. 2008	*Chaetogaster limnaei*	Planorbidae	USA	Yes: Host defence from trematodes
McKoy et al. 2011	*Chaetogaster limnaei limnaei*	Thiaridae	Jamaica	Yes: Host defence from trematodes
Zimmermann et al. 2011	*Chaetogaster limnaei limnaei*	Planorbidae	USA	Yes: Host defence from trematodes
Hopkins et al. 2013	*Chaetogaster limnaei limnaei*	Planorbidae	USA	Yes: Host defence from trematodes
Stoll et al. 2013	*Chaetogaster limnaei limnaei*	Physidae	Germany	Yes: Host growth and reproduction

Smythe et al. 2015	*Chaetogaster limnaei* (external and internal)	Physidae	USA	No
Höckendorff et al. 2015	*Chaetogaster limnaei limnaei*	Physidae, Lymnaeidae, Bithyniidae	Germany	Yes: Host growth
Hopkins et al. 2015	*Chaetogaster limnaei* (external)	Planorbidae	USA	No
Hopkins et al. 2016	*Chaetogaster limnaei (external)*	Planorbidae	USA	Yes: Host defence from trematodes
Mitchell and Leung 2016	*Chaetogaster limnaei*	Physidae, Lymnaeidae, Planorbidae	Australia	No
Stoll et al. 2017	*Chaetogaster limnaei limnaei*	Physidae, Lymnaeidae, Planorbidae, Acroloxidae, Bithyniidae	Germany	Yes: Host reproductive success
Al-Khalaifah 2018	*Chaetogaster limnaei*	Lymnaeidae	Kuwait	No
Muñiz-Pareja and Iturbe-Espinoza 2018	*Chaetogaster limnaei*	Lymnaeidae	Peru	Yes: Host defence from trematodes
Collado et al. 2019	*Chaetogaster limnaei*	Physidae	Chile	No
Liquin et al. 2021	*Chaetogaster limnaei*	Cyrenidae	Argentina	Yes: Host health (gill damage and host respiration)
Abdel-Redha and Al-Abbad 2021	*Chaetogaster limnaei*	Lymnaeidae	Iraq	No
Hopkins et al. 2022	*Chaetogaster limnaei*	Physidae, Planorbidae	USA	No
Liquin et al. 2022	*Chaetogaster limnaei*	Cyrenidae	Argentina	No
Outa et al. 2022	*Chaetogaster limnaei*	Lymnaeidae, Planorbidae, Thiaridae, Viviparidae,	Kenya	No

		Ampullariidae, Unionidae		
Hobart et al. 2022	*Chaetogaster limnaei*	Physidae, Lymnaeidae, Planorbidae	USA	Yes: Host: defence from trematodes
Mack et al. 2023	*Chaetogaster*	NA	North America and Europe	No

Chapter 2. Appendices

Appendix 2-1. Formula for Artificial Spring Water (ASW). Table first lists components of stock solutions and then describes the ratio of each stock solution needed to create 24L of ASW. Recipe was provided to me by Jacob Hambrook.

Stock Solutions	*Stock Solution A*	
	Ferric Chloride ($FeCl3 – 6H2O$)	0.25g
	MilliQ Water	1L
	Stock Solution B	
	Anhydrous Calcium Chloride	11g
	MilliQ Water	1L
	Stock Solution C	
	Potassium Phosphate Monobasic ($KH2PO4$)	34g
	MilliQ Water	500ml
	Bring to pH 7.2 (with NaOH)	
	Ammonium Sulphate	1.5g
	MilliQ Water	Bring to final volume of 1L
Final Solution	Stock solution A	12ml
	Stock Solution B	60ml
	Stock Solution C	60ml
	Stock Solution D	30ml
	Distilled Water	24L (final volume)

Appendix 2-2. Methods for water quality analyses. All analyses were performed by the Biogeochemical Analytical Service Laboratory (BASL) at the University of Alberta. Samples were taken from Lafarge ponds and the lab-bred snail colony. Information about methods was provided by BASL technicians. Abbreviations are as follows: Standard Methods for the Examination of Water and Wastewater (APHA), US Environmental Protection Agency Test Methods (US EPA), US Geological Survey (USGS). An asterisk'*' indicates methods are modified from reference.

Laboratory Method	Reference	Method	Instrument
Automated determination of total nitrogen and total dissolved nitrogen in surface and wastewaters by flow Injection analysis	US EPA	*TN/TDN - Method 353.2	Lachat QuickChem QC8500 FIA Automated Ion Analyzer
Determination of total phosphorus and total dissolved phosphorus in waters by flow injection analysis	APHA	*TP/TDP – Method 4500-P-G	Lachat QuickChem QC8500 FIA Automated Ion Analyzer
Automated determination of alkalinity, gran alkalinity, conductivity, and pH in water sample using the PC-Titrate instrumentation	APHA APHA USGS	*Alkalinity - Titration Method, 2320 B *pH - Electrometric Method, 4500-H+ B *Gran Alkalinity – Method Series 09-A6.6	Mantech PC-Titration Plus System
Determination of total Dissolved solids in water sample	US EPA	*TDS- Method 160.1	N/A
Determination of chlorophyll a in water by fluorometry		*Welschmeyer, N.A. 1994. Fluorometric Analysis of chlorophyll a in the presence of chlorophyll b and	Agilent Eclipse fluorescence spectrophotometer

	pheopigments. Limnol. Oceanogr., 39(8), 1994, 1985-1992. (Modified)

Appendix 2-3. List of GenBank sample depositors for tree construction (snail subset). See Chapter 2 for more details about chosen samples.

GenBank Accession	Attribution
KM612060	Hebert, P.D.N., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. and DeWaard, J.R.
MG421540	Dewaard, J.R.
MG423475	Dewaard, J.R.
MG422937	Dewaard, J.R.
GU680899	Collector: Glover, S. Specimen ID: Pip, E.
GU680874	Collector: Glover, S. Specimen ID: Pip, E.
MG421606	Dewaard, J.R.
MG422342	Dewaard, J.R.
MG422145	Dewaard, J.R.
MG421380	Dewaard, J.R.
AF419323	Remigio, E.A., Lepitzki, D.A.W., Lee, J.S. and Hebert, P.D.N.
AF346745	Remigio, E.A., Lepitzki, D.A.W., Lee, J.S. and Hebert, P.D.N.
AY651179	Wethington, A.R. and Guralnick, R.
MK308008	Aguilar, R., Ogburn, M.B. and Hines, A.H.
KT831388	Gordy, M.A., Kish, L., Tarrabain, M. and Hanington, P.C.
AY651200	Wethington, A.R. and Guralnick, R.
AF346741	Remigio, E.A., Lepitzki, D.A.W., Lee, J.S. and Hebert, P.D.N.
MG421410	Dewaard, J.R.
KP182986	Ng, T.H., Tan, S.K. and Yeo, D.C.
OM970095	Voroshilova, I.S.
KF737921	Albrecht, C., Foeller, K., Clewing, C., Hauffe, T. and Wilke, T.
MZ798294	Aryaiepour, M., Sarvi, S., Rokni, M.B., Pirestani, M., Mansoorian, A. and Molai, M.B.
OP566899	Paul, P. and Aditya, G.

KM206699	Al-Bdairi, A.B.M., Sraphet, S., Triwitayakorn, K., Al-Miali, H.M. and Mohammad, M.K.
KM612034	Hebert, P.D.N., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. and DeWaard, J.R.
KM611811	Hebert, P.D.N., Ratnasingham, S., Zakharov, E.V., Telfer, A.C., Levesque-Beaudin, V., Milton, M.A., Pedersen, S., Jannetta, P. and DeWaard, J.R.
MG421227	Dewaard, J.R.

Appendix 2-4. List of GenBank sample depositors for tree creation (*Chaetogaster* subset). See Chapter 2 for more details about chosen samples.

GenBank Accession	Attribution
OQ281711	Mack, J.M., Klinth, M., Martinsson, S., Lu, R., Stormer, H., Hanington, P., Proctor, H.C., Erseus, C. and Bely, A.E.
OQ281726	Mack, J.M., Klinth, M., Martinsson, S., Lu, R., Stormer, H., Hanington, P., Proctor, H.C., Erseus, C. and Bely, A.E.
OQ281710	Mack, J.M., Klinth, M., Martinsson, S., Lu, R., Stormer, H., Hanington, P., Proctor, H.C., Erseus, C. and Bely, A.E.
OQ281729	Mack, J.M., Klinth, M., Martinsson, S., Lu, R., Stormer, H., Hanington, P., Proctor, H.C., Erseus, C. and Bely, A.E.
KF952346	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952336	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952309	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952303	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952300	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
OQ281712	Mack, J.M., Klinth, M., Martinsson, S., Lu, R., Stormer, H., Hanington, P., Proctor, H.C., Erseus, C. and Bely, A.E.
KF952313	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952333	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952340	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952323	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.

KF952311	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952298	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
KF952326	Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R. and Litvaitis, M.K.
LN810268	Vivien, R., Wyler, S., Lafont, M. and Pawlowski, J.
JQ519897	Envall, I., Gustavsson, L. and Erseus, C.

Appendix 2-5. Raw survey data from Lafarge ponds 2021. Includes information on snail shell length and number of external *Chaetogaster* of 76 snails collected over two survey dates.

Snail ID	**Snail Length (mm)**	**No. external *Chaetogaster***
Collection date: August 16th, 2021 (Lafarge)		
1	8.74	3
2	6.77	4
3	6.93	1
4	7.35	7
5	7.4	1
6	6.83	1
7	12.93	20
8	12.26	13
9	12.2	3
10	13.2	14
11	13.08	16
12	14.48	20
13	12.08	12
14	13.43	21
15	11.07	13
16	15.4	15
17	12.13	11
18	12.85	8
19	12.88	10
20	9.34	7
21	7.95	5
22	9.73	6
23	15.8	15
24	7.93	6
25	9.51	10
26	7.65	5
27	5.8	4
28	7.28	5
29	11.67	16
30	6.95	5
31	9.55	5
32	5.86	1
33	9.15	8
34	13.4	11
35	9.08	4
36	7.62	4

37	5.65	3
38	6.93	3
39	7.17	5
40	6.63	4
Collection Date: August 30th, 2021 (Lafarge)		
41	6.21	3
42	7.25	5
43	6.05	7
44	7.97	3
45	6.6	2
46	4.95	1
47	5.51	1
48	8.4	3
49	8.32	3
50	9.34	4
51	5.65	2
52	6.25	1
53	5.44	3
54	10.18	10
55	9.81	4
56	5.5	4
57	4.34	0
58	5.74	0
59	5.81	1
60	6.6	1
61	9.99	2
62	6.23	3
63	8.63	5
64	6.05	6
65	5.16	1
66	5.46	6
67	7.38	1
68	6.26	1
69	5.09	3
70	7.63	1
71	6.3	3
72	4.7	2
73	6.82	3
74	5.87	6
75	7.55	3

76	5.14	5

Appendix 2-6. Raw survey data from Morinville (Heritage Lake) in 2021. Includes information on snail shell length and number of external *Chaetogaster* of 57 snails collected over two survey dates.

Snail	**Snail Length (mm)**	**No. of External *Chaetogaster***
Collection Date: August 23rd, 2021 (Morinville)		
1	9.52	18
2	12.51	2
3	12.4	29
4	11.71	17
5	12.39	17
6	11.72	15
7	12.92	19
8	12.75	9
9	13.06	11
10	12.84	15
11	12.97	7
12	14.57	10
13	13.53	7
14	12.8	24
15	7	2
16	11.12	18
17	11.02	12
18	7.32	2
19	13.12	18
20	13.38	12
21	11.88	29
22	12.11	16
23	12.79	15
24	12.88	20
25	10.65	2
26	11.74	18
27	12.44	9
Collection Date: September 26th, 2021 (Morinville)		
28	12.78	16
29	8.15	5
30	14.52	17
31	12.73	15
32	15.27	20
33	11.09	7
34	14.81	9

35	13.72	21
36	12.97	20
37	13.5	16
38	12.35	7
39	12.47	10
40	13.91	15
41	15.45	18
42	13.7	5
43	14.98	13
44	12.72	13
45	13.07	17
46	14.52	15
47	14.82	9
48	15.1	12
49	14.84	17
50	14.66	11
51	14.58	12
52	14.53	12
53	11.48	7
54	14.73	20
55	15.26	16
56	13.66	15
57	12.83	12

Appendix 2-7. Raw survey data from Lafarge Pond 1 in 2022. Includes information on snail shell length, number of external and internal *Chaetogaster*, the presence/absence of trematode infection (rediae/sporocysts), and the number of metacercariae of 260 snails collected over 13 survey dates.

