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**Impacts of Parasites on Reproductive Behaviour and Fitness of the Intertidal
Amphipod *Corophium volutator* (Pallas)**

by

Dean Gregory McCurdy

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy**

Department of Biology

Carleton University

Ottawa, Ontario

December 1999

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Impacts of Parasites on Reproductive Behaviour and Fitness of the Intertidal
Amphipod *Corophium volutator* (Pallas)

submitted by
Dean Gregory McCurdy

in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Biology

Thesis Supervisor

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Abstract

I investigated parasite- and host-mediated effects on host behavior and investment in reproduction using the intertidal amphipod *Corophium volutator* (Pallas) and two of its parasites; the nematode *Skrjabinoclava morrisoni* (Wong and Anderson) and the trematode *Gynaecotyla adunca* (Linton). In systems where parasites are transmitted to a final host when their intermediate host is eaten (trophic transmission), parasites should evolve to alter behaviour of intermediate hosts in ways to increase their likelihood of being ingested by final-host predators (the 'manipulation hypothesis'). However, life history theory predicts that hosts should respond to parasitism by altering their own behaviours or life histories to minimize costs of parasitism ('adaptive host response' hypothesis).

I tested predictions of the 'manipulation' and 'adaptive host response' hypotheses by comparing the timing and duration of reproductive events and anti-predator behaviours between parasitized and unparasitized amphipods. To design realistic tests, I first investigated the ecology and evolution of reproduction in unparasitized amphipods and the foraging ecology of shorebirds (major predators on *C. volutator* and final hosts of its parasites). I used results from these studies to determine the extent to which birds feed on amphipods at times when amphipods are expected to differ in their susceptibility to shorebird predation (daytime versus nighttime). Related to these investigations, I examined

responses of unparasitized amphipods to shorebird predators to provide a 'baseline' against which responses of parasitized amphipods could be compared.

After I investigated the natural history of shorebirds and *C. volutator*, I tested predictions about how parasites should affect amphipod behaviour and investment in reproduction. Using experiments and collections, I found evidence that both parasite- and host-mediated effects on host behaviour may occur within single parasite-host systems; particularly when the optimal timing for control over host behaviour (explored using an empirical model) differs between parasite and host.

Acknowledgments

Although a thesis is ultimately attributed to a single author, any undertaking as large as this requires the encouragement, co-operation, and support of others. My thesis is no exception. The names that follow are only some of the many people who made this endeavor possible. My apologies and thanks to any I have forgotten.

First, thanks to my supervisors, both past and present. Dr. Tom Herman fostered my interest in biology, encouraged me to take-on this project and to follow my curiosity. The use of his office as well as the laboratory, animal care, and storage facilities at Acadia University also was greatly appreciated. My thesis supervisor, Dr. Mark Forbes, was instrumental in helping me to shape and communicate my ideas. Mark could be counted on for almost anything (you're 'in charge' now Mark). The positive impact of Mark's advice, patience, and understanding will remain with me throughout my academic career. Dr. Sherman Boates shared his wealth of knowledge of *Corophium* and sandpipers and he was crucial in helping me with the design and execution of field and laboratory experiments. Thanks Sherman, for letting me make my own mistakes!

I often kept my field assistants 'in the dark' (literally) about what they were working on, but they nonetheless worked tirelessly during each my field seasons. In 1996, Andrew Barry helped to dissect shorebirds and nematodes and assisted with sampling done at night. In 1997, Carina Gjerdrum showed incredible skill and patience in dealing with an overwhelming amount of data ('...male on

surface...female antennae out...'). Also in 1997, Keiko Lui (a.k.a. La femme Lui) went for weeks without sleep to help with day and night sampling. In 1998, Matthew Lavers and Dan McPhee provided assistance in the laboratory and in the field (thanks for the Tai-Chi lessons guys!). On several occasions, my lab mates, Kerry Hecker, Nancy Léonard, and Christopher Yourth drove across the country only to be 'rewarded' with 18h-days of field work and 20,000 amphipods to sort! The other members of my lab, Sharleen Hawco, Brian Leung, Dave McCruer, and Gina Schalk could always be counted on to discuss statistics or debate philosophy with me. Students from the 'Weatherhead' lab (Michelle Gunness, Kelley Kissner, Sophie Sommerer) and the 'Fahrig' lab (Darren Bender, Julie Brennan, Tom Contreras, Michelle Lee) also provided insights on my project and on life as a graduate student and beyond). My parents, Iris McCurdy and David McCurdy and my brother Derek and his wife (Michelle) were instrumental in setting up laboratory experiments in 1996 and 1997. Finally, thanks to my wife Wai-Ming (Vivien) McCurdy for her advice and her unwavering belief in me and my ideas. Vivien also spent >1500 hours collecting, sorting, measuring, and squashing amphipods!

Along the way, several other people and institutions supported my research. My thesis advisors, Dr. David Currie and Dr. Myron Smith were enthusiastic about my project and provided insightful suggestions on many occasions. At Carleton University, Tim Cathart-Black of Instructional Media Services helped with film analysis. At Acadia University, Mr. Jim Wolford, Dr. Graham Daborn, and Dr. Glenys Gibson were always available to discuss my

project, identify infaunal invertebrates, and assist with photography. Financial support for this project was provided to me in the form of post-graduate scholarships from Carleton University and from the Natural Sciences and Engineering Research Council of Canada (NSERC) and by a NSERC grant to Dr. Mark Forbes. Financial and logistical support in Nova Scotia (field assistants, technicians, equipment, and vehicles) also was provided by the Nova Scotia Department of Natural Resources (Wildlife Division).

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GENERAL INTRODUCTION

PARASITE- VERSUS HOST-MEDIATED CONTROL OF HOST BEHAVIOUR

A parasite can be defined as any organism that relies on another organism as a source of energy and as habitat (Starr and Taggart 1998). Researchers have documented numerous direct and indirect impacts of parasites on hosts. Parasites directly impact their hosts as they feed on host tissue and/or reproduce on/in their hosts. Direct impacts of parasites on hosts include: reduced growth, reduced **reproductive success**, or increased morbidity or mortality (Price 1980). At the population level, direct effects of parasitism have been implicated in causing declines and crashes of host populations (e.g., Jensen and Mouritsen 1992). Researchers have only begun to investigate indirect impacts of parasites on hosts. Indirect impacts on hosts include: decreased attractiveness to mates (Hamilton and Zuk 1982), increased susceptibility to predation (Poulin 1994a; Moore 1995), alteration of host sex (Godfray and Werren 1996), and alteration of the outcome of competitive interactions (Thomas et al. 1999). Through direct and indirect effects, parasites may even regulate host populations through ecological time (Scott and Dobson 1989) and structure ecological communities (Minchella and Scott 1991; Thomas et al. 1998).

1. Manipulation of host behaviour by parasites

I tested specific predictions of an hypothesis related to an indirect effect of parasites on their hosts; parasite-induced alteration of host behaviour (hereafter referred to as parasite 'manipulation' of host behaviour). Parasite manipulation provides an excellent example of how parasites have evolved to maximize their own **fitness** at a cost to their hosts. When parasites manipulate host behaviour, they cause their hosts to behave in an unusual manner which can result in an increased likelihood that the parasites will complete their life cycles. The most celebrated cases of parasite manipulation have been observed in systems where the parasite is transmitted from an **intermediate host** to a **final host** via predation by the final host on the intermediate host (**trophic transmission**). By manipulating the behaviour of the intermediate host, the parasite renders this host more susceptible to predation by a final host. Parasite manipulation of host behaviour may also occur in parasites that do not rely on trophic transmission. For example, sandflies (*Lutzomyia vexator* (Coquillett)) infected by the malarial protozoan *Plasmodium mexicanum* frequent warmer substrates than uninfected flies (Fialho and Schall 1995). The altered thermal ecology of infected flies has no apparent benefit for the host, but enables the parasite to develop in time to infect vertebrate hosts.

Manipulations of host behaviour by parasites may be passive or active (Poulin 1994a). Passive manipulations usually occur at minimal cost to the parasite. For example, **metacercariae** of the trematode *Diplostomum*

spathacuem renders its host, the three-spined stickleback (*Gasterosteus aculeatus* L.) more susceptible to predation by birds simply by residing in the eye, which blinds the stickleback (Milinski 1990). Active alterations impose a direct cost to the parasite, such as producing and releasing a neurochemical to alter the behaviour of a host (Thompson and Kavaliers 1994). Because active alterations are costly, natural selection should optimize (not maximize) manipulative effort of such parasites (Poulin et al. 1994). Manipulative effort of individual parasites may be highest when: few parasites are found in individual hosts, hosts are short-lived, passive transmission rates are low, or parasite fecundity is low (Poulin 1994a).

A major problem with existing tests of the 'manipulation' hypothesis is that they fail to differentiate between behavioural changes caused by parasites and changes caused by hosts (Poulin 1995). Throughout my thesis, I consider the possibility that changes in the behaviour may be due to parasite manipulation, host response (below), or non-adaptive side-effects of infection. To test for parasite manipulation, I developed a series of fine-tuned predictions of the 'manipulation' hypothesis in a parasite-host system where trophic transmission occurs. I also provide the first test of this hypothesis using studies in the field and in the laboratory. My predictions were related to the timing of parasite manipulation, its duration, and its outcome on hosts of different ages and sexes. For example, parasites that rely on trophic transmission should only begin to alter behaviour of intermediate hosts when the parasites have developed to a stage

where they can be transmitted successfully to final hosts (Poulin et al. 1994). A parasite that alters host behaviour too early during its development would decrease its own fitness since pre-infective transmission will often kill the parasite (e.g., Rankin 1940). Similarly, parasites that rely on trophic transmission should only expend energy to manipulate behaviour of intermediate hosts when final hosts are present and are likely to feed on hosts with altered behaviour, particularly if manipulation is costly or if manipulation at an inappropriate time increases the likelihood of predation by inappropriate final hosts of the parasite.

2. Adaptive life history responses of hosts to parasitism

Conventional theory on parasite-host relationships suggests that hosts are constrained in their ability to resist parasites through evolutionary time (Minchella 1985). This is because selection pressure is likely stronger on parasites to infect hosts than on hosts to resist parasites (each parasite must compromise its host(s) to successfully complete its life cycle, whereas not all hosts become infected by parasites, and even if they do, fitness of infected hosts is usually >0). Despite this, hosts do attempt to minimize the impacts of parasitism on their reproductive success through a variety of defenses. Hosts may avoid parasites in space or time (Murray 1990) or exhibit behaviours that deter parasites from attacking (e.g., Forbes and Baker 1990; Hart 1994). When challenged by parasites, hosts may resist infections through the use of a physiological immune

system (e.g., Granath 1995) or through the use of chemical defenses (Baldwin and Preston 1999).

In systems where the cost of active resistance to parasites is high, it has been suggested that hosts may have evolved heritable life-history adaptations to parasitism ('adaptive host response' hypothesis; Minchella 1985; Forbes 1993). Life history responses should impose little or no cost to the host when not in use, making them particularly useful for hosts in systems where the likelihood that a host will become infected is low or variable. Responding to parasitism through variation in life history goes beyond simple tolerance of infections because it reflects a shift in the reproductive 'tactics' of the host. Infected hosts may alter allocation to early reproduction (that when first infected) versus future reproduction (that when the infections are older) in a way that maximizes the host's lifetime reproductive success (Forbes 1993). Infected hosts still suffer from reduced fitness relative to uninfected hosts, but less so than if they had not shifted their timing of reproduction. Alterations in life history by hosts may have positive or negative consequences for the parasites (Poulin et al. 1994).