Snail ID	**Snail Length (mm)**	**No. of external *Chaetogaster***	**No. of internal *Chaetogaster***	**Trematode infection**	**No. of Metacercariae**
Collection Date: July 5th, 2022 (Week 1)					
1	12.088	0	0	0	0
2	9.575	0	0	0	0
3	13.29	0	0	0	0
4	9.372	3	0	0	0
5	11.168	0	0	0	0
6	11.049	0	0	0	0
7	8.7	0	0	0	0
8	9.672	0	0	0	0
9	12.45	4	0	0	0
10	11.95	0	0	0	0
11	14.123	0	0	0	0
12	9.93	0	0	0	0
13	11.773	1	0	0	0
14	9.719	0	0	0	0
15	11.159	0	0	0	0
16	10.808	0	0	0	0
17	11.84	4	0	0	0
18	12.894	0	0	0	0
19	10.954	0	0	0	0
20	11.983	0	0	0	0
Collection Date: July 13th, 2022 (Week 2)					
21	12.868	3	0	0	0
22	13.714	2	0	0	0
23	12.862	0	0	0	0
24	12.285	3	0	0	0
25	15.383	0	0	0	0
26	13.905	1	0	0	0
27	15.344	5	0	0	0
28	14.679	3	0	0	0
29	12.735	1	0	0	0
30	10.141	2	0	0	0
31	13.317	3	0	0	0
32	14.225	4	0	0	0
33	13.019	0	0	0	0
34	12.71	3	0	0	0
35	10.814	5	0	0	0
36	12.987	3	0	0	0
37	8.507	0	0	1	0
38	12.436	1	0	0	0

39	13.192	1	0	0	0
40	14.244	0	0	0	0
Collection Date: July 20th, 2022 (Week 3)					
41	13.834	4	0	0	0
42	13.481	8	0	0	0
43	12.968	4	0	0	0
44	15.137	14	0	0	0
45	13.851	1	0	0	0
46	13.112	6	0	0	0
47	11.136	6	0	0	0
48	15.027	7	0	0	0
49	14.458	9	0	0	0
50	12.116	7	0	0	0
51	14.58	5	0	0	0
52	14.868	8	0	0	0
53	13.995	4	0	0	0
54	12.761	4	0	0	0
55	13.344	4	0	0	0
56	14.207	6	0	0	0
57	14.532	7	0	0	0
58	14.633	8	0	0	0
59	14.594	11	0	0	0
60	15.08	8	0	0	0
Collection Date: July 27th, 2022 (Week 4)					
61	5.046	2	0	0	0
62	13.888	2	0	0	0
63	5.029	2	0	0	0
64	5.38	3	0	0	0
65	13.195	17	0	0	0
66	NA	NA	NA	NA	NA
67	7.366	2	0	0	0
68	6.728	0	0	0	0
69	5.571	0	0	0	0
70	11.032	11	0	1	0
71	7.207	6	0	0	0
72	15.397	7	0	0	0
73	15.267	3	0	0	9
74	5.537	0	0	0	0
75	5.867	1	0	0	0
76	4.783	4	0	0	0
77	7.135	3	0	0	0
78	15.336	4	0	0	0
79	12.277	5	0	0	0
80	NA	0	0	0	2
Collection Date: August 4th, 2022 (Week 5)					
81	6.207	2	0	0	2

82	8.792	7	0	0	0
83	9.91	0	0	1	0
84	16.241	5	0	0	2
85	9.825	4	0	0	5
86	7.43	3	0	0	1
87	8.845	4	0	0	4
88	6.029	7	0	0	22
89	13.481	6	0	1	19
90	15.485	1	0	0	4
91	6.19	3	0	0	4
92	7.557	6	0	0	0
93	7.446	0	0	0	3
94	4.362	5	0	0	0
95	15.838	0	0	0	10
96	8.075	3	0	0	2
97	6.941	2	0	0	5
98	7.546	2	0	0	2
99	7.911	1	0	0	0
100	8.957	0	0	0	0
Collection Date: August 10th, 2022 (Week 6)					
101	6.427	0	0	0	0
102	5.868	1	0	0	0
103	8.238	0	0	0	2
104	7.8	0	0	0	5
105	5.82	0	0	0	0
106	8.234	0	0	0	0
107	13.974	7	0	0	0
108	7.658	1	0	0	2
109	8.447	1	0	0	0
110	15.225	9	0	0	0
111	8.316	1	0	1	1
112	6.48	0	0	0	0
113	7.347	1	0	0	1
114	7.192	2	0	0	1
115	5.834	0	0	0	0
116	4.75	1	0	0	2
117	9.212	5	0	0	0
118	7.326	0	0	0	0
119	6.48	2	0	0	2
120	6.82	0	0	0	0
Collection Date: August 17th, 2022 (Week 7)					
121	7.595	0	0	0	0
122	9.849	0	0	0	0
123	8.56	2	0	0	0
124	11.606	2	0	0	0
125	10.246	5	0	0	0

126	9.837	5	0	0	0
127	6.56	0	0	0	0
128	10.346	2	0	0	1
129	6.47	3	0	0	0
130	10.259	1	0	0	0
131	15.913	5	0	0	0
132	7.786	1	0	0	1
133	5.743	2	0	0	1
134	9.971	4	0	0	0
135	7.838	2	0	0	0
136	14.724	4	0	0	0
137	10.876	3	0	0	0
138	10.061	2	0	0	0
139	10.14	3	0	0	0
140	7.678	1	0	0	0
Collection Date: August 24th, 2022 (Week 8)					
141	10.648	5	0	0	0
142	12.479	1	0	0	0
143	11.754	3	0	0	0
144	12.961	4	0	1	0
145	8.662	0	0	0	3
146	14.864	6	0	0	0
147	9.968	0	0	0	0
148	9.834	4	0	0	0
149	14.567	4	0	0	0
150	10.578	2	0	0	0
151	12.905	5	0	0	2
152	11.287	6	0	0	0
153	9.415	1	0	1	0
154	10.768	3	0	0	0
155	12.307	4	0	0	0
156	12.67	6	0	0	0
157	9.823	5	0	0	0
158	11.664	3	0	0	0
159	10.944	1	0	0	0
160	14.979	4	0	0	0
Collection Date: September 1st, 2022 (Week 9)					
161	14.071	4	0	0	0
162	10.44	4	0	1	0
163	15.343	3	0	0	0
164	14.142	2	0	0	0
165	12.335	7	0	0	0
166	14.689	8	0	0	0
167	10.353	4	0	0	0
168	10.841	0	0	0	0
169	13.238	9	0	0	0

170	15.282	15	0	1	0
171	NA	NA	NA	NA	NA
172	12.129	4	0	1	0
173	14.505	4	0	1	0
174	15.828	21	0	0	0
175	13.973	5	0	0	11
176	13.787	3	0	0	0
177	NA	NA	NA	NA	NA
178	12.133	9	0	0	0
179	14.467	7	0	0	0
180	14.763	7	0	0	0
Collection Date: September 9th, 2022 (Week 10)					
181	7.077	6	0	0	0
182	9.86	9	0	0	0
183	13.932	6	0	1	39
184	15.682	22	0	0	0
185	15.072	18	0	0	0
186	15.244	18	0	0	0
187	8.63	3	0	0	0
188	16.913	6	0	0	0
189	11.266	4	0	0	0
190	10.861	5	0	0	0
191	15.072	10	0	0	0
192	13.815	4	0	1	0
193	15.662	7	0	0	0
194	8.786	5	0	0	0
195	15.403	8	0	0	0
196	12.243	4	0	0	0
197	17.683	7	0	0	0
198	13.519	4	0	0	0
199	16.725	9	0	0	0
200	13.434	7	0	0	0
Collection Date: September 13th, 2022 (Week 11)					
201	13.546	7	0	0	0
202	18.704	5	0	0	7
203	14.57	11	0	0	11
204	13.053	3	0	0	0
205	13.641	12	0	0	0
206	15.283	9	0	1	0
207	10.666	7	0	0	0
208	12.008	2	0	0	0
209	11.432	7	0	0	0
210	13.104	7	0	0	3
211	10.44	7	0	0	0
212	10.807	10	0	0	0
213	14.272	15	0	0	0

214	12.507	7	0	0	0
215	12.025	11	0	0	0
216	15.859	22	0	0	11
217	11.682	6	0	0	0
218	15.154	14	0	0	0
219	7.433	9	0	0	0
220	11.824	7	0	1	0
Collection Date: September 20th, 2022 (Week 12)					
221	NA	19	0	0	0
222	NA	10	6	0	0
223	15.492	22	0	0	0
224	NA	12	0	1	0
225	16.874	12	0	0	0
226	NA	9	0	0	0
227	NA	11	0	0	0
228	14.331	11	0	0	0
229	NA	14	0	0	0
230	NA	7	0	0	0
231	15.461	17	0	0	0
232	NA	10	0	0	0
233	15.949	19	0	0	0
234	NA	2	0	0	0
235	NA	12	0	0	0
236	NA	4	0	1	0
237	NA	6	0	0	0
238	NA	20	1	0	0
239	NA	7	0	0	0
240	NA	11	0	0	0
Collection Date: September 27th, 2022 (Week 13)					
241	14.025	13	0	0	12
242	15.604	16	0	0	0
243	17.055	19	0	0	0
244	11.29	12	0	0	0
245	15.199	20	0	0	2
246	7.746	3	0	0	0
247	13.867	14	3	0	0
248	13.31	4	0	0	0
249	14.954	46	0	0	0
250	13.737	7	0	0	0
251	13.049	6	0	0	0
252	14.505	8	0	0	0
253	15.606	23	0	0	0
254	15.047	1	0	0	0
255	18.656	19	0	0	0
256	15.335	14	0	0	0
257	13.573	10	0	0	0

258	11.907	11	0	0	0
259	16.077	10	0	0	0
260	15.33	17	0	0	0

Appendix 2-8. Raw survey data from Morinville (Heritage Lake) in 2022. Includes information on snail shell length, number of external and internal *Chaetogaster*, the presence/absence of trematode infection (rediae/sporocysts), and the number of metacercariae of 260 snails collected over 13 survey dates.

Snail ID	**Snail Length (mm)**	**No. of external *Chaetogaster***	**No. of internal *Chaetogaster***	**Trematode infection**	**No. of metacercariae**
Collection Date: July 5th, 2022 (Week 1)					
1	12.123	7	0	0	1
2	5.272	2	0	0	0
3	11.526	8	5	0	1
4	13.295	13	5	0	5
5	15.478	3	2	0	6
6	12.11	13	3	0	2
7	12.907	4	4	0	0
8	6.395	5	0	0	0
9	15.86	2	6	1	5
10	15.015	5	5	1	4
11	14.046	3	4	0	1
12	12.47	4	1	0	2
13	11.796	4	6	1	0
14	14.142	2	6	1	5
15	5.959	1	3	0	0
16	6.155	6	1	0	0
17	14.231	1	6	0	3
18	10.219	2	2	1	0
19	14.244	1	6	1	6
20	17.854	1	10	0	0
Collection Date: July 13th, 2022 (Week 2)					
21	9.62	4	0	0	0
22	5.811	8	0	0	0
23	11.099	5	0	0	0
24	10.152	23	0	0	0
25	8.11	3	3	0	0
26	7.981	9	2	0	0
27	6.43	1	0	0	0
28	6.872	5	0	0	0
29	7.441	12	0	0	0
30	7.364	1	0	0	0
31	8.028	7	0	0	0
32	7.617	0	0	0	0
33	5.968	3	0	0	0
34	5.373	12	0	0	0
35	9.23	12	0	0	0
36	7.748	4	0	0	0
37	8.287	7	0	0	0
38	6.859	7	0	0	0