For life history responses to be adaptive for hosts, they must result in higher fitness than if the host does not respond (Forbes 1993; Poulin 1995). The type of response should vary depending on the life cycles of the parasite and host. For example, in systems where parasites initially impose a high cost to hosts, but where hosts usually outlive their infections, hosts are expected to allocate few resources to reproduction until after their infections are gone (e.g.,

Ballabeni 1995). However, in systems where hosts become infected by parasites that have increasingly negative effects on their energy budgets (resulting from parasite growth or proliferation), then hosts are expected to respond when first infected (e.g., Minchella and LoVerde 1981; Polak and Starmer 1998). It has been suggested that adaptive host response precludes manipulation by parasites (Poulin et al. 1994). However, I will argue (Chapter 4) that both manipulation by parasites and responses by hosts may occur in a single parasite-host system, providing that the optimal times to control host behaviour differ between parasite and host.

3. Study system

I used the intertidal amphipod *Corophium volutator* and its nematode parasite *Skrjabinoclava morrisoni* (Wong and Anderson) and its trematode parasite *Gynaecotyla adunca* (Linton) as model parasite-host systems to test predictions of hypotheses related to parasite- and host-mediated changes in host life history and behaviour. *Corophium volutator* is well suited for these studies as it has a short life cycle (6 months to a year, depending on the season and on the population; Fish and Mills 1979) and it occurs at high densities ($>10,000$ /m²; Gratto et al. 1983). The species also is well understood ecologically, with respect to the effects of competition and predation (e.g., Boates 1980; Wilson 1989; Matthews et al. 1992). *Corophium volutator* is additionally important as it is likely a 'keystone' species in soft-bottom intertidal communities; this means that

impacts of parasites on populations of this species likely have consequences for other intertidal fauna. The effects of parasites on populations of *C. volutator* is of concern as parasites have been implicated in causing crashes of *C. volutator* populations in the Baltic Sea (Jensen and Mouritsen 1992; Bick 1994) and could be responsible for recent, as yet unexplained, declines of populations in parts of the Bay of Fundy (Shepherd et al. 1995).

4. Outline of thesis

My thesis is divided into 6 main chapters and a series of appendices. While I have attempted to minimize the use of complex terminology, I do employ terms which are commonly used by parasitologists and evolutionary biologists. These terms appear in bold at first usage and are defined in the Glossary of Terms (Appendix A). Most of the Chapters in my thesis represent an expansion of original publications. Rather than citing my publications throughout my thesis, I cite the chapter(s) that corresponds to each publication, as follows:

Chapter 1: McCurdy, D.G., Boates, J.S., and Forbes, M.R. Reproductive synchrony in the intertidal amphipod *Corophium volutator*. *Oikos* **88**: 301-308.

Chapter 2: McCurdy, D.G., Boates, J.S., and Forbes, M.R. (1997) Diurnal and nocturnal foraging by Semipalmated Sandpipers *Calidris pusilla*. *J. Avian Biol.* **28**: 253-256.

Chapter 3: McCurdy, D.G., Forbes, M.R., and Boates, J.S. (1999) Evidence that the parasitic nematode *Skrjabinoclava* manipulates host *Corophium* behavior to increase transmission to the sandpiper, *Calidris pusilla*. Behav. Ecol. **10**: 351-357.

Chapter 4: McCurdy, D.G., Forbes, M.R., and Boates, J.S. 1999. Testing alternative hypotheses for variation in amphipod life history in relation to parasitism. Int. J. Parasitol. **29**: 1001-1009.

Chapter 5: McCurdy, D.G., Forbes, M.R., and Boates, J.S. 1999. Male amphipods increase their mating effort before behavioural manipulation by trematodes. Can. J. Zool., in press.

Chapter 6: McCurdy, D.G., Boates, J.S., and Forbes, M.R. An empirical model on the optimal timing of reproduction for female amphipods infected by trematodes. Journal of Parasitology, submitted.

In Chapters 1 and 2, I investigated several aspects of the reproductive ecology of *C. volutator* and the foraging ecology of its major predator, the semipalmated sandpiper, *Calidris pusilla* L. (sandpipers also serve as a final hosts for nematode and trematode parasites of *C. volutator*). Although these chapters represent studies that were originally conducted to test other questions

related to the ecology and evolution of mating systems and foraging tactics (respectively), I used results from these studies to establish and test specific predictions of hypotheses related to parasite- versus host-mediated effects on host behaviour and investment in reproduction (Chapters 3-5).

In Chapter 1, I explored the evolution and ecology of **reproductive synchrony** in *C. volutator*. Specifically, I tested the 'mate-availability' hypothesis which predicts that females from populations that exhibit female-biased sex ratios (as found in *C. volutator*, Schneider et al. 1994) should reproduce asynchronously to maximize their likelihood of obtaining a mate. In testing assumptions of this hypothesis, I elucidated several aspects of the ecology of female *C. volutator* (the presence of sexual reproduction, the relationship between female moult and **receptivity**, survivorship compared to males). I also investigated mate-choice in this species.

In Chapter 2, I compared the levels of foraging pressure by semipalmated sandpipers on a population of *C. volutator* between daytime and at nighttime. Semipalmated sandpipers are major predators of *C. volutator* during the daytime (each may consume >20,000 / day; Hicklin and Smith 1979). However, little is known about the energetics of shorebirds that forage at night, and previous to my study, the level of predation pressure on *C. volutator* by sandpipers foraging at night was unknown. Using a new technique (counts of sandpiper droppings), I tested the 'supplemental' hypothesis which predicts that shorebirds may feed extensively at night if their energy requirements cannot be met during the day.

In Chapter 3, I used the nematode *Skrjabinoelava morrisoni* to test the 'manipulation' hypothesis in *C. volutator*. To do this, I made collections in the field to investigate anti-predator responses of parasitized versus unparasitized amphipods. I also conducted experiments in the laboratory to test specific predictions related to behaviours of parasitized versus unparasitized male amphipods (e.g., trail complexity, distance traveled, burrow-probing activity). In all cases, I related my results to the developmental stage of parasites (as stated above, parasites are only expected to manipulate behaviour once they develop to their infective stage). Finally, I assessed whether nematode parasites pose a survivorship cost to male amphipods.

In Chapters 4-6, I investigated parasitism of female and male *C. volutator* by the trematode *Gynaecotyla adunca*. Because females often invest more in individual matings than males, host responses of females can be measured more easily than in males (e.g., Meuleman 1972; Minchella and LoVerde 1981). Using female hosts made it possible for me to study complex life-history trade-offs to parasitism (Chapters 4,6). However, testing for host response in males also is important because natural selection may act differently on males and females to maximize their reproductive success (Zuk 1990), leading to sex-specific differences in effects of parasites on hosts (e.g., McCurdy et al. 1998). Furthermore, few tests of adaptive host response have been conducted in male hosts (see Polak and Starmer 1998 for an exception). Using both female and male hosts made it possible to further fine-tune predictions of hypotheses related

to parasite manipulation and adaptive host response. For example, unparasitized male *C. volutator* often crawl in search of mates, but unparasitized female amphipods usually remain burrowed in the substrate (Chapter 3). As a result, increased crawling by parasitized males may be interpreted as either parasite manipulation (increased transmission to foraging sandpipers) or as a host response (increased mate-searching effort). However, increased crawling in females is consistent only with manipulation by parasites (Chapters 3,4).

In Chapter 4, I used female *C. volutator* to test predictions of the 'manipulation' and 'adaptive host response' hypotheses by comparing behaviour (using collections in the field) and investment in reproduction (moulting and mating activity) of parasitized versus unparasitized amphipods. In addition to considering the developmental stage of parasites (as in Chapter 3), I also studied responses of amphipods of different ages (these 'young' versus 'old' females had a different amount of investment in reproduction when experimentally infected/uninfected by parasites). I then determined how different levels of parasitism influenced survivorship of females and discussed how parasitism may shift the trade-off between longevity and reproduction in infected amphipods.

In Chapter 5, I tested predictions of the 'manipulation' and 'adaptive host response' hypotheses using male *C. volutator*. Using experiments, I investigated how parasitism by new versus old (infective) infections affected male mating effort (likelihood of mating, reproductive success of mates). I also determined whether mating history of males affected the ability of males to respond to

parasitism in the laboratory. In addition, I examined the effects of parasitism on survivorship of males. Throughout, I interpreted behavioural changes in infected hosts in relation to the developmental stages of trematodes.

In Chapter 6, I explored the logic underlying the 'manipulation' and 'adaptive host response' hypotheses. I did this by constructing an empirical model for female *C. volutator* infected by *G. adunca* to examine the costs of altered behaviour for parasitized hosts and to examine the fitness consequences for female amphipods that reproduce at various times following infection by trematodes. Female amphipods were used because much was known about their reproductive ecology (Chapter 1) and effects of parasites on survivorship and reproductive success (Chapter 4).

I conclude my thesis by providing a general summary of the results obtained, conclusions reached in each chapter, and by providing suggestions for future studies of parasite 'manipulation' and host response. I also discuss the importance of *C. volutator* as a 'keystone' species in soft-bottom intertidal ecosystems and explore possible consequences of parasitism on populations of *C. volutator* and on soft-bottom intertidal ecosystems.

CHAPTER 1

REPRODUCTIVE SYNCHRONY IN FEMALE *COROPHIUM VOLUTATOR*

1. Introduction

Generally, organisms reproduce when environmental conditions are most favourable- for example, when food is readily available, when susceptibility to predation is low, or when opportunity for successful dispersal (of parents or their offspring) is high (Ims 1990; Stearns 1992). However, ecological and/or sociobiological factors may affect variation in timing of reproduction, leading either to exaggerated or lessened degrees of reproductive synchrony (Ims et al. 1988; Ims 1990). In extreme cases, all females mate or give birth at the same time (complete synchrony), whereas in other species, female mating and birthing schedules are more uniformly distributed through time (Ims 1990). In populations where sexual reproduction is present and the operational sex ratios are female-biased, females should reproduce asynchronously because synchronous females would 'swamp' males, decreasing an individual female's probability of successful fertilization (the 'mate-availability' hypothesis; Emlen and Oring 1977). The 'mate-availability' hypothesis has been supported for populations of the wood lemming *Myopus schisticolor* Lilljeborg, where females breed synchronously when sex ratios are 1:1, but breed asynchronously when sex ratios are female biased (Ims et al. 1988).

Spatial and temporal distributions of receptive females often are viewed as determinants of male reproductive adaptations to ensure successful mating (Shuster 1991a). However, for populations with extreme female-biased sex ratios, selective pressure can be greater on females than males to ensure mating. Such selection is expected to depend also on whether paternal investment is high or low (Gwynne 1991). I studied reproductive synchrony in the intertidal amphipod *Corophium volutator*, an ideal organism for study because *C. volutator* shows extreme female-biased sex ratios, in both juveniles and adults (Schneider et al. 1994).