39	5.648	2	0	0	0
40	7.507	5	0	0	0
Collection Date: July 20th, 2022 (Week 3)					
41	8.843	2	0	0	0
42	8.603	4	0	0	0
43	8.069	4	0	0	0
44	8.037	3	0	0	0
45	6.357	8	0	0	0
46	7.728	3	0	0	0
47	9.429	2	1	0	0
48	8.178	3	0	0	0
49	9.436	5	0	0	0
50	9.361	4	0	0	0
51	8.938	4	2	0	0
52	8.57	4	0	0	0
53	8.286	5	0	0	0
54	9.92	4	0	0	0
55	6.579	0	0	0	0
56	6.93	3	0	0	0
57	10.883	7	0	0	0
58	8.946	3	2	0	0
59	7.685	1	0	0	0
60	6.162	4	0	0	0
Collection Date: July 27th, 2022 (Week 4)					
61	8.126	0	2	0	0
62	9.371	1	0	0	0
63	10.692	0	0	0	0
64	8.443	1	0	0	0
65	8.762	1	0	0	0
66	10.476	4	2	0	0
67	7.62	2	0	0	0
68	11.077	0	0	0	0
69	10.427	1	0	0	0
70	10.294	1	0	0	0
71	10.384	0	0	0	0
72	8.648	0	0	0	0
73	11.509	0	0	0	0
74	8.943	0	0	0	0
75	9.388	0	0	0	0
76	10.749	0	0	0	0
77	8.847	0	2	0	0
78	10.981	0	5	0	0
79	8.82	1	0	0	0
80	7.247	0	0	0	0
Collection Date: August 4th, 2022 (Week 5)					
81	8.094	0	1	0	0

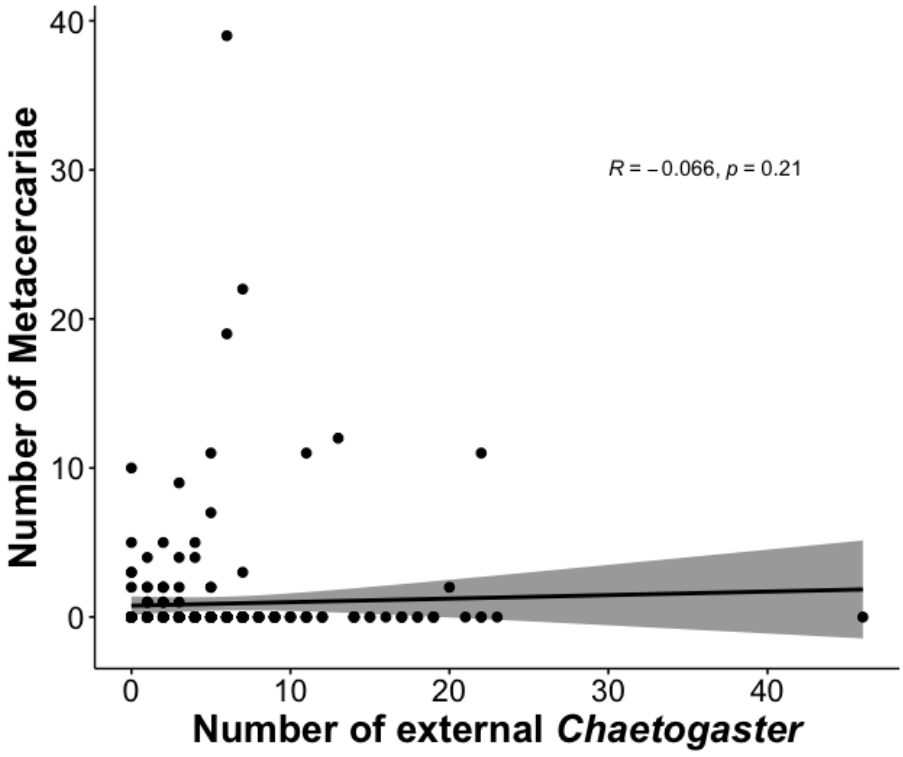
82	6.406	0	1	0	0
83	6.675	0	0	0	0
84	6.414	1	0	0	0
85	10.63	2	0	0	0
86	10.122	1	0	0	0
87	7.759	0	0	0	0
88	6.519	1	0	0	0
89	9.209	2	0	0	0
90	9.534	0	0	0	0
91	8.586	1	2	0	0
92	9.101	0	0	0	0
93	7.347	0	0	0	0
94	7.209	2	0	0	0
95	7.712	5	1	0	0
96	7.501	0	0	0	0
97	8.992	0	0	0	0
98	7.872	2	0	0	0
99	7.421	2	0	0	0
100	7.093	0	2	0	0
Collection Date: August 10th, 2022 (Week 6)					
101	8.277	5	0	0	0
102	7.371	2	0	0	0
103	7.891	3	0	0	0
104	8.215	1	0	0	0
105	8.247	2	0	0	0
106	11.305	3	0	0	0
107	8.023	5	1	0	0
108	7.102	3	0	0	0
109	8.718	0	0	0	0
110	12.431	3	3	0	0
111	10.68	2	0	0	0
112	6.267	0	2	0	0
113	8.252	1	0	0	0
114	6.572	3	0	0	0
115	8.479	3	1	0	0
116	8.134	2	2	0	0
117	8.179	2	1	0	0
118	6.415	0	0	0	0
119	8.304	3	2	0	0
120	7.872	3	2	0	0
Collection Date: August 17th, 2022 (Week 7)					
121	9.081	0	0	0	0
122	9.985	0	0	0	0
123	6.392	1	0	0	0
124	9.695	2	0	0	0
125	11.096	2	2	0	0

126	8.22	0	0	0	0
127	7.891	3	0	0	0
128	9.836	5	0	0	0
129	7.176	2	0	0	0
130	12.824	8	0	0	0
131	12.297	3	0	0	0
132	8.338	2	0	0	0
133	8.032	4	2	0	0
134	6.685	2	0	0	0
135	12.771	0	0	0	0
136	6.364	0	1	0	0
137	8.109	1	0	0	0
138	11.628	7	0	0	0
139	8.837	2	0	0	0
140	13.331	6	0	0	0
Collection Date: August 24th, 2022 (Week 8)					
141	11.397	6	0	0	0
142	11.054	3	2	0	0
143	9.968	3	0	0	0
144	11.065	3	0	0	0
145	7.7	1	2	0	0
146	6.704	3	0	0	0
147	8.224	4	0	0	0
148	6.966	0	0	0	1
149	NA	1	3	0	0
150	9.79	1	0	0	0
151	8.58	2	0	0	0
152	9.396	3	4	0	0
153	7.626	1	0	0	0
154	10.642	6	1	0	0
155	9.299	4	2	0	0
156	12.221	2	5	0	0
157	9.824	2	3	0	0
158	9.436	4	0	0	0
159	7.938	3	0	0	0
160	11.589	0	0	0	0
Collection Date: September 1st, 2022 (Week 9)					
161	9.857	3	3	0	0
162	14.069	3	2	0	0
163	11.895	1	0	0	0
164	12.314	4	0	0	0
165	9.877	3	0	0	0
166	12.326	5	0	0	0
167	10.461	3	0	0	0
168	6.108	1	0	0	0
169	12.801	3	0	0	0

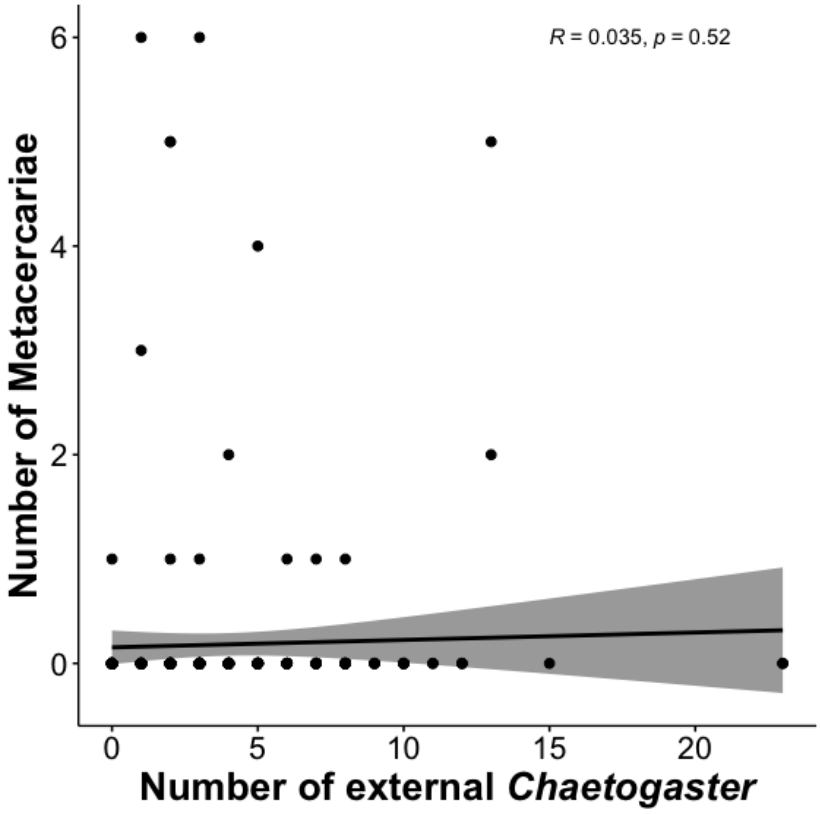
170	9.459	2	0	0	0
171	11.843	3	3	0	0
172	11.606	3	3	0	0
173	13.8	2	0	0	0
174	8.51	2	0	0	0
175	14.963	2	0	0	0
176	9.084	6	3	0	0
177	12.744	2	0	0	0
178	13.992	5	5	0	0
179	10.703	5	1	0	0
180	11.329	2	0	0	0
Collection Date: September 9th, 2022 (Week 10)					
181	10.218	4	0	0	0
182	8.072	5	4	0	0
183	11.321	8	2	0	0
184	12.546	6	0	0	0
185	12.372	4	1	0	0
186	13.25	8	3	0	0
187	13.951	6	2	0	0
188	9.499	4	0	0	0
189	12.499	6	0	0	0
190	12.562	3	1	0	0
191	8.726	4	3	0	0
192	11.123	2	0	0	0
193	11.881	5	2	0	0
194	11.402	3	3	0	0
195	11.55	2	1	0	0
196	8.941	8	1	0	0
197	11.253	5	3	0	0
198	10.211	8	2	0	0
199	11.02	2	3	0	0
200	12.884	8	2	0	0
Collection Date: September 13th, 2022 (Week 11)					
201	10.831	5	3	0	0
202	12.184	4	5	0	0
203	11.993	7	2	0	0
204	10.952	7	4	0	0
205	11.01	4	1	0	0
206	12.998	10	3	0	0
207	12.253	9	2	0	0
208	11.232	2	1	0	0
209	9.617	7	5	0	0
210	10.203	5	0	0	0
211	11.689	11	3	0	0
212	10.094	11	3	0	0
213	11.566	5	0	0	0

214	12.507	10	0	0	0
215	12.171	5	4	0	0
216	10.764	5	0	0	0
217	11.482	9	3	0	0
218	11.308	6	2	0	0
219	10.62	10	0	0	0
220	11.806	5	0	0	0
Collection Date: September 20th, 2022 (Week 12)					
221	NA	13	0	0	0
222	NA	5	3	0	0
223	NA	11	1	0	0
224	NA	8	2	0	2
225	11.965	1	4	0	0
226	NA	11	2	0	0
227	NA	20	4	0	0
228	NA	8	4	0	0
229	NA	9	3	0	0
230	9.081	8	0	0	0
231	NA	11	3	0	0
232	11.035	10	3	0	0
233	NA	5	0	0	0
234	NA	9	1	0	0
235	NA	10	3	0	0
236	NA	5	3	0	0
237	8.604	5	1	0	0
238	NA	12	0	0	0
239	NA	6	4	0	0
240	NA	3	5	0	0
Collection Date: September 27th, 2022 (Week 13)					
241	9.954	7	3	0	0
242	11.44	2	3	0	0
243	11.15	5	5	0	0
244	12.521	8	10	0	0
245	11.862	9	7	0	0
246	13.416	8	0	1	0
247	9.994	4	1	0	0
248	11.004	8	2	0	0
249	11.912	2	3	0	1
250	13.444	6	2	0	0
251	14.441	5	0	0	0
252	12.966	6	0	0	1
253	10.471	15	6	0	0
254	11.384	8	2	0	0
255	13.232	8	0	0	0
256	11.387	10	0	0	0
257	14.761	23	3	0	0

258	12.382	10	3	0	0
259	12.259	5	8	0	0
260	11.579	4	5	0	0



Appendix 2-9. Correlation analysis from 241 snails from Lafarge in 2022. Test was completed between the number of metacercariae and external *Chaetogaster* per snail. A 95% confidence interval was provided along with the correlation coefficient and p value. No association was found between the two variables (R = -0.066, p =0.21). See Table 2-2 for a summary of collection dates.



Appendix 2-10. Correlation analysis from 243 snails from Morinville in 2022. The test was completed between the number of metacercariae and external *Chaetogaster* per snail. A 95% confidence interval was provided along with the correlation coefficient and p value. No association was found between the two variables (R = 0.035 p =0.52).

Appendix 2-11. CO1 sequences from snail and worm specimens sequenced in this thesis. Samples are organized first by organism into physid snails (n = 24) and *Chaetogaster* worms (n = 17) and then by specimen ID. Please see the Methods in Chapter Two for a detailed discussion on how these sequences were obtained. Sequences have been trimmed to 612 base pairs (snails) or 626 base pairs (*Chaetogaster*).

Specimen	**CO1 Sequences**
Snail Sequences	
VAF-2S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG CTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGACCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-3S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG CTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGACCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-5S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTATTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-6S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTATTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTATTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-7S	GTGGATTGGTTGGTACTGGATTAAGTTTATTAATTCGTTTAGAACTAGGAACAACGTTAGTTTTGTTAGAT GAACATTTTTATAATGTTATTGTAACAGCTCATGCATTTGTTATAATTTTTTTTTATGGTTATACCTATAATA ATTGGTGGATTTGGGAATTGAATGGTACCTATATTAATTTGGGGCACCTGATATAAGATTTCCTCGAATAA ATAACATAAGATTTTGGTTATTACCTCCATCTTTCATTTTATTATTATGTTCTTCTATAGTCGAAGGGGGGG TAGGTACTGGTTGAACAGTTTATCCCCCATTATCTGGTCCTATTGCTCATTCTGGGTCATCCGTTGACTTAG CTATTTTTTCTTTACATTTAGCAGGATTATCTTCAATTTTAGGTGCTATTAACTTTATTACAACAATTTTTAA CATACGATCTCCAGGAATCACTTTGGAACGGATAAGTTTATTTGTTTGGTCAGTTCTTATTACTGCATTTTT ATTACTACTGTCATTACCTGTACTGGCTGGGGCAATTACAATGTTATTAACAGATCGAAATTTTAATACTA GTTTTTTTGATCCAAGAGGTGGTGGAGACCCAATTTTA
VAF-8S	GTGGATTGGTTGGTACTGGATTAAGTTTATTAATTCGTTTAGAACTAGGAACAACGTTAGTTTTGTTAGAT GAACATTTTTATAATGTTATTGTAACAGCTCATGCATTTGTTATAATTTTTTTTTATGGTTATACCTATAATA ATTGGTGGATTTGGGAATTGAATGGTACCTATATTAATTTGGGGCACCTGATATAAGATTTCCTCGAATAA ATAACATAAGATTTTGGTTATTACCTCCATCTTTCATTTTATTATTATGTTCTTCTATAGTCGAAGGGGGGG TAGGTACTGGTTGAACAGTTTATCCCCCATTATCTGGTCCTATTGCTCATTCTGGGTCATCCGTTGACTTAG

	CTATTTTTTCTTTACATTTAGCAGGATTATCTTCAATTTTAGGTGCTATTAACTTCATTACAACAATTTTTAA CATACGATCTCCAGGAATCACTTTGGAACGGATAAGTTTATTTGTTTGGTCAGTTCTTATTACTGCATTTTT ATTACTACTGTCATTACCTGTACTGGCTGGGGCAATTACAATGTTATTAACAGATCGAAATTTTAATACTA GTTTTTTTGATCCAAGAGGTGGTGGAGACCCAATTTTA
VAF-9S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAC GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG CTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAATTTTAATACTAG ATTTTTTGATCCAAGTGGTGGTGGGGATCCCATTTTA
VAF-10S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-11S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-12S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-13S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-14S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
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	ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTGGGTGCTCCGGATATAAGTTTCCCACGAATA AATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGG GCTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTA GCTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTA ATATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTT TATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAATTTTAATACTA GATTTTTTGATCCAAGTGGTGGTGGGGATCCCATTTTA
VAF-27S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAC GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG CTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAATTTTAATACTAG ATTTTTTGATCCAAGTGGTGGTGGGGATCCCATTTTA
VAF-28S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAC GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG CTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAATTTTAATACTAG ATTTTTTGATCCAAGTGGTGGTGGGGATCCCATTTTA
VAF-29S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGAAACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATA AATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGG GTTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTA GCTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTA ATATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTT TATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTA GATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-30S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAC GAACATTTTTACAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGAAACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATA AATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGG GTAGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTA GCTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTA ATATACGATCTCCAGGTATTACTTTAGAACGAATAAGTTTATTTGTTTGATCAGTGTTAATTACTGCATTTT TATTACTACTATCTTTACCTGTTTTAGCTGGAGCTATTACAATGCTACTTACAGATCGAAACTTTAATACTA GATTTTTTGATCCAAGTGGTGGTGGGGATCCTATTTTA
VAF-31S	GTGGTTTAGTTGGTACAGGTTTAAGTTTACTAATTCGATTAGAGTTGGGAACTACATTAGTTTTATTAGAT GAACATTTTTATAATGTTATTGTTACAGCACATGCTTTTGTTATAATTTTTTTTATAGTAATACCTATAATA ATTGGGGGATTTGGGAATTGAATAGTTCCGATACTAATTTGGTGCTCCGGATATAAGTTTCCCACGAATAA ATAATATAAGATTTTGATTACTACCTCCATCTTTTATTTTATTATTGTGTAGTTCAATAGTTGAAGGTGGGG TTGGTACTGGTTGAACAGTTTACCCTCCTTTATCTGGGCCTATTGCACACTCTGGGTCTTCAGTTGATTTAG CTATTTTTTCATTACATTTAGCTGGTTTATCTTCAATTTTAGGTGCAATTAATTTTATTACAACTATTTTTAA TATACGATCTCCAGGTATCACTTTAGAACGAATAAGTTTATTTGTCTGATCAGTGTTAATTACTGCATTTTT ATTACTATTATCTTTACCTGTTTTAGCTGGAGCTATCACAATGCTACTTACAGATCGAAACTTTAATACTAG ATTTTTTGATCCTAGTGGTGGTGGGGATCCTATTTTA
VAF-44S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT

	TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
VAF-45S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
VAF-46S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
VAF-47S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
VAF-48S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
VAF-49S	GTGGATTGGTCGGTACAGGTTTAAGCTTGTTAATTCGTTTGGAATTAGGAACATCTCTTGTACTGTTGGAT GAACATTTTTATAATGTAATTGTTACAGCACATGCTTTTGTAATGATTTTTTTTATAGTTATACCTATAATA ATTGGAGGGTTTGGGAATTGAATAGTACCTATATTAATTTGGTGCTCCCGATATAAGATTTCCTCGGATAA ATAATATAAGATTTTGACTTTTACCGCCTTCATTTATCTTATTATTATGTAGGTCTATAGTTGAGGGTGGAG TTGGAACTGGGTGAACTGTTTACCCCCCTCTATCAGGACCTGTAGCTCACTCTGGTTCATCAGTAGATCTT GCTATTTTCTCATTACACTTAGCTGGGTTATCATCTATTCTAGGTGCTATTAATTTTATTACTACCATTTTTA ATATACGTTCACCTGGTATTACACTGGAACGAATAAGCTTATTTGTTTGATCGGTGTTAATTACTGCATTTT TATTATTATTGTCATTGCCTGTTTTAGCAGGGGCTATTACTATACTATTAACTGATCGAAATTTTAATACTA GGTTCTTTGATCCAAGAGGGGGGGGAGACCCTATTCTA
Chaetogaster Sequences	
VAF-19W	GTGAGCGGGAATAATCGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAGTTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATATCAAC
VAF-20W	GTGAGCGGGAATAATCGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAGTTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC

	TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATATCAAC
VAF-22W	GTGAGCAGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-24W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-33W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-34W	GTGAGCAGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-35W	GTGAGCAGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-36W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC

VAF-37W	GTGAGCAGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-38W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-39W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAGCCAGGATCATTC CTAGGAAGAGACCAACTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGATTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCTCCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGAATAAAACTAGAACGACTTCCATTATTTGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTTTTATCACTTCCAGTGCTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-40W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-41W	GTGGGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCGGGATCATTC CTAGGAAGAGACCAATTATATAATACTATAGTTACTGCACACGCATTTTTAATAATTTTCTTCTTAGTTAT ACCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCGGACATAGCATTTC CTCGACTTAACAATCTAAGATTTTGGCTATTACCACCATCACTTATTCTATTAGTTTCCTCTGCAGCAGTAG AAAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTC TGTAGACTTAGCTATTTTCTCCCTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTGAACTTCATTAC TACTGTAATTAACATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCCTATGAGCAGTGTTAT TAACAGTAACTCTACTTCTTTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGT AACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-43W	GTGGGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCGGGATCATTC CTAGGAAGAGACCAATTATATAATACTATAGTTACTGCACACGCATTTTTAATAATTTTCTTCTTAGTTAT ACCAGTATTTATTGGGGGATTTGGAAATTGACTAATTCCTCTAATACTAGGTGCACCGGACATAGCATTTC CTCGACTTAACAATCTAAGATTTTGGCTATTACCACCATCACTTATTCTATTAGTTTCCTCTGCAGCAGTAG AAAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTC TGTAGACTTAGCTATTTTCTCCCTACATCTTGCAGGTGCATCATCTATTCTAGGGGCACTGAACTTCATTAC TACTGTAATTAACATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCCTATGAGCAGTGTTAT TAACAGTAACTCTACTTCTTTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGT AACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-50W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT

	ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-51W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC
VAF-52W	GTGAGCGGGAATAATTGGAACAGGAACTAGAATAATCATTCGTATTGAACTAGCTCAACCAGGATCATTC CTAGGAAGAGACCAATTATATAATACTCTAGTTACTGCACACGCATTTTTAATAATTTTCTTTTTAGTTATA CCAGTATTTATTGGGGGATTTGGTAATTGACTAATTCCTCTAATACTAGGTGCACCAGACATAGCATTTCC TCGACTTAATAATCTAAGATTTTGACTATTACCACCATCATTAATTCTATTAATTTCCTCTGCAGCAGTAGA AAAAGGAGCTGGAACTGGGTGAACAGTATATCCACCACTCTCAAGAAACCTGGCACATGCTGGACCTTCT GTAGACTTAGCTATTTTCTCCTTACATCTTGCAGGTGCATCATCTATTCTAGGAGCACTAAACTTCATTACT ACTGTAATTAATATACGATGAAATGGGATAAAACTAGAACGACTTCCATTATTCGTATGAGCAGTGTTATT AACAGTAATTCTACTTCTCTTATCACTTCCAGTACTTGCTGGGGCAATTACCATACTATTAACAGACCGTA ACCTAAATACTTCATTCTTCGACCCTGCAGGAGGAGGTGATCCGATCTTATACCAAC

Appendix 2-12. Neighbor-joining distance matrix (snail subset). Groups with 5% or higher p-distances (i.e., branch length of 0.05 or higher) were considered different, these values are highlighted in bold. My samples are also colour coded to reflect the colour scheme in Figure 2-17. A total of 24 CO1 sequences came from my samples, and 27 sequences from GenBank, See Methods in Chapter 2 for further details.