Corophium volutator occurs in distinct patches within the muddy intertidal zone (Matthews et al. 1992). Studies on reproductive behaviour of this species, to date, have focused primarily on males (e.g., Boates et al. 1995; Forbes et al. 1996). Matthews et al. (1992) found reproductive synchrony, measured over months, among female *C. volutator* living on a mudflat where predatory semipalmated sandpipers were present, but not for amphipods living on a mudflat where sandpipers were absent. Breeding synchrony, however, can be measured at several scales (see Matthews et al. 1992 for a study at the mudflat and seasonal scales). I expected that female *C. volutator* would show asynchronous breeding on the mudflat lacking sandpiper predators because only intrinsic factors relevant to this species' biology dictate the degree of asynchrony: namely, the degree of biased sex ratios and the degree of flexibility in 'decisions' about receptivity of females relative to their moult cycle. If reproductive

synchrony was present, I expected females to show other adaptations to ensure matings because males were expected to be limiting. Because reproduction may be constrained by environmental factors, other than seasonal predation pressures, I also chose to examine patterns of female moult in relation to tidal and lunar cycles (spring tides occur during new and full moons and neap tides occur during first and last quarter moons). Tidal and lunar cycles affect the timing of reproductive events in crustaceans (e.g., Klapow 1976; Moore 1981), including mate-searching activity and brood release in *C. volutator*, at least over a large spatial scale (Fish and Mills 1979, but see Boates and Smith 1989). Finally, I interpret how patterns of female breeding ability may affect reproductive strategies of male *C. volutator*.

I started by testing assumptions of the 'mate-availability' hypothesis for my study species. These assumptions also were relevant for tests of the 'manipulation' and 'adaptive host response' hypotheses (Chapters 3-6). First, I examined whether males were required for female reproduction (evidence of sexual reproduction). To do this, I simply determined whether newly-moulted females could become **ovigerous** in the absence of a male (female amphipods cannot store sperm across a moult; Sexton 1935). My testing of this assumption was necessary because asexual reproduction occurs in a related species; *Corophium bonnellii* Milne Edwards (Moore 1981; Powell and Moore 1991). Second, I examined whether female moult coincided with receptivity in *C. volutator*, as shown in other crustaceans (reviewed in Forbes et al. 1996). To test

this assumption, I manipulated the delay between female moult and mating opportunity to determine how long females remain receptive following a moult. If delays are minimal, then females moulting synchronously do, in fact, breed with a high degree of synchrony. I further assessed costs to delayed mating by comparing clutch sizes of females that were given the opportunity to mate soon versus long after their moult. While this was not a central premise to the 'mate-availability' hypothesis, another researcher has shown that even short delays in mating can result in decreased fecundity (Borowsky 1988), a cost that would further constrain breeding synchrony. Finally, I compared survivorship of females and males in the laboratory. If male-biased mortality exists, this could result in seasonal changes in operational sex ratios. If mate-availability is a consideration for females of this species, then breeding synchrony should be lower at times when sex ratios are more female-biased.

After satisfying the above assumptions (see results), I tested my first prediction of the 'mate-availability' hypothesis by quantifying the frequency distribution of female moults at both large and small spatial and temporal scales. Because this species shows sex ratios of 5-15 females/male (Schneider et al. 1994), I predicted that females should spread out their reproductive activity in order to increase their probability of securing a mate (Ims 1990), particularly if females are receptive only for short durations over their lives.

I tested for two other ways that female *C. volutator* may maximize their mating opportunities. First, I compared the frequency of female and male moults.

I predicted that females might increase their opportunities for reproduction by moulting more frequently than males, especially if reproduction is constrained by female moult, as seen in other amphipods (Borowsky 1991). Females might instead reduce their time-to-next-moult when cohabiting with a potential mate (females and males share burrows in nature; Forbes et al. 1996). Such adaptations are expected if it is apparent that environmental constraints reduce the time available for reproduction.

2. Methods

Study species

Several aspects of the natural history of *C. volutator* are relevant to this study. Juveniles (<5 mm, tip of rostrum-end of telson length; Appendix B) and adult females (5-11 mm in length) usually remain in 1-10 cm U-shaped burrows which they construct in sand/silt sediments. Adult males (5-11 mm) do not make burrows (Meadows 1964); instead they crawl during ebb tides in search of females, with which they may later co-habit (Boates et al. 1995). Female-biased sex ratios cannot be explained solely by differential predation on crawling males, as such biases also occur in juveniles, which are not subjected to male-biased predation (Schneider et al. 1994). Additionally, female sex-ratio biases occur for this species at sites where sandpiper predators are absent (Boates et al. 1995).

Study site and collection methods

On 1 and 2 August 1997, I collected *C. volutator* from the mudflat at Blomidon, Nova Scotia (Appendix C). I chose Blomidon because of high densities of *C. volutator* (>20,000/m²) and because of previous information on reproductive behaviour and demography of *C. volutator* there (Boates and Smith 1989; Matthews et al. 1992; Forbes et al. 1996). Furthermore, *C. volutator* at Blomidon are neither preyed on by semipalmated sandpipers nor parasitized by larval helminths, both of which influence reproductive activity of *C. volutator* (Boates et al. 1995; Chapters 3-5).

I collected female amphipods from 10 discrete patches spaced at 50-m intervals starting 30 m from the shoreline at Blomidon (patches of *C. volutator* at Blomidon are distinctly observable as small mounds surrounded by less disturbed sediments). Collections were limited to ca. 10 x 10 m within each patch. I chose to sample at the patch level because this is the approximate scale at which males usually mate-search (Forbes et al. 1996) and thus, the scale at which females should compete for access to 'local' mates. From these collections, I assessed the degree of moulting synchrony at the patch level for amphipods transferred to a laboratory setting. Thus, patch became a label for 'local area'.

At each patch, I removed 10-15 100 cm² cores and gently sieved (0.85-mm mesh) these cores in nearby tidal pools. I then placed sieved amphipods in 500-ml jars containing seawater (max. 100 females/jar), for transport to Acadia University (25 km drive away). Using live-specimen forceps, I also collected males that were mate-searching within the patch of *C. volutator* located 80 m from the shoreline, where males were most abundant. I used these males in experiments (males were sorted and transported as described above for females). Once in the laboratory, I aerated water in each jar until *C. volutator* were processed (within 1 day).

In the laboratory, I sorted through the contents of each jar and chose non-ovigerous females and males for use in experiments and for observations on female moulting and breeding activity. I only retained non-ovigerous, recently-

mature females (5-7 mm, rostrum-telson length) so that females used in experiments had not yet bred. I only chose adult, reproductively-active males (≥ 5 mm in length).

Testing assumptions and predictions of the 'mate-availability' hypothesis

To determine the relationship between female moult and breeding ability, I individually housed 200 males in 20 x 150-mm culture tubes containing 30 ml of artificial seawater (Instant Ocean). The bottom of each tube was lined with 1 cm of fresh, filtered (0.42 mm mesh) mud from Blomidon. I added 100 $\mu\text{g/ml}$ of Streptomycin sulfate and Penicillin-G to each tube because these antibiotics reduce amphipod mortality and enhance growth (Pelletier and Chapman 1996). The ambient temperature in the laboratory was between 15-17°C, similar to the average substrate temperatures of nearby mudflats (Piccolo et al. 1993). Every 3 days, I exchanged 10 ml of seawater from each tube and fed each amphipod the diatom *Thalassiosira* sp. (200,000 cells/ml). On 5 August, each male was removed from his culture tube and placed with a single female that had moulted within the previous 3 days. Subsequently, each pair was observed daily and the reproductive condition of the female was recorded (ovigerous or non-ovigerous). After 3 days, I removed all females, measured their body lengths (rostrum-telson), and counted the number of embryos in the brood pouches of ovigerous females. I also recorded the presence of male *exuvia* during this time to determine if their ability to mate was timed to their moult.

To determine if the presence of a male was required for a female to reproduce and to observe the temporal distribution of moulting by females, I housed 1042 adult, non-ovigerous female *C. volutator* in the laboratory (collection, housing, and maintenance as described above). Each day, I recorded the presence of any female exuvia. Once a female moulted, she was removed and used to assess the relationship between female moult and ability to breed (above).

To compare frequency of moult and survivorship between females and males and to assess the effect of male presence on a female's likelihood of moulting, I collected *C. volutator* from Blomidon (as described above) and housed 400 pairs of adult males and non-ovigerous females in 400 separate culture tubes (conditions also as described above). Each day, I checked for the presence of male and female exuvia and recorded any mortality given any of the following conditions: the end of the experiment (after 23 days), when one member of the pair died, or when the brood of an ovigerous female was first released. I later removed the pair and measured the body length of each amphipod.

Analyses

To determine if female moults were aggregated in time, I applied a χ^2 test to compare the observed distribution of female moults to a uniform random distribution of moults. To determine if females were more likely to moult or

release broods during spring tides (those increasing in magnitude) or neap tides (those decreasing in magnitude), I applied the 'actual tidal method' (Reaka 1976; Appendix D). I used 'initial lunar analysis' and 'divided lunar analysis' to examine the relationship between female moult and the phases of the lunar cycle. I used parametric tests when the assumptions of homogeneous variance and normal distributions were not violated (assessed using Levene and Shapiro-Wilk's tests, respectively; Zar 1996). However, to compare median dates of moult between females and males and to compare median dates of moult between females that were housed alone versus those that were paired with a male, I applied a non-parametric Mann-Whitney U test. To compare clutch sizes of females and the timing of female moult in relation to distance of patch from the shoreline, I applied Kruskal-Wallis' and Spearman Rank tests, respectively.

To compare survivorship between females and males, I used Cox's *F*-test (Cox 1964). This test also allows for the inclusion of incomplete observations (i.e., individuals that were removed from the experiment for reasons unrelated to survivorship, such as amphipods accidentally damaged by an observer). To determine if male body size or the number of matings a male had obtained influenced male survivorship, I used Cox's Proportional Hazard Regression (Cox 1972). I used Cox's regression to relate **hazard rate** (the probability per time unit that an individual that has survived to the beginning of the respective interval will die in that interval) to body size and mating history of males. I conducted all analyses using the software package Statistica (StatSoft Inc. 1993).

3. Results

Testing assumptions of the 'mate-availability' hypothesis

None of the 1042 females housed alone became ovigerous in the absence of a male, even after moulting (females housed alone after their second moult in the laboratory still remained non-ovigerous). Furthermore, of the 400 females that I paired with males, no female became ovigerous unless she had previously moulted. Specifically, females did not become ovigerous after 96 h following their moult. In fact, fewer females became ovigerous as time since moulting and housing with a male increased (i.e., from 1-2, 2-3, 3-4 days; $X^2_{[2]}=13.2$, $n=195$, $P<0.005$; Figure 1-1). For females that mated, there were no significant differences among their clutch sizes across the three time intervals ($H_{[2,94]}=1.5$, NS). Furthermore, of all females that could be observed (a few females remained in their burrows and could not be assigned within 24 h to ovigerous or non-ovigerous categories), those that became ovigerous did so within a few minutes to 24 h of being paired with a male.