	Snail ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	VAF-29S	Lafarge																	
2	VAF-14S	Lafarge	0.0018																
3	VAF-12S	Lafarge	0.0018	0.0000															
4	VAF-10S	Lafarge	0.0018	0.0000	0.0000														
5	KM612060	*Physella ancillaria*	0.0018	0.0000	0.0000	0.0000													
6	VAF-11S	Lafarge	0.0018	0.0000	0.0000	0.0000	0.0000												
7	VAF-13S	Lafarge	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000											
8	VAF-31S	Lafarge	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000										
9	MG422145	*Physella ancillaria*	0.0035	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018									
10	MG422342	*Physella ancillaria*	0.0035	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000								
11	MG422937	*Physella ancillaria*	0.0035	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000							
12	MG423475	*Physella ancillaria*	0.0035	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000	0.0000						
13	MG421540	*Physella ancillaria*	0.0035	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000	0.0000	0.0000					
14	VAF-3S	Morinville	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053				
15	VAF-2S	Morinville	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053	0.0000			
16	VAF-6S	Morinville	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070		
17	VAF-5S	Morinville	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0000	
18	AF346745	*Physella wrighti*	0.0088	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0088	0.0088	0.0088	0.0088	0.0088	0.0105	0.0105	0.0105	0.0105
19	AF419323	*Physella wrighti*	0.0088	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0088	0.0088	0.0088	0.0088	0.0088	0.0105	0.0105	0.0105	0.0105
20	MG421380	*Physella ancillaria*	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0070	0.0070
21	MG421606	*Physella ancillaria*	0.0053	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0035	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0070	0.0070
22	VAF-28S	Lafarge	0.0070	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0070	0.0070	0.0070	0.0053	0.0053	0.0088	0.0088
23	VAF-26S	Lafarge	0.0088	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0070	0.0088	0.0088	0.0088	0.0088	0.0088	0.0070	0.0070	0.0105	0.0105
24	VAF-27S	Lafarge	0.0070	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0070	0.0070	0.0070	0.0053	0.0053	0.0088	0.0088
25	VAF-9S	Lafarge	0.0070	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0070	0.0070	0.0070	0.0070	0.0070	0.0053	0.0053	0.0088	0.0088
26	AF346741	*Physella gyrina*	0.0123	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0123	0.0123	0.0123	0.0123	0.0123	0.0141	0.0141	0.0141	0.0141
27	KT831388	*Physella gyrina*	0.0123	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0123	0.0123	0.0123	0.0123	0.0123	0.0141	0.0141	0.0141	0.0141
28	MG421410	*Physella ancillaria*	0.0141	0.0123	0.0123	0.0123	0.0123	0.0123	0.0123	0.0123	0.0141	0.0141	0.0141	0.0141	0.0141	0.0158	0.0158	0.0158	0.0158
29	VAF-30S	Lafarge	0.0123	0.0140	0.0140	0.0140	0.0141	0.0140	0.0140	0.0140	0.0158	0.0158	0.0158	0.0158	0.0158	0.0175	0.0175	0.0175	0.0175
30	AY651200	*Physella gyrina*	0.0123	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0123	0.0123	0.0123	0.0123	0.0123	0.0141	0.0141	0.0141	0.0141
31	MK308008	*Physella gyrina*	0.0123	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0105	0.0123	0.0123	0.0123	0.0123	0.0123	0.0141	0.0141	0.0141	0.0141
32	AY651179	*Physella wolfiana*	0.0105	0.0088	0.0088	0.0088	0.0088	0.0088	0.0088	0.0088	0.0105	0.0105	0.0105	0.0105	0.0105	0.0123	0.0123	0.0123	0.0123
33	GU680874	*Physa jennessi*	**0.1353**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1353**	**0.1353**	**0.1336**	**0.1336**
34	VAF-8S	Lafarge	**0.1368**	**0.1351**	**0.1351**	**0.1351**	**0.1353**	**0.1351**	**0.1351**	**0.1351**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1368**	**0.1368**	**0.1351**	**0.1351**
35	GU680899	*Physa jennessi*	**0.1353**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1353**	**0.1353**	**0.1336**	**0.1336**
36	VAF-7S	Lafarge	**0.1351**	**0.1333**	**0.1333**	**0.1333**	**0.1336**	**0.1333**	**0.1333**	**0.1333**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1318**	**0.1351**	**0.1351**	**0.1333**	**0.1333**
37	VAF-49S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
38	VAF-48S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
39	VAF-47S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
40	VAF-46S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
41	VAF-45S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
42	VAF-44S	Lab bred	**0.1632**	**0.1614**	**0.1614**	**0.1614**	**0.1617**	**0.1614**	**0.1614**	**0.1614**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1614**	**0.1614**	**0.1579**	**0.1579**
43	KM206699	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
44	OP566899	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
45	MZ798294	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
46	KF737921	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
47	OM970095	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
48	KP182986	*Physella acuta*	**0.1617**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1599**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1582**	**0.1599**	**0.1599**	**0.1564**	**0.1564**
49	KM611811	*Aplexa elongata*	**0.1757**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1775**	**0.1775**	**0.1740**	**0.1740**
50	KM612034	*Aplexa elongata*	**0.1740**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1705**	**0.1705**	**0.1705**	**0.1705**	**0.1705**	**0.1757**	**0.1757**	**0.1722**	**0.1722**
51	MG421227	*Aplexa elongata*	**0.1757**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1740**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1722**	**0.1775**	**0.1775**	**0.1740**	**0.1740**

	Snail ID		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	VAF-29S	Lafarge																	
2	VAF-14S	Lafarge																	
3	VAF-12S	Lafarge																	
4	VAF-10S	Lafarge																	
5	KM612060	*Physella ancillaria*																	
6	VAF-11S	Lafarge																	
7	VAF-13S	Lafarge																	
8	VAF-31S	Lafarge																	
9	MG422145	*Physella ancillaria*																	
10	MG422342	*Physella ancillaria*																	
11	MG422937	*Physella ancillaria*																	
12	MG423475	*Physella ancillaria*																	
13	MG421540	*Physella ancillaria*																	
14	VAF-3S	Morinville																	
15	VAF-2S	Morinville																	
16	VAF-6S	Morinville																	
17	VAF-5S	Morinville																	
18	AF346745	*Physella wrighti*																	
19	AF419323	*Physella wrighti*	0.0035																
20	MG421380	*Physella ancillaria*	0.0035	0.0035															
21	MG421606	*Physella ancillaria*	0.0035	0.0035	0.0000														
22	VAF-28S	Lafarge	0.0053	0.0053	0.0018	0.0018													
23	VAF-26S	Lafarge	0.0070	0.0070	0.0035	0.0035	0.0018												
24	VAF-27S	Lafarge	0.0053	0.0053	0.0018	0.0018	0.0000	0.0018											
25	VAF-9S	Lafarge	0.0053	0.0053	0.0018	0.0018	0.0000	0.0018	0.0000										
26	AF346741	*Physella gyrina*	0.0141	0.0141	0.0105	0.0105	0.0123	0.0141	0.0123	0.0123									
27	KT831388	*Physella gyrina*	0.0141	0.0141	0.0105	0.0105	0.0123	0.0141	0.0123	0.0123	0.0000								
28	MG421410	*Physella ancillaria*	0.0158	0.0158	0.0123	0.0123	0.0141	0.0158	0.0141	0.0141	0.0018	0.0018							
29	VAF-30S	Lafarge	0.0176	0.0176	0.0141	0.0141	0.0158	0.0175	0.0158	0.0158	0.0035	0.0035	0.0053						
30	AY651200	*Physella gyrina*	0.0141	0.0141	0.0105	0.0105	0.0123	0.0141	0.0123	0.0123	0.0035	0.0035	0.0053	0.0070					
31	MK308008	*Physella gyrina*	0.0141	0.0141	0.0105	0.0105	0.0123	0.0141	0.0123	0.0123	0.0035	0.0035	0.0053	0.0070	0.0035				
32	AY651179	*Physella wolfiana*	0.0123	0.0123	0.0088	0.0088	0.0105	0.0123	0.0105	0.0105	0.0018	0.0018	0.0035	0.0053	0.0053	0.0053			
33	GU680874	*Physa jennessi*	**0.1371**	**0.1371**	**0.1336**	**0.1336**	**0.1353**	**0.1371**	**0.1353**	**0.1353**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1371**	**0.1353**		
34	VAF-8S	Lafarge	**0.1388**	**0.1388**	**0.1353**	**0.1353**	**0.1368**	**0.1386**	**0.1368**	**0.1368**	**0.1353**	**0.1353**	**0.1353**	**0.1351**	**0.1353**	**0.1388**	**0.1371**	0.0018	
35	GU680899	*Physa jennessi*	**0.1371**	**0.1371**	**0.1336**	**0.1336**	**0.1353**	**0.1371**	**0.1353**	**0.1353**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1336**	**0.1371**	**0.1353**	0.0000	0.0018
36	VAF-7S	Lafarge	**0.1371**	**0.1371**	**0.1336**	**0.1336**	**0.1351**	**0.1368**	**0.1351**	**0.1351**	**0.1336**	**0.1336**	**0.1336**	**0.1333**	**0.1336**	**0.1371**	**0.1353**	0.0000	0.0018
37	VAF-49S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
38	VAF-48S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
39	VAF-47S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
40	VAF-46S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
41	VAF-45S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
42	VAF-44S	Lab bred	**0.1634**	**0.1652**	**0.1617**	**0.1617**	**0.1632**	**0.1649**	**0.1632**	**0.1632**	**0.1617**	**0.1617**	**0.1599**	**0.1649**	**0.1652**	**0.1652**	**0.1599**	**0.1757**	**0.1772**
43	KM206699	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
44	OP566899	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
45	MZ798294	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
46	KF737921	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
47	OM970095	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
48	KP182986	*Physella acuta*	**0.1617**	**0.1634**	**0.1599**	**0.1599**	**0.1617**	**0.1634**	**0.1617**	**0.1617**	**0.1599**	**0.1599**	**0.1582**	**0.1634**	**0.1634**	**0.1634**	**0.1582**	**0.1740**	**0.1757**
49	KM611811	*Aplexa elongata*	**0.1722**	**0.1722**	**0.1705**	**0.1705**	**0.1722**	**0.1740**	**0.1722**	**0.1722**	**0.1705**	**0.1705**	**0.1722**	**0.1705**	**0.1740**	**0.1722**	**0.1687**	**0.1757**	**0.1775**
50	KM612034	*Aplexa elongata*	**0.1705**	**0.1705**	**0.1687**	**0.1687**	**0.1705**	**0.1722**	**0.1705**	**0.1705**	**0.1687**	**0.1687**	**0.1705**	**0.1687**	**0.1722**	**0.1705**	**0.1670**	**0.1740**	**0.1757**
51	MG421227	*Aplexa elongata*	**0.1722**	**0.1722**	**0.1705**	**0.1705**	**0.1722**	**0.1740**	**0.1722**	**0.1722**	**0.1705**	**0.1705**	**0.1722**	**0.1705**	**0.1740**	**0.1722**	**0.1687**	**0.1757**	**0.1775**

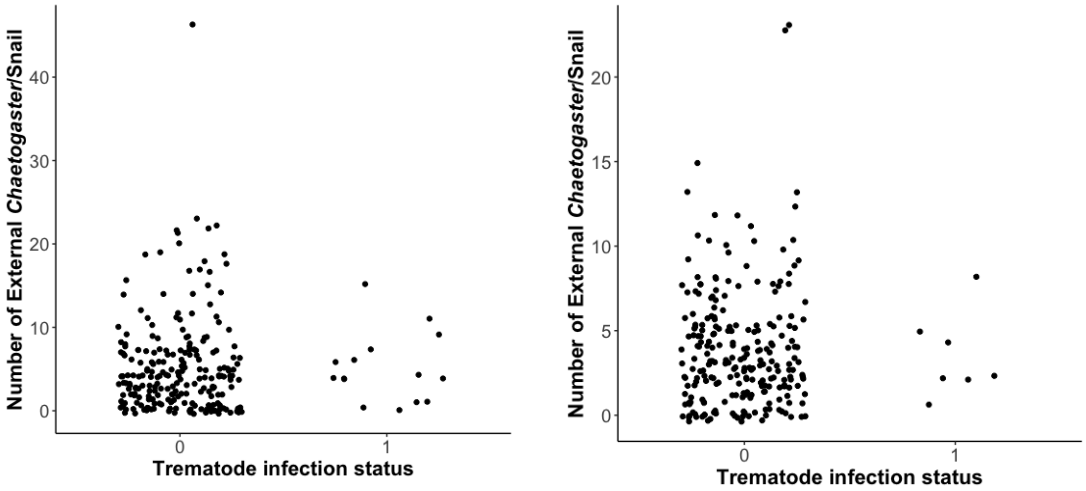
	Snail ID		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	VAF-29S	Lafarge																	
2	VAF-14S	Lafarge																	
3	VAF-12S	Lafarge																	
4	VAF-10S	Lafarge																	
5	KM612060	*Physella ancillaria*																	
6	VAF-11S	Lafarge																	
7	VAF-13S	Lafarge																	
8	VAF-31S	Lafarge																	
9	MG422145	*Physella ancillaria*																	
10	MG422342	*Physella ancillaria*																	
11	MG422937	*Physella ancillaria*																	
12	MG423475	*Physella ancillaria*																	
13	MG421540	*Physella ancillaria*																	
14	VAF-3S	Morinville																	
15	VAF-2S	Morinville																	
16	VAF-6S	Morinville																	
17	VAF-5S	Morinville																	
18	AF346745	*Physella wrighti*																	
19	AF419323	*Physella wrighti*																	
20	MG421380	*Physella ancillaria*																	
21	MG421606	*Physella ancillaria*																	
22	VAF-28S	Lafarge																	
23	VAF-26S	Lafarge																	
24	VAF-27S	Lafarge																	
25	VAF-9S	Lafarge																	
26	AF346741	*Physella gyrina*																	
27	KT831388	*Physella gyrina*																	
28	MG421410	*Physella ancillaria*																	
29	VAF-30S	Lafarge																	
30	AY651200	*Physella gyrina*																	
31	MK308008	*Physella gyrina*																	
32	AY651179	*Physella wolfiana*																	
33	GU680874	*Physa jennessi*																	
34	VAF-8S	Lafarge																	
35	GU680899	*Physa jennessi*																	
36	VAF-7S	Lafarge	0.0000																
37	VAF-49S	Lab bred	**0.1757**	**0.1754**															
38	VAF-48S	Lab bred	**0.1757**	**0.1754**	0.0000														
39	VAF-47S	Lab bred	**0.1757**	**0.1754**	0.0000	0.0000													
40	VAF-46S	Lab bred	**0.1757**	**0.1754**	0.0000	0.0000	0.0000												
41	VAF-45S	Lab bred	**0.1757**	**0.1754**	0.0000	0.0000	0.0000	0.0000											
42	VAF-44S	Lab bred	**0.1757**	**0.1754**	0.0000	0.0000	0.0000	0.0000	0.0000										
43	KM206699	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018									
44	OP566899	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000								
45	MZ798294	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000							
46	KF737921	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000	0.0000						
47	OM970095	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000	0.0000	0.0000					
48	KP182986	*Physella acuta*	**0.1740**	**0.1740**	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000	0.0000	0.0000	0.0000				
49	KM611811	*Aplexa elongata*	**0.1757**	**0.1757**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**	**0.1828**			
50	KM612034	*Aplexa elongata*	**0.1740**	**0.1740**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	**0.1810**	0.0018		
51	MG421227	*Aplexa elongata*	**0.1757**	**0.1757**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	**0.1863**	0.0070	0.0053	

Appendix 2-13. Neighbor-joining distance matrix (*Chaetogaster* subset). Groups with 5% or higher p-distances (i.e., branch length of 0.05 or higher) were considered different, these values are highlighted in bold. My samples are also colour coded to reflect the colour scheme in Figure 2-18. A total of 17 CO1 sequences came from my samples, and 19 sequences from GenBank, See Methods in Chapter 2 for further details.

	Worm ID		1	2	3	4	5	6	7	8	9	10	11	12
1	VAF-51W	Morinville												
2	VAF-50W	Morinville	0.0000											
3	KF952333	*Chaetogaster limnaei*	0.0000	0.0000										
4	VAF-40W	Morinville	0.0000	0.0000	0.0000									
5	KF952303	*Chaetogaster limnaei*	0.0000	0.0000	0.0000	0.0000								
6	VAF-36W	Lafarge	0.0000	0.0000	0.0000	0.0000	0.0000							
7	KF952346	*Chaetogaster limnaei*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000						
8	VAF-33W	Lafarge	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000					
9	KF952326	*Chaetogaster limnaei*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
10	VAF-52W	Morinville	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000			
11	KF952298	*Chaetogaster limnaei*	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018		
12	KF952311	*Chaetogaster limnaei*	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	
13	OQ281726	*Chaetogaster* sp. 22	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0000	0.0000
14	OQ281711	*Chaetogaster* sp. 22	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0018	0.0018
15	VAF-20W	Lafarge	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0018	0.0018
16	VAF-19W	Lafarge	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0018	0.0018
17	KF952323	*Chaetogaster limnaei*	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
18	KF952340	*Chaetogaster limnaei*	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
19	KF952313	*Chaetogaster limnaei*	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
20	KF952300	*Chaetogaster limnaei*	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
21	KF952309	*Chaetogaster limnaei*	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
22	OQ281729	*Chaetogaster* sp. 22	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0269	0.0269
23	VAF-43W	Morinville (Internal)	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
24	VAF-41W	Morinville (Internal)	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305	0.0287	0.0287
25	OQ281712	*Chaetogaster* sp. 22	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0287	0.0269	0.0269
26	OQ281710	*Chaetogaster* sp. 22	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
27	VAF-39W	Morinville	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
28	VAF-38W	Morinville	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
29	VAF-37W	Lafarge	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
30	VAF-35W	Lafarge	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
31	VAF-34W	Lafarge	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
32	VAF-24W	Morinville	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
33	VAF-22W	Lafarge	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0180	0.0162	0.0162
34	KF952336	*Chaetogaster limnaei*	0.0251	0.0251	0.0251	0.0251	0.0251	0.0251	0.0251	0.0251	0.0251	0.0251	0.0233	0.0233
35	JQ519897	*Chaetogaster diaphanus*	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1670**	**0.1652**	**0.1652**
36	LN810268	*Chaetogaster diaphanus*	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1706**	**0.1724**	**0.1724**

	Worm ID		13	14	15	16	17	18	19	20	21	22	23	24
1	VAF-51W	Morinville												
2	VAF-50W	Morinville												
3	KF952333	*Chaetogaster limnaei*												
4	VAF-40W	Morinville												
5	KF952303	*Chaetogaster limnaei*												
6	VAF-36W	Lafarge												
7	KF952346	*Chaetogaster limnaei*												
8	VAF-33W	Lafarge												
9	KF952326	*Chaetogaster limnaei*												
10	VAF-52W	Morinville												
11	KF952298	*Chaetogaster limnaei*												
12	KF952311	*Chaetogaster limnaei*												
13	OQ281726	*Chaetogaster* sp. 22												
14	OQ281711	*Chaetogaster* sp. 22	0.0018											
15	VAF-20W	Lafarge	0.0018	0.0036										
16	VAF-19W	Lafarge	0.0018	0.0036	0.0000									
17	KF952323	*Chaetogaster limnaei*	0.0287	0.0305	0.0305	0.0305								
18	KF952340	*Chaetogaster limnaei*	0.0287	0.0305	0.0305	0.0305	0.0000							
19	KF952313	*Chaetogaster limnaei*	0.0287	0.0305	0.0305	0.0305	0.0000	0.0000						
20	KF952300	*Chaetogaster limnaei*	0.0287	0.0305	0.0305	0.0305	0.0000	0.0000	0.0000					
21	KF952309	*Chaetogaster limnaei*	0.0287	0.0305	0.0305	0.0305	0.0000	0.0000	0.0000	0.0000				
22	OQ281729	*Chaetogaster* sp. 22	0.0269	0.0287	0.0287	0.0287	0.0018	0.0018	0.0018	0.0018	0.0018			
23	VAF-43W	Morinville (Internal)	0.0287	0.0305	0.0305	0.0305	0.0144	0.0144	0.0144	0.0144	0.0144	0.0126		
24	VAF-41W	Morinville (Internal)	0.0287	0.0305	0.0305	0.0305	0.0144	0.0144	0.0144	0.0144	0.0144	0.0126	0.0000	
25	OQ281712	*Chaetogaster* sp. 22	0.0269	0.0287	0.0287	0.0287	0.0126	0.0126	0.0126	0.0126	0.0126	0.0108	0.0018	0.0018
26	OQ281710	*Chaetogaster* sp. 22	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0341	0.0341
27	VAF-39W	Morinville	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
28	VAF-38W	Morinville	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
29	VAF-37W	Lafarge	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
30	VAF-35W	Lafarge	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
31	VAF-34W	Lafarge	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
32	VAF-24W	Morinville	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
33	VAF-22W	Lafarge	0.0162	0.0180	0.0180	0.0180	0.0341	0.0341	0.0341	0.0341	0.0341	0.0323	0.0377	0.0377
34	KF952336	*Chaetogaster limnaei*	0.0233	0.0251	0.0251	0.0251	0.0413	0.0413	0.0413	0.0413	0.0413	0.0395	0.0449	0.0449
35	JQ519897	*Chaetogaster diaphanus*	**0.1652**	**0.1670**	**0.1652**	**0.1652**	**0.1741**	**0.1741**	**0.1741**	**0.1741**	**0.1741**	**0.1741**	**0.1795**	**0.1795**
36	LN810268	*Chaetogaster diaphanus*	**0.1724**	**0.1741**	**0.1724**	**0.1724**	**0.1813**	**0.1813**	**0.1813**	**0.1813**	**0.1813**	**0.1813**	**0.1867**	**0.1867**

	Worm ID		25	26	27	28	29	30	31	32	33	34	35	36
1	VAF-51W	Morinville												
2	VAF-50W	Morinville												
3	KF952333	*Chaetogaster limnaei*												
4	VAF-40W	Morinville												
5	KF952303	*Chaetogaster limnaei*												
6	VAF-36W	Lafarge												
7	KF952346	*Chaetogaster limnaei*												
8	VAF-33W	Lafarge												
9	KF952326	*Chaetogaster limnaei*												
10	VAF-52W	Morinville												
11	KF952298	*Chaetogaster limnaei*												
12	KF952311	*Chaetogaster limnaei*												
13	OQ281726	*Chaetogaster* sp. 22												
14	OQ281711	*Chaetogaster* sp. 22												
15	VAF-20W	Lafarge												
16	VAF-19W	Lafarge												
17	KF952323	*Chaetogaster limnaei*												
18	KF952340	*Chaetogaster limnaei*												
19	KF952313	*Chaetogaster limnaei*												
20	KF952300	*Chaetogaster limnaei*												
21	KF952309	*Chaetogaster limnaei*												
22	OQ281729	*Chaetogaster* sp. 22												
23	VAF-43W	Morinville (Internal)												
24	VAF-41W	Morinville (Internal)												
25	OQ281712	*Chaetogaster* sp. 22												
26	OQ281710	*Chaetogaster* sp. 22	0.0323											
27	VAF-39W	Morinville	0.0359	0.0251										
28	VAF-38W	Morinville	0.0359	0.0251	0.0000									
29	VAF-37W	Lafarge	0.0359	0.0251	0.0000	0.0000								
30	VAF-35W	Lafarge	0.0359	0.0251	0.0000	0.0000	0.0000							
31	VAF-34W	Lafarge	0.0359	0.0251	0.0000	0.0000	0.0000	0.0000						
32	VAF-24W	Morinville	0.0359	0.0251	0.0000	0.0000	0.0000	0.0000	0.0000					
33	VAF-22W	Lafarge	0.0359	0.0251	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
34	KF952336	*Chaetogaster limnaei*	0.0431	0.0305	0.0072	0.0072	0.0072	0.0072	0.0072	0.0072	0.0072			
35	JQ519897	*Chaetogaster diaphanus*	**0.1777**	**0.1598**	**0.1526**	**0.1526**	**0.1526**	**0.1526**	**0.1526**	**0.1526**	**0.1526**	**0.1490**		
36	LN810268	*Chaetogaster diaphanus*	**0.1849**	**0.1670**	**0.1580**	**0.1580**	**0.1580**	**0.1580**	**0.1580**	**0.1580**	**0.1580**	**0.1544**	0.0108	



Appendix 2-14. The number of *Chaetogaster*/snail according to trematode infection status. Trematode infection (sporocysts/rediae) is denoted at as present (1) or absent (1). Graph on the left displays results from snails collected at Lafarge in 2022 (n = 241) while the graph on the right displays results from Morinville collected in 2022 (n = 243). Data has been jittered to allow increase readability. See Chapter 2 for more details on the collection and dissection of the snails.

Chapter 3. Appendices

Appendix 3-1. Test of snail length measurements repeatability in ImageJ. A total of 15 snails were photographed four days apart and then measured in ImageJ. The average difference in size (disregarding direction of change) was 0.832 mm in length.

Snail	Length on Aug 31st, 2022 (mm)	Length on Sept 3rd, 2022 (mm)	Total Change (mm)	Total Change (mm) (absolute value)
1	6.159	5.394	-0.765	0.765
2	4.284	6.097	1.813	1.813
3	5.267	6.756	1.489	1.489
4	8.347	6.855	-1.492	1.492
5	5.023	6.075	1.052	1.052
6	5.512	5.049	-0.463	0.463
7	5.867	6.042	0.175	0.175
8	6.501	7.556	1.055	1.055
9	4.994	4.423	-0.571	0.571
10	4.806	5.519	0.713	0.713
11	4.325	4.133	-0.192	0.192
12	5.459	4.156	-1.303	1.303
13	5.403	5.047	-0.356	0.356
14	5.56	5.427	-0.133	0.133
15	4.712	3.8	-0.912	0.912

Appendix 3-2. Change in length of snails used in Fitness Experiment 1 (original data). Ignoring direction of change, the average difference in snail length from start to finish is 0.476 mm. Excluding the snails that died early in the experiment (given a value of NA), a total of 26 snails with *Chaetogaster* were measured along with 29 snails without *Chaetogaster*.