Mortality was extremely low for females; whether housed alone or paired with a male, whereas mortality of males was significantly higher (Cox's $F=16.5$, $P<0.0001$; Figure 1-2). Smaller (younger) males lived longer than larger (older) males (there was a positive relationship between hazard rate and male body size; model: $X^2_{[2]}=35.98$, $n=390$, $P<0.0001$; male body size: $\beta=0.76$, $t=6.55$, $P<0.0001$). Mortality of males did not differ between males that mated and those that did not ($\beta=0.203$, $t=1.05$, NS).

Testing predictions of the 'mate-availability' hypothesis

Overall, female moult differed from complete asynchrony ($\chi^2_{[26]}=168.7$, $n=1042$, $P<0.0001$). Specifically, females were more likely to moult during spring tides ($n=664$) than during neap tides ($n=378$) ($\chi^2_{[1]}=78.5$, $n=1042$, $P<0.0001$; Figure 1-3). Female moult was also related to lunar phenomena. Using 'initial lunar analysis', I observed that more females moulted leading up to the apex of the new moon ($n=435$) than during the first quarter ($n=209$), full ($n=218$), or last quarter moons ($n=180$) ($\chi^2_{[3]}=158.9$, $n=1042$, $P<0.0001$). 'Divided lunar analysis' indicated that most females moulted around the time of the first quarter phase of the moon ($n=297$), many around the time of the full ($n=271$) and new ($n=254$) moons and the fewest around the last quarter ($n=220$) ($\chi^2_{[3]}=12.0$, $n=1042$, $P<0.005$).

Moulting synchrony was influenced by patch location. Specifically, females collected from patches located low within the intertidal zone were more likely to moult during the first spring tide (apex on 3 August), while those collected from the highest patches tended to moult during the following spring tide (apex on 18 August; overall, there was a negative relationship between moult date and distance from shore; Spearman's $R=-0.169$, $n=1042$, $P<0.0001$; Figure 1-4). Thus, synchrony in female moult is best described at an intermediate scale between the mudflat level and patch levels; the aggregate patch level. When broken down by patch, there was no longer a single lunar phase during which

most females moulted (the first spring tide in August occurred around a new moon, while the second spring tide occurred around a full moon).

I observed that females were 8 times more likely to moult than males ($\chi^2_{[1]}=120.4$, $n=137$, $P<0.0001$; Figure 1-5). This comparison only included females and males collected from the same patch and housed together in the laboratory. However, males that moulted did so at the same time as most females (median dates of moult did not differ between the sexes; $U=393.5$, $n=81$, NS). I found no significant difference between the median date of moult for females that were housed alone prior to moult and those that were paired with a male over the same time period ($U=80335.5$, $n=1252$, NS). The median ($\pm$ lower and upper quartiles) intermoult interval for females was 25.0 (21.0, 26.0) days ($n=27$). No males moulted twice over the course of my experiments.

Figure 1-1. Proportions ($\pm 95\%$ confidence levels) of female *Corophium volutator* that became ovigerous when housed with a male in the laboratory following moults at time intervals of 1-2 days, >2-3 days, and >3-4 days, respectively. Female amphipods were collected at Blomidon, Nova Scotia. Sample sizes are shown above the bars.

Figure 1-2. Cumulative proportions of female (dashed line) and male (solid line) *Corophium volutator* surviving in the laboratory over the month of August, 1997. Amphipods were collected at Blomidon, Nova Scotia.

Figure 1-3. Overall temporal distribution of moults for 1042 non-ovigerous, adult female *Corophium volutator* in the laboratory in August, 1997. Apices for each phase of the moon also are shown (new and full moons correspond to spring tides, whereas first and last quarter moons correspond to neap tides). Amphipods were collected at Blomidon, Nova Scotia.

Figure 1-4. Temporal distributions of female moult for non-ovigerous, adult female *Corophium volutator* collected at 50-m intervals along the intertidal zone at Blomidon, Nova Scotia and housed in the laboratory in August, 1997. Locations of females that moulted on 9 and 10 of August were not determined.

Figure 1-5. Proportions ($\pm 95\%$ confidence levels) of female and male *Corophium volutator* that moulted in the laboratory over the month of August, 1997. Sample sizes are shown above the bars. Amphipods were collected at Blomidon, Nova Scotia.

4. Discussion

Assumptions of the 'mate-availability' hypothesis

Female *C. volutator* did not appear to reproduce asexually, whereas *C. bonnellii* females readily become ovigerous in the absence of males (Moore 1981).

Pseudogamy also is unlikely as there is genetic evidence for paternity in broods of *C. volutator* collected from Blomidon (Parsons 1995).

I found that female *C. volutator* were receptive to mating only for a short time following their moult, similar to other amphipods (Elwood et al. 1987; Borowsky 1988, 1991). Such refractory periods are believed to be caused by hardening of the brood pouch interfering with successful fertilization or reduction in waterborne pheromones reducing mate attraction (Borowsky 1991). Borowsky (1988) also found that the number of juveniles produced per brood decreased by 19% for the amphipod *Gammarus palustris* (Bousfield) when copulation was delayed by only six hours following a female's moult. I did not find a similar effect on recently fertilized (Stage 'A'; Peer et al. 1986) eggs for female *C. volutator*. However, delays between moult and copulation can result in females skipping a reproductive event entirely. In addition, my results suggest that females that delay their moult may further reduce their opportunities for mating because males suffer higher mortality than females over time.

Predictions of the 'mate-availability' hypothesis

In a female-biased population of amphipods, I observed that males were required for females to reproduce, that breeding ability was related to timing of the females' moult, and that females were receptive for relatively short periods of time. Thus, I expected that females should moult asynchronously to increase their likelihood of obtaining a mate (Knowlton 1979; Ims et al. 1988). However, I observed that females from similar patches moulted more or less synchronously in the laboratory. If anything, actual levels of synchrony may be higher in natural populations of *C. volutator* as laboratory studies may underestimate the degree of reproductive synchrony present (Klapow 1976). Reproducing synchronously should pose a disadvantage for female *C. volutator* by decreasing their probability of successfully finding and mating with a male; a costly strategy given that females are only receptive for a few days each month.

So why do female *Corophium volutator* reproduce synchronously? There is a seasonal constraint on females as the breeding season lasts ca. 4 months per year in the upper Bay of Fundy (Matthews et al. 1992). However, any disadvantage of reproducing a few days before or after the optimal date(s) should be more than offset by the advantage of reproducing at a time when few other females are receptive, particularly given that males are able to assess the ability of females to breed (Forbes et al. 1996). That female amphipods at a nearby mudflat, where sandpipers are present, begin to reproduce 2-3 weeks earlier than at Blomidon (Matthews et al. 1992) suggests that reproduction is not

necessarily limited to a narrow period of time. Thus, it appears that seasonal constraints alone cannot account for the high degree of synchrony I observed.

It also is unlikely that other hypotheses used to explain reproductive synchrony in intertidal organisms can explain the degree of moult synchrony I observed. Reaka's (1976) modification of Hamilton's (1971) 'selfish herd' hypothesis suggests that females should synchronize their reproductive activity around the same time as their neighbors to avoid being susceptible to predation by congeners or cannibalism by conspecifics during moult. However, this strategy is unlikely to be relevant for female *C. volutator* as they usually remain in their burrows (Boates et al. 1995). Furthermore, I have observed no evidence of predation on burrowed adult *C. volutator* by conspecifics or by other common macroinvertebrate intertidal fauna in natural populations or in the laboratory (D.G. McCurdy and J.S. Boates pers. obs. over 20 years of study). Klapow (1972, 1976) interpreted a high degree of synchronous moulting in the intertidal isopod *Excirolana chiltoni* in relation to tidal cycle as an adaptation to prevent females from being stranded above the mean high water mark (Klapow 1972, 1976). However, for female *C. volutator*, the risk of being stranded above the high tide line would seemingly be low because females usually remain in their burrows and because *C. volutator* are found in the mid-intertidal zone and not within a narrow vertical range, as *E. chiltoni*.

I believe a more likely explanation for the tidal pattern of moult is that it is timed to provide the best opportunities for dispersal of receptive or newly-mated

females and/or the eventual release of their broods. Support for the idea that dispersal of adults may explain reproductive synchrony was found by Meadows and Reid (1966) and Hughes (1988) who observed that adult and ovigerous female *C. volutator* (respectively) were more likely to swim during spring tides than during neap tides. Hughes (1988) argued that swimming was not directly linked to reproductive activity because swimming was not apparent over the entire breeding season. However, my observation that reproduction is highly synchronous may explain why Hughes (1988) did not consistently observe swimming by *C. volutator* over the breeding season. Support for the idea that moulting during spring tides relates to the future timing of dispersal of offspring comes from studies by Watkin (1941), Fish and Mills (1979), Gratto et al. (1983), and Peer et al. (1986), who observed that females tend to release broods during spring tides and from Lawrie and Raffaelli (1998), who observed that juveniles periodically swim during the progression from neap to spring tides. However, because other studies have found that recently-emerged juvenile *C. volutator* remain near the maternal burrow (Meadows and Reid 1966; Hughes 1988), it is unclear what advantage moulting during spring tides would have for recently-emerged progeny. Furthermore, Forbes et al. (1996) found evidence that female *C. volutator* releasing broods are close to moult, suggesting that females dispersing with mature broods may be close to their next moult. Thus, while further study of dispersal behaviour by adult and juvenile *C. volutator* is required, particularly at smaller scales, it appears that synchrony in female moult may be

linked to dispersal opportunities for amphipods; probably of adults rather than their offspring.

Although female amphipods are expected to skip reproductive bouts in the population I studied, females moulted 8 times more frequently than males; this should increase their chance of mating, at least once over their lifetimes. In other words, frequent moulting by females may compensate (at least partially) for the loss of mating opportunities caused by constraints which lead to synchronous reproduction (above). I did not find that female *C. volutator* reduce their time-to-moult in the presence of a potential mate. This was unexpected because studies on other species of amphipods have shown that a female may reduce her time-to-moult in the presence of a male (Kinne 1959; Nair and Anger 1979). This inability to hasten onset of moulting may be due to the high energetic costs associated with female moult; moulting crustaceans often are unable to feed (Shuster 1991b).

Implications for male reproduction

Reproductive synchrony in females has numerous consequences for males.

Although I found that males did not moult as frequently as females, my observation that males tended to moult at the same time as females was unexpected because it suggests that some males expend energy on moulting during the few days per month that females are receptive. This may indicate that moulting is not costly for males (as they most likely mate immediately after they

moult), or that the timing of male moult is also constrained (perhaps by similar factor(s) that constrain female moult).

My results also suggest that mating opportunities for males vary widely over the tidal cycle and among different levels of the intertidal zone. While male *C. volutator* may increase their level of mate-searching around a portion of the tidal cycle (Fish and Mills 1979), high levels of mate-searching occur throughout the spring and summer (Boates and Smith 1989; Boates et al. 1995). The maintenance of such high levels of mate-searching suggests that males may defend harems of females so that they can mate with them as soon as females become receptive. Such **harem-defense polygyny** has previously been observed in a siphonoecetine amphipod (Just 1988) and may also explain high levels of male-male aggression in *C. volutator*, despite female-biased sex ratios (Forbes et al. 1996).

Implications for testing adaptive host response

My results have consequences for life history responses to parasitism by male and female *C. volutator*. For female *C. volutator*, life history responses may be constrained because of the requirement for sexual reproduction. The female-biased sex ratios in this amphipod (Schneider et al. 1994), the requirement for females to moult to become receptive, the limited time that females are receptive, and the long time between moults place further constraints on females. One possibility is that females decrease their time to moult when parasitized (tested in Chapter 4). For

males, the high degree of synchrony in reproduction of females may limit their ability to respond to parasitism by altering their timing of reproduction. However, given that male *C. volutator* appear to exhibit mate-choice (Forbes et al. 1996), parasitized males may compensate for limited mating opportunities by increasing the investment they make to individual receptive females (e.g., fertilizing more eggs of females; tested in Chapter 5).

CHAPTER 2

DIURNAL AND NOCTURNAL FORAGING BY SEMIPALMATED SANDPIPERS (*CALIDRIS PUSILLA*) ON *COROPHIUM VOLUTATOR*

1. Introduction

Nocturnal foraging by shorebirds has been observed repeatedly (Dodd and Colwell 1996 and citations therein). However, little is known about the sensory ecology and energetics of shorebirds feeding at night, particularly for migratory species such as the semipalmated sandpipers (Boates 1980; Manseau and Ferron 1991). During their stop-over in the Bay of Fundy, sandpipers feed during the day almost exclusively on the intertidal amphipod *Corophium volutator* to fuel their 4300 km fall migration to South America (Harrington and Morrison 1979). Individual birds may consume >20,000 *C. volutator* per daily tidal cycle (Hicklin and Smith 1979). Boates (1980) determined that sandpipers feeding on *C. volutator* must deposit 10 g of fat and calculated that (at feeding rates of 30, 25, and 20 *C. volutator* per minute) sandpipers would have to ingest *C. volutator* larger than 4 mm or birds would face negative daily energy budgets. Despite the fact that sandpipers are size-selective (Goss-Custard 1969; Boates 1980), relatively few adult *C. volutator* (≥ 5 mm) are found by mid-August when sandpipers are present (Peer et al. 1986; Matthews et al. 1992). Thus, they may face negative energy budgets at that time.

In the Minas Basin of the Bay of Fundy, Boates (1980) observed some sandpipers feeding at night whereas Manseau and Ferron (1991) observed that counts of sandpipers on a mudflat rich in *C. volutator* were lower at night than during the day. Despite such apparent reductions in the number of nocturnally-feeding birds, Dugan (1981) and Mouritsen (1994) showed that activity levels of *C. volutator* may be higher at night and closer to the surface; thus prey are potentially more available. McNeil et al. (1992) suggested that shorebirds may feed at night to meet their energy requirements when energy demands are high (the 'supplemental' hypothesis).

The objective of this study was to compare nocturnal and diurnal foraging by migrant semipalmated sandpipers on *C. volutator*. To accomplish this, predation pressure by sandpipers was assessed indirectly by measuring the densities of their droppings to determine their amount of successful prey capture (or overall ingestion rate) as these factors are closely related in individual birds (Boates 1980). This technique has been used to study foraging pressure by mammals (Overton 1971) and I compared it to other methods (such as counting birds and measuring peck rates) that have been used to determine shorebird activity and foraging pressure on prey (e.g., Boates 1980).

2. Methods

I conducted studies at the Starrs Point mudflat located within the Hemispheric Shorebird Reserve in the Southern Bight of the Minas Basin, Kings County, Nova Scotia, Canada. The actual study area was a triangular-shaped mudflat approximately 6 km² (Appendix C). As it tapers seaward, its substrate changes from silt to firm mud, and finally to sand (Gratto 1978). The extremely high semi-diurnal tides which cross the mudflat range from 7.5 m during neap tides to over 15 m on spring tides and are the highest tides in the world (Canadian Hydrographic Service 1998). The mudflat between the shoreline and the beginning of the sandy area supports densities of *C. volutator* up to 60,000 per m², primarily along its numerous tidal channels (Boates 1980). This zone is rich in *C. volutator* and is unaffected by lunar-related changes in tidal range as it lies between spring and neap tide levels. Samples were taken on neap tides as this is the only time that the flats are exposed for much of the night. Starrs Point was chosen because it supports the highest densities of sandpipers in the area (Hicklin 1980).

To count droppings left by birds, 4 pairs of 10 x 10 m plots were staked out along the mudflat at ca. 500-m intervals perpendicular to shore. Pairs of plots were placed ca. 80 m apart to minimize disturbance to any birds feeding in the area during censuses. The first 6 plots (A-F) were bounded on each side by tidal channels while plots G and H were located in the middle of shallow tidal channels. All plots were sampled for *C. volutator* by removing a 100 cm²

substrate sample (5-10 cm deep to the bottom of the oxygenated layer) from the center of each quadrat on 8 August 1996. Amphipods were then classified as juveniles or adults ($<$ or $\geq$ 5 mm rostrum-telson length, respectively); adults were sexed following Schneider et al. (1994).

Bird droppings were counted in each plot on 26 August 1996 between 03:40 and 04:50 Atlantic Standard Time (AST; night) and 16:05 and 17:15 AST (day). The 12-hour and 25-minute advance corresponds to the semi-diurnal advance in the tidal cycle which ensures equal exposure time for both sampling periods. Each plot had been exposed for about 3 hours which allowed the birds to complete most of their feeding before the tide recovered the mudflat. I observed that droppings wash away completely on the rising tide (as do the shallow tracks of the sandpipers) so any droppings found within each plot were deposited during the time of exposure. The only shorebirds that leave droppings of this size are semipalmated and least Sandpipers *C. minutilla* (found in mixed flocks, but the latter are far outnumbered by the former; Hicklin and Smith 1984).

ANCOVA was used to determine if densities of droppings differed from day to night after correcting for any relationship between distance from shore and density of droppings. A $\log_{10}(x+1)$ transformation was used to correct for a lack of homogeneity of variance. A second-order polynomial term for distance from shore was added to correct for a lack of independence in the residuals (Zar 1996).

3. Results

Sandpipers began to arrive at Starrs Point on 11 July, and by 14 July, the number of birds foraging on the mudflat by day had increased from a few hundred to over five thousand. Substrate samples taken on 8 August showed high densities of *C. volutator* (Figure 2-1) and, by that time, all of the study plots had been visited by sandpipers on at least 10 occasions.

Using ANCOVA, I found that the relationship between distance from shore and density of droppings was not significantly different between day and night samples (interaction term distance x day/night $F=0.72$, NS, and distance² x day/night $F=1.41$, NS). On re-running the model without the interaction term, I found that the densities of droppings produced during the day and at night were again not significantly different ($F=0.42$, NS). However, I did find an overall positive relationship between distance from shore and the density of droppings (model, $r=0.76$, $n=16$, $P<0.005$; distance, $F=4.94$, $P<0.05$; distance², $F=7.02$, $P<0.05$; Figure 2-2).

Densities of *C. volutator* (recorded on 8 August) and densities of droppings were not related (day, $r=0.35$, $n=8$, NS; night, $r=0.35$, $n=8$, NS). There also was no significant relationship between *C. volutator* density and distance from shore ($r=0.20$, $n=8$, NS, Figure 2-1). However, during the day and at night few sandpipers were seen feeding in areas adjacent to the plots (where *C. volutator* densities were at least an order of magnitude lower than in the plots) and only sparse numbers of droppings were observed in such areas.

Figure 2-1. The relationship between densities of burrowed *Corophium volutator* (number per core sample: 100 cm² x 5-10 cm depth) and distance from shore (m) for eight plots (A-H) at Starrs Point, Nova Scotia (measured during the day on 8 August, 1996).

Figure 2-2. The relationship between densities of droppings left by semipalmated sandpipers (number of droppings per m²) and distance from shore (m) (curve is based on all data points). Droppings were counted during the day and at night on 26 August, 1996.

4. Discussion

I believe that my finding of little or no differences in sandpiper foraging activity during the day and at night is significant, although this study quantifies feeding on only one night and counts of sandpipers at night can be highly variable (Manseau and Ferron 1991). Unlike counts of birds, counts of droppings represent a cumulative amount of feeding over the entire tidal cycle and shorebirds consistently return to the same plots on successive tides (Boates 1980).

During the day, successful capture rates of *C. volutator* and defecation rates are related (Boates 1980) and feeding rates are limited only by digestion rate, which is dependent on environmental temperatures (Klaassen et al. 1990, but see Zwarts and Dirksen 1990). There is no reason to believe that assimilation efficiency of prey differs between day and night since air temperatures on the mudflat are similar during the day and at night (Manseau and Ferron 1991). Thus, I conclude that either there were: more birds feeding at night less successfully, the same number of birds present with a similar rate of feeding success, or fewer birds present with a higher capture rate. Regardless of the actual numbers of birds present, densities of droppings indicated that predation pressure on *C. volutator* was at least as great within the plots at night.

The second-order relationship I observed between density of droppings and distance from shore may be best explained by the shape of the mudflat itself. The mudflat narrows sharply around Plots G and H and as a result, sandpipers

feeding on the ebbing tide aggregate in high densities there (Boates 1980). The finding that densities of droppings were unrelated to prey densities in the plots supports results of Boates (1980) and Wilson (1990). These studies found that, above a threshold density (approximately 2000 per m²), densities of feeding sandpipers and *C. volutator* are not related. All of my plots had densities >8300 burrowed *C. volutator* per m².

I believe that the counts of droppings used in my study provide a more useful index of shorebird feeding on the mudflat during the day and night than do direct counts of birds or measures of peck rates. Bird counts using lights may cause disturbance in some cases (Goss-Custard 1969) and they record only the number of birds present at one moment. For peck rates to be useful, the relationship between peck rate and successful capture, and the behaviour of the prey must be known. Mouritsen (1994) found that Dunlins (*Calidris alpina* Schinzii) preferred to feed on a mudflat rich in *C. volutator* at night and that burrowed amphipods were closer to the surface; thus prey may not be equally available over time. He also found that during the day, Dunlins pecked the mud while at night probing was more common. For other species of shorebirds, McNeil et al. (1995) observed that prey availability in tropical wintering areas was greater at night than during the day while Zwarts (1990) observed differences in the foraging strategies of Whimbrels (*Numenius phaeopus* L.) feeding on fiddler crabs (*Uca tangeri* Eydoux) during the day and at night. Dugan (1981) suggested, and Boates and Smith (1989) found evidence, that foraging success

of semipalmated sandpipers was greater when peck rates were lower. However, Pienkowski (1980), Manseau and Ferron (1991), and Mouritsen (1994) interpreted lower peck rates, particularly at night, to mean the opposite. Clearly, peck rates may be unreliable; particularly those used to compare feeding at different times.

With respect to nocturnal foraging by semipalmated sandpipers, my results support the 'supplemental' hypothesis of nocturnal feeding in shorebirds (McNeil et al. 1992). That is, shorebirds feed at night to meet their energetic requirements. In most cases, this has been used to explain winter foraging by birds in temperate climates (Goss-Custard 1970; Heppleston 1971) and foraging in tropical wintering areas (Zwarts et al. 1990; McNeil et al. 1995). In my study, fat deposition for the fall migration was probably the reason for foraging at night. I expected that feeding success would differ between night and day. This is particularly so considering that sandpipers rely at least partly on visual cues to locate prey (Boates 1980; Pienkowski 1981) and although they may switch to tactile foraging at night (McNeil and Robert 1988; Robert and McNeil 1989) sandpipers supplementing their diet by feeding at night would most likely have different rates of success in locating prey at night, unless it was more available (25 August was a dark night as the moon was not visible until after the counts of droppings were completed). If numbers of birds were the same between day and night, then success rate would not differ between day and night and night foraging would be equally important to energetics of migrant sandpipers.