Snail ID	**Start Length (mm)**	**End Length (mm)**	**Total Change (mm)**
Chaetogaster(+)			
1	6.074	NA	NA
2	5.337	6.945	1.608
3	5.66	4.812	-0.848
4	5.47	5.478	0.008
5	5.271	5.198	-0.073
6	6.279	6.066	-0.213
7	6.467	6.161	-0.306
8	5.875	5.800	-0.075
9	3.528	3.963	0.435
10	4.208	4.461	0.253
11	5.499	5.324	-0.175
12	5.233	5.728	0.495
13	5.398	NA	NA
14	5.185	4.925	-0.26
15	4.167	4.323	0.156
16	5.641	5.504	-0.137
17	5.769	6.311	0.542
18	3.953	4.254	0.301
19	3.522	3.904	0.382
20	4.895	4.946	0.051
21	3.39	3.801	0.411
22	3.912	4.810	0.898
23	5.633	6.384	0.751
24	7.709	6.959	-0.75
25	6.739	6.902	0.163
26	4.224	5.097	0.873
27	3.677	4.422	0.745
28	4.393	4.369	-0.024
Chaetogaster(-)			
29	6.73	NA	NA
30	5.744	5.718	-0.026
31	7.615	7.968	0.353
32	7.44	6.314	-1.126
33	5.374	6.312	0.938

34	5.041	5.410	0.369
35	5.979	5.110	-0.869
36	5.237	5.205	-0.032
37	4.915	4.865	-0.05
38	5.119	5.358	0.239
39	5.419	4.260	-1.159
40	6.906	6.754	-0.152
41	6.417	6.761	0.344
42	5.067	4.525	-0.542
43	5.157	5.484	0.327
44	5.324	5.537	0.213
45	5.116	4.708	-0.408
46	5.099	4.335	-0.764
47	4.3	4.100	-0.2
48	5.701	5.984	0.283
49	5.306	5.712	0.406
50	5.526	5.143	-0.383
51	5.293	5.765	0.472
52	5.289	4.561	-0.728
53	5.228	5.611	0.383
54	5.96	5.309	-0.651
55	5.404	6.060	0.656
56	4.343	5.348	1.005
57	6.24	4.972	-1.268
58	4.195	5.099	0.904

Appendix 3-3. Egg production by lab-bred snails in Fitness Experiment 1. Raw data including information on each of the 58 snails tested (original treatment, status at the end of experiment and initial shell length), and well as number of eggs laid each week and whether the data from that snail was included in the analysis. Treatments include *Chaetogaster*(-) as 'C(-)' and *Chaetogaster*(+) and 'C(+)'. End status considers *Chaetogaster*(-) as 'C(-)', kept *Chaetogaster* as 'KC' and lost *Chaetogaster* as 'LC'. Columns labelled 1-6 relate to weeks 1-6 of the experiment respectively. A zero in egg production indicates that the snail was alive but produced no eggs, while an 'NA' indicates that the snail was dead in that week. Please see the Methods section of Chapter 3 for more details on this experiment.

Snail ID	Treatment	End Status	Snail Length (mm)	Week						Analysis
				1	2	3	4	5	6	
1	C(-)	C(-)	6.73	NA	NA	NA	NA	NA	NA	exclude
2	C(-)	C(-)	5.744	0	0	3	0	7	21	include
3	C(-)	C(-)	7.615	15	25	0	8	22	12	include
4	C(-)	C(-)	7.44	0	0	13	0	0	0	include
5	C(-)	C(-)	5.374	0	0	0	0	0	3	include
6	C(-)	C(-)	5.041	0	0	0	0	8	30	include
7	C(-)	C(-)	5.979	0	0	14	0	11	32	include
8	C(-)	C(-)	5.237	0	5	0	0	20	12	include
9	C(-)	C(-)	4.915	0	0	11	0	31	8	include
10	C(-)	C(-)	5.119	0	0	19	0	10	16	include
11	C(-)	C(-)	5.419	0	0	0	0	0	0	include
12	C(-)	C(-)	6.906	0	0	0	0	0	19	include
13	C(-)	C(-)	6.417	15	27	8	0	3	11	include
14	C(-)	C(-)	5.067	0	0	11	0	6	11	include
15	C(-)	C(-)	5.157	5	0	24	4	0	3	include
16	C(-)	C(-)	5.324	0	0	18	0	0	7	include
17	C(-)	C(-)	5.116	0	0	11	0	0	3	include
18	C(-)	C(-)	5.099	0	0	0	0	0	0	include
19	C(-)	C(-)	4.3	0	0	0	0	0	0	include
20	C(-)	C(-)	5.701	0	14	4	0	20	38	include
21	C(-)	C(-)	5.306	0	15	0	0	0	13	include
22	C(-)	C(-)	5.526	0	23	10	0	3	8	include
23	C(-)	C(-)	5.293	7	40	4	0	0	24	include
24	C(-)	C(-)	5.289	0	6	0	0	0	0	include
25	C(-)	C(-)	5.228	0	0	0	24	0	4	include
26	C(-)	C(-)	5.96	0	0	0	NA	NA	NA	include
27	C(-)	C(-)	5.404	0	8	0	2	0	0	include
28	C(-)	C(-)	4.343	13	9	0	0	0	1	include
29	C(-)	C(-)	6.24	0	0	15	1	0	7	include
30	C(-)	C(-)	4.195	0	0	0	0	0	0	include
31	C(+)	KC	6.074	39	0	NA	NA	NA	NA	exclude
32	C(+)	KC	5.337	0	14	0	0	3	5	include
33	C(+)	KC	5.66	7	7	5	0	0	0	include
34	C(+)	LC	5.47	0	0	0	0	0	0	include

35	C(+)	KC	5.271	24	0	22	0	NA	NA	include
36	C(+)	LC	6.279	39	0	0	NA	NA	NA	include
37	C(+)	KC	6.467	55	18	NA	NA	NA	NA	exclude
38	C(+)	KC	5.875	7	31	0	2	0	2	include
39	C(+)	LC	3.528	0	0	0	0	0	0	include
40	C(+)	LC	4.208	0	0	0	0	0	0	include
41	C(+)	KC	5.499	0	0	0	NA	NA	NA	include
42	C(+)	LC	5.233	0	0	0	9	0	0	include
43	C(+)	LC	5.398	NA	NA	NA	NA	NA	NA	exclude
44	C(+)	LC	5.185	0	0	0	0	6	0	include
45	C(+)	LC	4.167	0	0	0	0	0	0	include
46	C(+)	LC	5.641	0	0	0	0	0	NA	include
47	C(+)	LC	5.769	0	0	3	0	0	0	include
48	C(+)	LC	3.953	0	0	0	0	0	0	include
49	C(+)	LC	3.522	7	20	23	18	0	5	include
50	C(+)	KC	4.895	0	0	NA	NA	NA	NA	exclude
51	C(+)	LC	3.39	0	0	0	0	0	0	include
52	C(+)	LC	3.912	0	14	0	0	3	14	include
53	C(+)	LC	5.633	0	0	NA	NA	NA	NA	exclude
54	C(+)	KC	7.709	18	0	0	0	0	0	include
55	C(+)	KC	6.739	36	0	0	0	12	0	include
56	C(+)	LC	4.224	0	0	0	2	0	13	include
57	C(+)	LC	3.677	0	0	0	0	0	0	include
58	C(+)	KC	4.393	0	0	0	0	0	0	include

Appendix 3-4. Egg production by lab-bred snails in Fitness Experiment 2. Raw data including information on each of the 70 snails tested (original treatment, status at the end of experiment and initial shell length), and well as number of eggs laid each week and whether the data from that snail was included in the analysis. Treatments include *Chaetogaster*(-) as 'C(-)' and *Chaetogaster*(+) and 'C(+)'. End status considers *Chaetogaster*(-) as 'C(-)', kept *Chaetogaster* as 'KC' and lost *Chaetogaster* as 'LC'. Columns labelled 1-6 relate to weeks 1-6 of the experiment respectively. A zero in egg production indicates that the snail was alive but produced no eggs, while an 'NA' indicates that the snail was dead in that week. Please see the Methods section of Chapter 3 for more details on this experiment.

Snail ID	**Treatment**	**End Status**	**Snail Length (mm)**	**Week**						**Analysis**
				1	**2**	**3**	**4**	**5**	**6**	
1	C(-)	C(-)	5.353	0	0	0	0	15	40	include
2	C(-)	C(-)	5.438	19	26	61	23	22	33	include
3	C(-)	C(-)	5.389	0	0	0	0	0	0	include
4	C(-)	C(-)	5.972	13	62	81	38	13	36	include
5	C(-)	C(-)	4.474	0	0	0	0	0	0	include
6	C(-)	C(-)	5.53	10	37	73	34	0	13	include
7	C(-)	C(-)	5.764	0	0	0	0	0	0	include
8	C(-)	C(-)	6.527	0	0	0	0	0	0	include
9	C(-)	C(-)	5.948	0	0	0	0	0	0	include
10	C(-)	C(-)	5.822	27	28	49	25	24	15	include
11	C(-)	C(-)	6.166	9	38	69	59	19	41	include
12	C(-)	C(-)	5.198	0	0	0	0	0	1	include
13	C(-)	C(-)	6.711	23	36	62	60	19	68	include
14	C(-)	C(-)	6.481	0	20	44	16	24	14	include
15	C(-)	C(-)	5.891	15	38	47	48	44	20	include
16	C(-)	C(-)	7.29	0	18	22	16	8	0	include
17	C(-)	C(-)	6.099	0	0	0	0	0	0	include
18	C(-)	C(-)	6.574	0	0	0	0	0	0	include
19	C(-)	C(-)	5.314	9	22	53	39	31	40	include
20	C(-)	C(-)	6.365	25	9	80	68	18	17	include
21	C(-)	C(-)	6.01	14	50	57	55	15	23	include
22	C(-)	C(-)	6.974	0	13	90	72	6	26	include
23	C(-)	C(-)	5.071	5	7	33	36	27	15	include
24	C(-)	C(-)	6.783	7	11	29	49	22	0	include
25	C(-)	C(-)	5.039	0	0	0	0	0	0	include
26	C(-)	C(-)	4.144	0	0	0	0	0	0	include
27	C(-)	C(-)	5.81	21	28	60	106	24	34	include
28	C(-)	C(-)	5.36	0	0	0	0	0	0	include
29	C(-)	C(-)	6.279	0	0	0	0	0	0	include
30	C(-)	C(-)	5.324	0	0	0	NA	NA	NA	exclude
31	C(+)	LC	7.138	0	33	0	NA	NA	NA	include
32	C(+)	KC	5.861	0	0	0	0	0	0	include
33	C(+)	KC	5.777	0	4	45	44	22	35	include
34	C(+)	KC	5.534	0	0	0	0	0	0	include

35	C(+)	LC	5.579	5	0	25	25	10	12	include
36	C(+)	KC	6.132	8	5	38	42	39	21	include
37	C(+)	KC	5.55	0	0	0	0	0	0	include
38	C(+)	KC	5.709	0	0	28	60	15	11	include
39	C(+)	LC	6.335	0	0	0	0	0	0	include
40	C(+)	LC	5.95	0	0	0	0	0	0	include
41	C(+)	LC	6.088	0	0	46	39	46	16	include
42	C(+)	LC	4.392	0	0	28	8	0	1	include
43	C(+)	KC	5.54	0	0	0	38	6	1	include
44	C(+)	KC	6.031	0	0	0	15	7	9	include
45	C(+)	LC	6.874	8	17	98	NA	NA	NA	include
46	C(+)	KC	5.912	0	0	0	0	0	0	include
47	C(+)	LC	5.7	0	0	0	0	33	42	include
48	C(+)	LC	6.521	0	5	33	50	29	31	include
49	C(+)	KC	5.041	0	3	20	30	13	4	include
50	C(+)	LC	7.77	0	10	0	56	2	23	include
51	C(+)	KC	6.204	10	14	0	43	21	29	include
52	C(+)	KC	7.334	0	47	70	67	46	25	include
53	C(+)	LC	5.147	0	0	0	0	0	0	include
54	C(+)	KC	5.371	0	0	0	0	0	0	include
55	C(+)	LC	5.126	6	9	19	NA	NA	NA	include
56	C(+)	LC	5.112	0	0	0	0	0	0	include
57	C(+)	LC	4.804	0	0	43	87	17	27	include
58	C(+)	LC	5.202	0	0	0	0	0	0	include
59	C(+)	KC	5.254	0	0	0	0	0	0	include
60	C(+)	LC	4.796	0	0	0	0	NA	NA	include
61	C(+)	KC	5.445	0	0	0	0	0	0	include
62	C(+)	KC	5.123	0	0	0	0	0	0	include
63	C(+)	LC	4.785	0	0	0	0	0	0	include
64	C(+)	LC	5.495	0	0	0	NA	NA	NA	include
65	C(+)	LC	5.202	0	0	0	0	0	0	include
66	C(+)	KC	4.975	0	0	0	0	0	0	include
67	C(+)	KC	5.969	0	0	37	42	11	3	include
68	C(+)	KC	5.314	0	21	102	42	14	7	include
69	C(+)	KC	4.188	0	0	12	52	0	1	include
70	C(+)	LC	4.812	0	0	0	0	0	0	include

Chapter 4. Appendices

Appendix 4-1. Raw data from Movement Assay 1 for field-caught snails from Lafarge. Table includes information on snail shell length, number of internal and external *Chaetogaster*, trematode infection (rediae/sporocysts) and distance travelled in one hour of 36 snails in six trials. See Methods in Chapter 4 for details.