As mentioned in the General Introduction, I used information on diurnal versus nocturnal foraging by sandpipers on *C. volutator* to make fine-tuned predictions related to parasite manipulation and host response. In the Chapters that follow, I outline how day-night comparisons in behaviour of infected versus uninfected amphipods can be used to test between parasite- and host-mediated explanations for changes in amphipod behaviour. My finding that predation pressure was as great at night as during the day means that transmission of parasites from intermediate-host amphipods to final-host sandpipers may occur at both times. However, amphipods that exhibit certain behaviours (e.g., crawling on the surface of the mud) would be expected to differ in their susceptibility to sandpiper predators between day and night (sandpipers forage visually during the day and blindly probe the mud at night; Robert and McNeil 1989; Mouritsen 1994).

CHAPTER 3

EVIDENCE THAT THE PARASITIC NEMATODE *SKRJABINOCLAVA MORRISONI* MANIPULATES BEHAVIOUR OF ITS INTERMEDIATE HOST, *COROPHIUM VOLUTATOR*

1. Introduction

In recent years, ecologists and evolutionary biologists have unraveled the complex interplay between some parasites and their hosts. For example, some parasites appear to alter the behaviour of intermediate hosts, in ways to increase their likelihood of transmission to final hosts (Holmes and Bethel 1972; Moore 1995). However, there can be costs to manipulating host behaviour (e.g., production of chemicals that interfere with 'normal' host behaviour; Helluy and Holmes 1990). Consequently, natural selection should optimize (not maximize) the timing and amount of manipulative effort of parasites (the 'manipulation' hypothesis). Energy not used to manipulate host behaviour could be saved or channeled into other fitness traits, such as parasite growth or fecundity (Poulin 1994a).

Researchers have detailed predictions concerning timing of manipulation of host behaviour (Combes 1991; Poulin 1994a; Poulin et al. 1994). Parasites should begin to manipulate their hosts only once they have developed sufficiently to be transmitted successfully to the final host, as being ingested prior to development of the infective stage of the parasite would result in the parasite's

death. Manipulative effort also should be likely when parasites, prey, and predators have limited temporal overlap (e.g., migrant birds visiting staging areas for a few to several weeks).

A major difficulty in testing for adaptive manipulation of host behaviour is in determining whether any behavioural changes in hosts are due to the parasite, the host, both parasite and host, or side-effects which are adaptive for neither host or parasite (Milinski 1990; Poulin et al. 1994; Poulin 1995). Using meta-analysis, Poulin (1994b) showed that parasites, including *acanthocephalans* and nematodes, appeared to alter behaviour of their hosts. However, he found that manipulation occurred less often than expected (i.e., when it otherwise would have been adaptive for the parasite). Poulin's work suggests that failure to elicit changes in host behaviour may be due to phylogenetic constraints on the parasite. This problem is compounded by the fact that there are few tests of the manipulation hypotheses in natural systems in which the ecologies of intermediate and final hosts are well understood. Factors such as the timing of changes in (anti-predator) behaviour must be known in relation to development of parasites, in order to adequately test for constraints or adaptations of parasites.

My general objective was to test predictions of the 'manipulation' hypothesis. In particular, I tested whether an *acuaroid* nematode (*Skrjabinoelava morrisoni*) could increase its likelihood of transmission to its final host (a sandpiper, *Calidris pusilla*) by altering the behaviour of its intermediate host, the amphipod *Corophium volutator*. To this end, I observed behaviour of infected and

uninfected amphipods in both the field and laboratory. I also determined if infected *C. volutator* show different behavioural responses in daylight versus darkness (e.g., increased versus decreased frequency of crawling, respectively). I hypothesized that nematodes should interfere with normal anti-predator responses. However, these responses may be context-dependent. For instance, nematodes which increase crawling activity of amphipods when predators are unable to use visual cues (at night) would not increase the intermediate host's probability of being ingested.

Rationale of study

For several reasons, the amphipod-nematode-sandpiper system I studied is ideal for investigating possible manipulation of host behaviour by parasites. First, *C. volutator* averages 86% of the diet of sandpipers as they stage in the Bay of Fundy (Hicklin and Smith 1979). Each bird may consume >20,000 amphipods per day (Hicklin and Smith 1979). Second, sandpipers prefer to feed on crawling amphipods during daylight hours (usually mate-searching males; Boates and Smith 1989), leading to the prediction that parasites should increase crawling activity of male *C. volutator* at that time to increase their likelihood of being ingested by a final host. Although the ecology of *S. morrisoni* is relatively unknown, laboratory studies have shown that *C. volutator* is an intermediate host for this nematode (Wong and Anderson 1988; Appendix E). *Skrjabinoclava morrisoni* completes its life cycle in semipalmated sandpipers; the larvae migrate

to the proventriculus where they mature, mate, and release eggs (Wong and Anderson 1987). Presumably, nematodes are ingested by sandpipers during their fall migration through the Bay of Fundy (Wong and Anderson 1990): this supposition can be tested indirectly by examining when natural infections of nematodes appear. In doing this study, I provide the first description of the distribution of *S. morrisoni* in natural populations of *C. volutator*. This is needed to address the extent of temporal overlap between nematodes, *Corophium*, and sandpipers.

Specific predictions

If behavioural alteration increases the likelihood of a nematode being ingested by a sandpiper, then late-stage larvae of nematodes that are capable of infecting final hosts should be found disproportionately in crawling amphipods. If male amphipods infected with early-stage larvae show more crawling than uninfected males, then this is unlikely to be a parasite adaptation, but may be a host adaptation (e.g., to optimize mate-searching effort if parasites affect future survival (Forbes 1993)), or it may be a non-adaptive side effect of parasitism.

I also compared crawling activity of parasitized and unparasitized amphipods in daylight and in darkness. Little is known about the ecology of *C. volutator* at night. However, sandpipers may feed at night (Chapter 2), although they switch from visual to tactile cues to locate their prey at night (i.e., pecking versus probing; Robert and McNeil 1989; Mouritsen 1994). As mentioned above,

if crawling activity is greater at night in infected amphipods, then parasites may not increase their transmission rates at that time.

I also addressed whether nematodes affect other amphipod behaviours or amphipod survival, in the laboratory. These other behaviours were unrelated or tangentially related to the probability of ingestion by final hosts. I expected that parasitized males, compared with uninfected controls, might travel a shorter distance per unit time, and/or attempt to enter fewer (artificial) burrows of potential mates. I also examined trail complexity of male amphipods. Trail complexity can decrease if an organism is under stress (Alados et al. 1996); thus, simpler (linear) trails may be a side effect of parasitism, although sandpipers may follow linear trails better than complex trails. Finally, I predicted that parasitized amphipods should be positively phototactic while crawling, but not while swimming, as this could make them susceptible to predation by fish (Imrie and Daborn 1981); these are inappropriate final hosts of *S. morrisoni* (Wong and Anderson 1987).

2. Methods

Field Studies

I collected *C. volutator* from the mudflat at Starrs Point, Nova Scotia, Canada (Appendix C). This 4-km wide mudflat is located within the Hemispheric Shorebird Reserve in the Southern Bight of the Minas Basin and is exposed twice daily to tides ranging from 7.5-15 m (Canadian Hydrographic Service 1998). I chose to sample at Starrs Point because it supports large populations of *C. volutator* (Boates 1980). In addition, large flocks of migratory sandpipers arrive in mid-summer to forage at this site (Matthews et al. 1992; Boates et al. 1995). This allowed me to compare crawling activity of amphipods and prevalence of their nematode parasites before versus after the arrival of the sandpipers, and also when predation by sandpipers on *crawling* amphipods was high versus negligible (daytime versus nighttime hours, respectively). I made frequent trips to Starrs Point and to nearby roosting sites throughout the month of July to determine when migrating sandpipers arrived in 1996, 1997, and 1998. Over the last 20 years, sandpipers have arrived from mid to late July (J.S. Boates pers. comm.).

I made 23 visits to Starrs Point (May to August) over three years to compare prevalence of nematodes in crawling versus non-crawling hosts, both during the day (1110-1450 Atlantic Standard Time) and at night (2205-0235). All trips were made on neap tides, when the mudflat is exposed for extensive periods at night (Boates 1980). I sampled within eight 10 x 10 m plots marked

with stakes and colored tape. Each plot contained a large sub-population of *C. volutator* in previous studies (Boates et al. 1995; Figure 2-1). Plots were placed in four pairs (members of pairs were 15-30 m apart) along tidal channels at 500 m intervals. This spacing allowed me sufficient time to reach each plot prior to its being uncovered by the tide.

I sampled for infected amphipods primarily within the four plots located lowest in the intertidal zone (where densities of foraging sandpipers were high; Figure 2-2). Sandpipers did not forage in plots, while plots were being sampled. I collected both crawling males and females only in areas that had been uncovered by the tide within 15 minutes. Each adult amphipod (≥ 5.0 mm, rostrum-telson length) was placed in a 3 ml microtube filled with seawater. I then collected two burrowed amphipods that were within 10 cm of the crawling amphipod and were of the same sex and approximate size (within 2 mm). I placed both of these amphipods in an adjoining microtube. All three amphipods were subsequently squashed to determine if nematodes were present (viewed at 40x magnification). Parasites were identified as early-stage larvae (Stages I and II) or late-stage larvae (Stage III) using characters described by Wong and Anderson (1988) (Appendix E). Although Wong and Anderson (1988) did not describe Stage IV larvae from amphipods exposed in the laboratory, I occasionally found larvae in amphipods which, based on their size and the extent to which the spicules of males were developed, were best described as Stage IV. I also considered these as late-stage larvae.

I counted crawling male and female amphipods during the day and at night (using flashlights) in 1997. To sample crawling activity before sandpiper arrival (28 June-1 July), I placed a 0.5 x 0.5 m quadrat randomly within each plot just as the water receded from it. For day trips after sandpiper arrival (27-30 July), I used a larger 1 x 1 m quadrat because *C. volutator* is known to greatly reduce its crawling activity at this time (Boates et al. 1995) and I wanted to collect at least some daytime crawling amphipods from quadrats for comparison. Amphipods that emerged to crawl during the first 5 minutes after the tide receded were collected using live-specimen forceps (most amphipods that crawled emerge almost immediately and rarely emerged after 5 minutes; crawling also does not appear to be influenced by the presence of human collectors; Boates and Smith 1989; Boates et al. 1995; pers. obs.). In the laboratory, all amphipods were sexed using the criteria of Schneider et al. (1994) and classified as either juveniles (<5.0 mm) or adults (5.0-11.0 mm).

Laboratory Studies

To infect *C. volutator* with *S. morrisoni* experimentally in the laboratory, I collected 91 adult male amphipods from the mudflat at Blomidon, Nova Scotia (Appendix C). Blomidon is close to Starrs Point (<10 km; Appendix C), but is not visited by shorebirds (Matthews et al. 1992). In addition, amphipods at this site were not infected by nematodes (Chapter 1). Thus, I was able to avoid potentially confounding effects of natural infections. I collected amphipods on 30 July 1996

and housed them in a 1-l container with aerated artificial seawater at 18‰ (20°C).

To obtain nematode eggs, ten adult *C. pusilla* were shot on 31 July 1996. I dissected each bird in the field and removed the section of its digestive tract containing the proventriculus and placed it in avian saline (8 g NaCl/l distilled water). In the laboratory, I plucked all nematodes from the proventriculus. I then cut each worm into four pieces and placed these in a microtube containing avian saline and shards of cover slips. I agitated the microtube on a vortex shaker for 5 minutes. This method effectively teased out and separated the eggs which I then placed in a 1-l container filled with artificial seawater.

To help me ensure adequate numbers of infected males, I took 72 amphipod males and added them to the solution containing the nematode eggs. I then added the cultured diatom *Thalassiosira* sp. (25,000 cells/ml) to elicit the feeding response of amphipods (as the nematode eggs must be ingested in order to develop; Wong and Anderson 1988). I exposed many males because I expected only some of them to become infected and I also needed to track the development of the parasites, which required that I periodically dissect an exposed amphipod. Obviously, these dissected amphipods could not be used in experimental trials. The remaining 19 males were housed in a separate container, fed *Thalassiosira* sp., but were not exposed to nematode eggs.

After 36 h, each male was removed and placed in an individual culture tube containing a non-ovigerous adult female *C. volutator* (housing methods

described in Chapter 1). The females provided males with well-maintained burrows (only females construct burrows; Meadows 1964). Each culture tube was aerated constantly and 10 ml of water was changed every 3 days, at which time amphipods were fed diatoms (methods described in Chapter 1). I observed whether males were on the surface or in their burrows at the beginning of the experiment (3 days post infection/post non-infection) and prior to removal for other experiments (following 17 days).

On 18 August, I removed all males from their culture tubes and used them in a series of experiments. First, each male was individually placed on a steel pan (30-cm diameter) lined with 1 cm of fresh mud. Seventy-two holes (0.2-0.3 cm diameter) were uniformly excavated in the mud to simulate entrances to the burrows of female amphipods. I then placed each male in the center of the pan facing a random direction and allowed him to crawl for 5 minutes, during which time his path was filmed. I gently rinsed the surface of the pan with seawater between male placements to ensure that artificial burrows remained in the same locations and to minimize any influences of the previous male. I recorded trail complexity, total distance traveled, time spent crawling, the number of artificial burrows probed, and the number of burrows passed over (not probed).

I recorded phototactic responses of exposed and unexposed males while they were crawling and swimming on 19 and 20 August (17 and 18 days post infection/post non-infection, respectively). I used phototactic chambers based on Moore's (1983) design. Chambers were constructed using two 25-cm Pyrex pie

plates. One plate was placed upside down on top of the other and half of the chamber was painted black. For experiments on phototactic responses of crawling amphipods, I lined the bottom of the chambers with fresh mud. For experiments on phototactic responses of amphipods while swimming, I filled the bottom of the chamber with artificial seawater (18‰). In each case, I placed a cardboard divider between the light and dark sides of the plate, with a 1-cm space remaining between the bottom of the divider and surface of the bottom plate. This space was needed to ensure that amphipods had easy access to either side of the plate. During each trial, I placed males, either exposed or not exposed to nematode eggs, individually into either the light or dark side of a chamber. I allowed males to crawl for 2.5 minutes or swim for 1 minute, after which I recorded the location of each male. Following this, I squashed each male to look for *S. morrisoni*.

Analyses

I used χ^2 tests of independence to analyze data on prevalence of nematodes in crawling and non-crawling amphipods, except where one or more of the expected frequencies was <6 , in which cases I used Fisher exact tests (Zar 1996). I applied power analysis to all non-significant χ^2 comparisons to ensure adequate statistical power (Cohen 1988). I transformed $(\ln(x+1))$ counts of crawling amphipods to correct for normality and a lack of homogeneity of variance, assessed using Kolmogorov-Smirnov and Bartlett's tests, respectively

(Zar 1996). Using parametric analyses of variance, I then compared crawling activity between day versus night and before versus after sandpiper arrival. The inferences I draw from this study apply specifically to nematodes and amphipods from Starrs Point. Thus, I considered samples from each plot as independent.

I used fractal analysis to compare the complexity of trails of parasitized and unparasitized *C. volutator*. Specifically, I used With's (1994) variation of the dividers method (Dicke and Burrough 1988; Appendix F). I used a multiple analysis of variance (MANOVA) to examine whether multivariate measures of behaviour based on trail complexity, total path length, time spent crawling, number of artificial burrows probed, and number of burrows not probed, differed between parasitized and unparasitized amphipods. Each response variable was recorded over a single 5-minute period. I used MANOVA over a series of univariate tests to reduce the probability of finding a significant result by chance and because I expected that the dependent variables were correlated. Due to the relatively small sample size of the control group ($n < 20$), I used univariate tests to verify that data met assumptions of normality and homogeneity of variance (Mardia 1980; Scheiner 1993). The statistical software package Statistica (Statsoft Inc. 1993) was used for all analyses, following Zar (1996).

3. Results

Nematodes and crawling amphipods (field results)

I start with results on crawling activity of uninfected male and female amphipods during day and night as these results influence some of the predictions concerning crawling of infected animals. I found no significant difference in the mean number of male amphipods crawling between day and night before sandpiper arrival in 1997 ($F_{[1,28]}=1.98$, NS). However, there was a significant decrease in crawling daytime activity of males following sandpiper arrival ($F_{[1,28]}=44.59$, $P<0.0001$; Figure 3-1). In comparison, the mean number of nighttime crawling males did not change following sandpiper arrival ($F_{[1,28]}=1.35$, NS). Accordingly, the numbers of crawling males were far higher at night than during the day, following sandpiper arrival ($F_{[1,28]}=53.10$, $P<0.0001$). As with males, I found no significant difference between the mean number of crawling females during the day and night, before sandpiper arrival ($F_{[1,28]}=1.28$, NS; Figure 3-1). After sandpiper arrival, however, more females were observed crawling at night than during the day ($F_{[1,28]}=16.21$, $P<0.0005$).

To obtain data on the distribution of *S. morrisoni* in natural populations of *C. volutator*, I examined 1153 crawling amphipods and 2060 burrowed amphipods (Table 3-1). I found that nematodes were present only in samples collected from mid-late August in all three years of study. Within these time periods during the day, I found that nematodes were more likely to be found in crawling amphipods than burrowed amphipods (males: Fisher exact $P_{[1]}<0.005$,

$n=263$; females: Fisher exact $P_{[1]} < 0.02$, $n=213$). On trips at night during this time, however, I found no significant difference in prevalence of nematodes between amphipods that were crawling versus burrowed within the substrate (males: $\chi^2_{[1]} = 0.26$, $n=378$, NS; females: Fisher exact $P_{[1]} = 1$, $n=369$). Male amphipods were no more likely to be infected by nematodes than female amphipods ($\chi^2_{[1]} = 0.52$, $n=3213$, NS).

In considering developmental stages of parasites, I found that during the day, late-stage (infective) nematode larvae were more often found in amphipods that were crawling on the surface than in burrowed amphipods (Fisher exact $P_{[1]} < 0.0001$, $n=503$; Table 3-1). However, early-stage nematode larvae were no more likely to be found in crawling than in burrowed amphipods (Fisher exact $P_{[1]} = 0.30$, $n=503$). There was no significant difference in prevalence of early- or late-stage larvae in crawling versus burrowed amphipods at night (late-stage larvae: $\chi^2_{[1]} = 0.03$, $n=771$, NS; early-stage larvae: Fisher exact $P_{[1]} = 0.33$, $n=754$). For all non-significant χ^2 tests, power (1-B) to detect a significant difference for a 'moderate' effect size ($w=0.3$; Cohen 1988) exceeded 0.95.

Behaviour and survival of male amphipods (laboratory results)

In the laboratory experiment, all 72 males exposed to eggs of *S. morrisoni* became infected by third-stage larvae ($\bar{x} \pm \text{S.E.} = 13.7 \pm 0.8$ larvae). By examining exposed males that died during the experiment, I determined that it took ca. 13 days for nematodes to develop into Stage III larvae in the laboratory. Nine males

died during the course of the experiment and all were infected by nematodes; however, survivorship over the experiment did not differ significantly between the infected and uninfected amphipods ($\chi^2_{[1]}=2.64$, $n=85$, NS). In addition, there was no significant difference in the size of infected and uninfected males at the end of the experiment ($t_{[78]}=0.74$, NS). In examining behaviour, I found no significant difference in the number of parasitized versus unparasitized males on the surface at the start of the experiment (3 days post infection/post non-infection: $\chi^2_{[1]}=1.39$, $n=85$, NS), but significantly more parasitized than unparasitized males were recorded on the surface when checked 18 days post infection/post non-infection ($\chi^2_{[1]}=7.56$, $n=82$, $P<0.01$; Figure 3-2). By this time, nematodes had developed into their infective stage.

From film analysis, I found no significant differences between parasitized and unparasitized males in mean complexity or total length of trails, time spent crawling, number of burrows probed, and number of burrows passed over during a 5-minute period (Wilk's lambda=0.95, $n=83$, NS; Table 3-2). With respect to phototactic responses, I found no significant difference between parasitized and unparasitized amphipods while crawling ($\chi^2_{[1]}=0.03$, $n=77$, NS; 87% of parasitized and 88% of unparasitized amphipods used the dark side of the chamber) or swimming ($\chi^2_{[1]}=1.24$, $n=80$, NS; 84% of parasitized and 72% of unparasitized amphipods used the light side of the chamber).

Table 3-1. The numbers of male and female *Corophium volutator* infected by the nematode parasite *Skrjabinoclava morrisoni* versus the numbers examined for crawling and burrowed samples collected from the mudflat at Starrs Point, Nova Scotia during the day and at night.

Date collected	Time	Crawling no. infected (examined)		Burrowed no. infected (examined)	
		Females	Males	Females	Males
08/08/96	day	0 (1)	0 (5)	0 (32)	0 (34)
09/08/96	night	0 (7)	0 (9)	0	0
* 23/08/96	day	2 (5)	1 (4)	0 (10)	0 (8)
* 24/08/96	night	0 (3)	0 (4)	0 (6)	0 (9)
27/05/97	day	0 (9)	0 (1)	0 (18)	0 (1)
16/06/97	day	0 (96)	0 (118)	0 (198)	0 (215)
28/06/97	day	0 (16)	0 (16)	0 (30)	0 (32)
30/06/97	night	0 (16)	0 (16)	0 (29)	0 (32)
01/07/97	day	0 (15)	0 (17)	0 (29)	0 (34)
01/07/97	night	0 (16)	0 (16)	0 (31)	0 (32)
27/07/97	day	0 (15)	0 (16)	0 (32)	0 (32)
29/07/97	night	0 (14)	0 (16)	0 (33)	0 (31)
30/07/97	day	0 (12)	0 (11)	0 (31)	0 (32)
30/07/97	night	0 (16)	0 (16)	0 (32)	0 (31)
* 13/08/97	day	0 (7)	1 (22)	1 (12)	1 (44)
* 18/08/97	day	3 (10)	5 (32)	0 (17)	1 (41)
* 21/08/97	day	1 (15)	3 (14)	2 (29)	0 (31)
* 24/08/97	night	2 (11)	1 (19)	0 (25)	2 (32)
13/06/98	day	0 (53)	0 (1)	0 (53)	0 (0)
23/06/98	day	0 (48)	0 (49)	0 (52)	0 (45)
22/07/98	day	0 (48)	0 (52)	0 (50)	0 (48)
* 17/08/98	day	2 (36)	2 (20)	0 (72)	2 (47)
* 19/08/98	night	2 (106)	5 (104)	8 (218)	7 (210)

*Samples used for χ^2 tests (dates when nematodes first appeared and subsequent dates within the same year).