Snail ID	Trial	Snail Length (mm)	No. of external *Chaetogaster*	No. of internal *Chaetogaster*	Trematode infection	Distance (cm)
1	1	6.21	3	0	0	145.725
2	1	7.25	5	0	1	29.1065
3	1	6.05	7	0	1	27.931
4	1	7.97	3	0	0	17.9342
5	1	6.6	2	0	0	25.2328
6	1	4.95	1	0	0	69.2691
7	2	5.51	1	0	0	31.7164
8	2	8.4	3	2	1	43.363
9	2	8.32	3	0	0	43.178
10	2	9.34	4	0	0	36.7103
11	2	5.65	2	0	1	24.4283
12	2	6.25	1	0	1	75.7959
13	3	5.44	3	0	0	31.6102
14	3	10.18	10	0	0	20.7071
15	3	9.81	4	0	0	21.2631
16	3	5.5	4	0	1	41.8811
17	3	4.34	0	0	0	112.303
18	3	5.74	0	0	0	36.9918
19	4	5.81	1	0	0	22.6371
20	4	6.6	1	0	0	55.8679
21	4	9.99	2	0	0	32.0631
22	4	6.23	3	0	0	125.444
23	4	8.63	5	0	0	26.6463
24	4	6.05	6	0	0	23.2456
25	5	5.16	1	0	0	81.8629
26	5	5.46	6	0	1	100.264
27	5	7.38	1	0	0	26.3875
28	5	6.26	1	0	1	280.529
29	5	5.09	3	0	1	34.2089
30	5	7.63	1	0	0	35.4173
31	6	6.3	3	0	0	215.7
32	6	4.7	2	0	0	40.3577
33	6	6.82	3	0	0	90.1504
34	6	5.87	6	0	0	27.7304
35	6	7.55	3	0	0	29.5889
36	6	5.14	5	0	0	30.7166

Appendix 4-2. Raw data from Movement Assay 2 for field-caught snails from Morinville. Table includes information on snail shell length, number of internal and external *Chaetogaster*, trematode infection (rediae/sporocysts), number of metacercariae and distance travelled in one hour of 30 snails in five trials. See Methods in Chapter 4 for details.

Snail ID	**Trial**	**Snail Length (mm)**	**No. of external *Chaetogaster***	**No. of internal *Chaetogaster***	**Trematode infection**	**No. of metacercariae**	**Distance (cm)**
1	1	12.78	16	4	0	27	270.615
2	1	8.15	5	4	0	2	372.905
3	1	14.52	17	3	0	31	499.891
4	1	12.73	15	3	0	6	310.521
5	1	15.27	20	2	0	27	383.725
6	1	11.09	7	5	0	5	477.789
7	2	14.81	9	5	0	30	397.543
8	2	13.72	21	6	0	26	1067.82
9	2	12.97	20	1	0	4	295.357
10	2	13.5	16	4	0	4	5269.98
11	2	12.35	7	2	0	15	67.6647
12	2	12.47	10	5	0	14	383.19
13	3	13.91	15	3	0	24	371.787
14	3	15.45	18	11	1	6	347.99
15	3	13.7	5	2	1	5	456.783
16	3	14.98	13	9	0	7	353.63
17	3	12.72	13	7	0	9	211.073
18	3	13.07	17	3	0	4	197.133
19	4	14.52	15	2	0	10	231.945
20	4	14.82	9	9	0	51	215.464
21	4	15.1	12	8	0	28	271.399
22	4	14.84	17	8	0	9	262.698
23	4	14.66	11	3	0	10	409.574
24	4	14.58	12	0	0	5	463.094
25	5	14.53	12	6	0	12	201.759
26	5	11.48	7	4	0	1	325.262
27	5	14.73	20	0	0	48	205.59
28	5	15.26	16	5	0	22	393.753
29	5	13.66	15	NA	1	NA	217.873
30	5	12.83	12	4	0	15	215.62

Appendix 4-3. Raw Preference Experiment data. Information on each of the 60 snails tested in the preference experiment, including: the date of the trial, snail treatment, number of external *Chaetogaster* found on the snail, and snail shell length. Here *Chaetogaster*(-) snails are denoted as C(-) and *Chaetogaster*(+) are denoted C(+).The position of each snail at each time check (1-20) in the arena is also given. Position 1 = bait snails with no *Chaetogaster*, position 2 = middle (no choice), position 3 = bait snail with *Chaetogaster*.

Date	**Snail ID**	**Treatment**	**No. of external *Chaetogaster***	**Snail length (mm)**	**Time Check**																	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
12-Mar	1	C(-)	0	6.56	2	1	1	2	3	3	3	3	3	3	3	3	3	3	2	2	1	1	1	
12-Mar	2	C(-)	0	5.71	1	1	1	1	1	1	2	2	1	1	1	1	1	2	2	3	3	3	3	3
12-Mar	3	C(+)	15	5.57	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12-Mar	4	C(-)	0	6.03	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	1	1
12-Mar	5	C(+)	13	6.35	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
12-Mar	6	C(-)	0	6.95	2	2	1	1	1	1	1	1	2	2	3	3	3	3	3	3	3	3	3	3
12-Mar	7	C(+)	9	5.2	1	1	1	1	1	2	2	3	3	3	3	3	3	3	3	3	2	2	2	1
12-Mar	8	C(+)	31	7.17	2	3	3	3	3	3	3	3	2	2	2	2	2	1	1	1	1	1	1	2
12-Mar	9	C(-)	0	6.5	3	3	3	3	3	3	2	1	1	1	1	2	2	3	3	3	2	2	1	2
12-Mar	10	C(+)	14	5.52	1	1	1	1	1	2	3	3	3	3	3	3	3	2	2	1	1	1	1	1
12-Mar	11	C(-)	0	6.18	2	2	2	1	1	2	3	3	1	1	2	3	3	3	3	3	3	3	3	3
12-Mar	12	C(+)	14	6.23	2	2	3	3	3	3	3	3	2	1	1	1	1	1	1	1	1	1	1	1
13-Mar	13	C(+)	13	5.71	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
13-Mar	14	C(-)	0	7.04	3	2	2	3	3	3	1	2	3	3	3	2	2	2	3	3	3	3	3	3
13-Mar	15	C(+)	11	5.54	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13-Mar	16	C(+)	14	6.92	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
13-Mar	17	C(+)	23	6.24	2	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
13-Mar	18	C(-)	0	6.25	2	2	2	1	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3
13-Mar	19	C(-)	0	7.18	1	1	2	2	3	2	1	1	1	1	1	1	2	2	2	1	1	2	3	3
13-Mar	20	C(+)	8	5.45	2	2	2	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
13-Mar	21	C(-)	0	4.92	1	1	1	1	2	3	3	3	3	3	3	3	3	3	1	1	1	1	1	1
13-Mar	22	C(+)	17	6.11	2	1	1	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3	2	2
13-Mar	23	C(-)	0	6.85	2	3	2	3	2	2	3	1	3	2	1	1	1	1	2	3	3	3	3	3
13-Mar	24	C(-)	0	6.92	1	2	1	1	1	2	3	3	3	2	1	1	2	3	3	3	2	1	1	2
15-Mar	25	C(+)	19	5.4	2	3	2	2	1	1	2	3	3	2	1	1	1	1	1	1	1	1	1	1
15-Mar	26	C(+)	13	4.94	2	1	2	2	3	2	1	1	1	1	1	1	1	1	1	1	1	1	2	2
15-Mar	27	C(+)	17	5.17	2	2	1	1	2	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1

15-Mar	28	C(+)	12	5.51	2	2	3	2	2	3	2	2	2	2	2	3	2	1	2	2	3	3	3	2
15-Mar	29	C(-)	0	6.89	1	1	2	1	2	3	3	1	1	3	2	1	2	1	1	2	3	1	1	1
15-Mar	30	C(-)	0	5.97	1	1	2	3	3	1	1	2	2	3	3	3	1	1	1	1	1	1	3	3
15-Mar	31	C(-)	0	6.01	2	3	3	1	2	1	1	1	1	1	1	3	1	1	1	1	1	1	1	1
15-Mar	32	C(+)	9	5.84	3	3	3	2	3	2	3	2	3	3	3	2	3	3	3	3	2	3	3	3
15-Mar	33	C(-)	0	6.4	3	2	1	1	3	3	3	3	3	3	1	1	1	2	1	2	3	3	3	3
15-Mar	34	C(+)	13	4.09	2	2	2	1	1	2	2	2	3	3	3	3	3	3	3	3	3	3	3	2
15-Mar	35	C(-)	0	6.41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15-Mar	36	C(-)	0	6.37	3	3	3	3	3	3	2	1	1	1	1	2	3	3	2	1	1	1	2	2
16-Mar	37	C(-)	0	6.15	2	3	3	3	2	2	3	3	3	2	2	3	3	3	3	3	3	3	3	3
16-Mar	38	C(+)	11	5.65	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
16-Mar	39	C(-)	0	5.54	3	3	3	2	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1
16-Mar	40	C(-)	0	5.64	1	1	1	1	1	1	1	1	2	3	3	3	2	2	1	3	3	3	2	2
16-Mar	41	C(-)	0	6.54	1	1	1	3	2	1	1	2	2	1	1	1	1	1	2	1	1	1	2	3
16-Mar	42	C(+)	14	5.44	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3
16-Mar	43	C(-)	0	5.63	2	2	2	3	2	1	1	1	2	1	1	1	2	2	3	3	1	1	1	1
16-Mar	44	C(-)	0	4.89	1	1	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
16-Mar	45	C(+)	18	5.16	2	3	3	3	3	2	2	1	1	1	1	1	1	2	2	2	3	3	3	3
16-Mar	46	C(+)	23	6.1	1	1	1	1	1	1	2	2	3	2	1	1	1	1	1	1	1	1	1	2
16-Mar	47	C(+)	17	5.22	3	3	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	2	1	1
16-Mar	48	C(+)	24	5.34	1	1	1	2	2	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1
17-Mar	49	C(+)	11	5.55	2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	3	3	3	3
17-Mar	50	C(-)	0	6.87	2	2	2	2	3	3	3	3	3	2	2	1	1	1	1	1	1	1	1	1
17-Mar	51	C(+)	15	5.91	2	2	2	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3
17-Mar	52	C(+)	12	7.68	2	2	2	3	3	3	2	2	2	2	1	1	1	1	1	1	1	1	1	1
17-Mar	53	C(+)	10	4.64	2	2	3	3	3	3	3	3	2	2	2	1	1	2	2	2	3	3	3	3
17-Mar	54	C(-)	0	7.33	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
17-Mar	55	C(+)	15	4.68	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
17-Mar	56	C(-)	0	6.24	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
17-Mar	57	C(+)	14	6.03	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17-Mar	58	C(-)	0	5.81	2	2	2	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
17-Mar	59	C(-)	0	5.92	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2
17-Mar	60	C(-)	0	4.5	1	1	1	2	3	3	3	3	3	3	3	2	3	3	3	3	3	3	2	1