Table 3-2. Comparison of univariate response variables for male *Corophium volutator* parasitized by the nematode *Skrjabinoclava morrisoni* (n=65) and uninfected male controls (n=18). Crawling responses of males were observed 18 days post infection/post non-infection.

Variable	Parasitized $\bar{x} \pm (\text{S.E.})$	Unparasitized $\bar{x} \pm (\text{S.E.})$	<i>t</i>	<i>P</i>
Fractal dimension (D)	1.3 (0.01)	1.3 (0.02)	0.3	NS
Trail length (cm)	88.3 (3.6)	90.5 (6.0)	0.3	NS
Time spent crawling (s)	274.7 (5.0)	278.5 (5.9)	0.4	NS
No. probes	2.6 (0.2)	2.3 (0.3)	0.7	NS
No. passed by	5.7 (0.4)	6.9 (0.7)	1.6	NS

Figure 3-1. Mean ($\pm$ one standard error) transformed numbers of adult male and female *Corophium volutator* observed crawling on the Starrs Point mudflat during the day ($\square$) and at night ($\blacksquare$). The first four dates in each graph (2-8 ebb tides after June 27; the date of a last quarter moon) represent days and nights prior to the arrival of migrating sandpiper predators. The last four sampling dates (58-64 ebb tides after June 27) followed the arrival of migrating sandpipers. Each mean is based on eight samples.

Figure 3-2. The proportions ($\pm 95\%$ confidence levels) of parasitized versus unparasitized male *Corophium volutator* found on the surface of mud-lined test tubes at 3 and 18 days post-infection/post non-infection by the nematode *Skrjabinoclava morrisoni*. Sample sizes are shown above the bars.

Prop. male amphipods on the surface

4. Discussion

Nematodes and crawling amphipods (field results)

My results verify that *C. volutator* is an intermediate host for *S. morrisoni* in natural populations. Parasites infected only the summer generation of amphipods (June-September) and did not overwinter in the fall generation. Either these amphipods were not infected or, if infected, amphipods did not survive to be sampled in the spring. Developmental time for nematodes in amphipods was 4-5 weeks in the field because sandpipers began to arrive in mid-late July and late-stage larval parasites did not begin to show up in field collections until mid-August. This means that the parasite only has a short time to complete its life cycle because most of the migratory sandpipers remain in the Bay of Fundy for only 4-6 weeks (Boates 1980). Furthermore, there is limited opportunity for transmission of nematodes as most of the potential intermediate hosts are from the summer generation which does not survive until the return of sandpipers, the following summer (Peer et al. 1986; Matthews et al. 1992). I never found nematodes in amphipods collected at a nearby mudflat (Blomidon) where sandpipers do not feed ($n=516$), suggesting that sandpipers are the only final hosts for *S. morrisoni*.

I suggest that adaptive manipulation by parasites, and not host response, was responsible for the increased daytime presence of infected hosts on the surface of the mud. Manipulation of host behaviour is likely for this system because passive transmission rates for parasites that infect amphipods that

remain burrowed are likely low, as overall numbers of burrowed amphipods at Starrs Point are very high ($>10,000/\text{m}^2$; Chapter 2). My results further support the 'manipulation' hypothesis because I found that only late-stage/infective parasites were more often found in crawling than in burrowed male and female amphipods, during the day. The fact that infected females were more likely to crawl than uninfected ones provides strong evidence in support of parasite manipulation because uninfected females rarely crawl ($<1\%$, Boates et al. 1995 and this study) and such increases are likely non-adaptive for females (Forbes et al. 1996). It is unlikely that increased crawling in amphipods was the result of infections in amphipods that regularly crawled (i.e., for reasons unrelated to parasitism) because prevalence of nematodes did not differ between males and females, despite that fact that a higher proportion of males crawled than females.

I predicted context-dependent changes in parasite manipulation of host behaviour (i.e., parasites should not manipulate crawling effort at night because this could be maladaptive for the parasite). My results are consistent with this because males and females were no more likely to be found crawling than burrowed, at night.

Behaviour and survival of male amphipods (laboratory results)

My laboratory results also support the idea that nematode parasites adaptively cause amphipods to be more susceptible to predation by birds. Specifically, I found that infected males were more likely to be on the surface, but only after the

parasites had developed to their infective stage. Similar results were found in laboratory experiments by Mouritsen and Jensen (1997) who observed that *C. volutator* infected by the trematode *Maritrema subdolum* Jägerskiöld were observed more often on the surface of the mud than burrowed within the substrate (although these researchers suggested that pathological side-effects of infection might also explain increased activity on the surface). I did not find other differences in crawling activity of males even though amphipods had intensities of infection that were much higher than those recorded from my field collections. I also found no difference in phototactic behaviour between infected and uninfected amphipods housed in the laboratory. One possible explanation for this is that parasites manipulate responses of amphipods to natural cues which were absent in the laboratory (e.g., sandpiper feeding cues or tidal cues can affect responses by *C. volutator*). Alternatively, manipulation of only one behaviour that increases the susceptibility of amphipods to final host predators would be an efficient use of resources for the parasite, if specific manipulations are costly. Additionally, the relation between these other behavioural changes and susceptibility to predation was less obvious and any alterations could reflect host decisions or side-effects of infection rather than parasite manipulation.

Nematode larvae reached their infective stage (Stage III) faster in the laboratory (13 days) than in the field (4-5 weeks). My results agree with those of Wong and Anderson (1988) who also found that development time of *S. morrisoni* in amphipods was 13 days. The difference in development time

between the laboratory and the field likely relates to differences in ambient temperatures. Laboratory studies (Wong and Anderson 1988; this study) have used a constant temperature of 20°C while substrate temperatures at Starrs Point are usually below this (Piccolo et al. 1993). These results highlight the fact that behavioural changes are tuned to development of nematodes, even when field and laboratory conditions vary greatly.

Finally, my results indicate that nematodes do not affect other fitness measures of male amphipods, beyond increasing the amphipod's susceptibility to predation. Specifically, I found no difference in survivorship of male amphipods over 20 days and no difference in the final body size between parasitized and unparasitized amphipods. This is not surprising as killing the host before reaching maturity would mean death for the parasite (although killing the host after maturity may not as amphipods also come to the surface when they are near death; pers. obs.). The fact that sandpipers continue to feed on crawling amphipods, despite the increased risk of ingesting parasites, suggests that the costs of parasitism by nematodes may be lower to the birds than the benefits of consuming readily visible prey (Lafferty 1992). My results suggest that manipulation of even simple aspects of host behaviour may allow parasites with **complex life cycles** to persist in situations where there is little temporal overlap between intermediate and final hosts.

CHAPTER 4

TESTING ALTERNATIVE HYPOTHESES FOR VARIATION IN LIFE HISTORY OF FEMALE *COROPHIUM VOLUTATOR* INFECTED BY THE TREMATODE *GYNAECOTYLA ADUNCA*

1. Introduction

Many studies have documented life history differences between parasitized and unparasitized hosts within populations. There is convincing evidence that some of these changes are the result of manipulation of host behaviour by parasites (e.g., Moore 1995; Chapter 3). Parasite-mediated changes in host behaviour often have been found in intermediate hosts, where parasites manipulate behaviour of intermediate hosts in ways to increase transmission to their final hosts (the 'manipulation' hypothesis; Poulin 1994a; Moore 1995). Such studies beg the question as to why the host appears to be a passive vehicle for parasite transmission, rather than entering into a conflict over limited energy or time budgets with the parasite (Keymer and Read 1991). Other studies have suggested that hosts should alter their investment in current reproduction once parasitized, especially if hosts receive reliable cues that they are parasitized, and providing there are costs to not responding with alterations (Minchella 1985; Forbes 1993). Such host-mediated responses may even be less costly than maintaining active immune responses (Forbes 1993). The type of host response also should depend on the type of parasite-host association. For

example, if parasites continue to grow inside hosts and exert a high mortality cost over time, then parasitized hosts should increase investment in current reproduction because they will realise higher fitness gains from this investment than from 'waiting' to invest later. In this chapter, I am interested in investigating the extent to which both manipulation by parasites and responses of hosts to parasitism explain differences between parasitized and unparasitized hosts with respect to behaviour and investment in reproduction.

Hypotheses concerning parasite- and host-mediated changes in host behaviour and reproduction differ in one key element, i.e., the former suggests parasite control over energy or time budgets of hosts, the latter suggests that hosts are in control. I used the **digenean** trematode parasite *Gynaecotyla adunca* and females of its second intermediate host, the amphipod *Corophium volutator* to determine whether either manipulation by parasites or adaptive responses of hosts could explain behaviour and life-history variation in infected hosts. Typically, any change in behaviour or life history traits of infected hosts is viewed either: a parasite adaptation to exploit hosts to their own ends, a host adaptation to minimize the costs of parasites', or a non-adaptive side-effect of parasitism (Poulin 1995). These hypotheses are normally viewed as mutually exclusive (Poulin et al. 1994), but need not be. The following example will help illustrate this point. Behavioural or physiological fever could help vertebrates battle infection by certain parasites (e.g., *Plasmodium* and *Trypanosoma*) may have evolved in that context. Fever responses, however, may not be effective

against all possible pathogens and may use up time or energy that could be channelled into other immunological responses. Fever can be viewed as either a host adaptation or a response actually favouring the parasite (although not necessarily resulting from a specific parasite adaptation) or simply a non-adaptive side-effect of infection (Poulin 1995; Freeman and Herron 1998). Hypotheses about the function of fever need not be mutually exclusive when the context in which fever evolved versus the context in which it is currently expressed are considered. My approach was to consider the conditions (namely, age of host, timing of infection) under which changes were observed in behaviour or life history traits of infected amphipods to determine whether hypotheses for parasite manipulation and host responses were, in fact, mutually exclusive for my study system.

Before detailing specific predictions of both hypotheses for my study system, I first review the relevant natural history of *C. volutator* and *G. adunca*. *Gynaecotyla adunca* reproduces asexually in the mud snail, *Ilyanassa obsoleta* (Say) producing **sporocysts** (Stunkard 1983; Appendix G). Each sporocyst then releases **cercariae**, which leave the snail and penetrate a second-intermediate host (usually an amphipod; Rankin 1940). Once inside the second-intermediate host the cercariae shed their tails (and are now called metacercariae) and encyst. The metacercariae complete their life cycle when the second-intermediate host is ingested by a shorebird (Rankin 1940). Female *C. volutator* usually inhabit burrows during the time that burrows are uncovered by the tide.

However, a very small proportion (<1%) of females leave their burrows to crawl on the surface of mudflats (Chapter 3).

Predictions for parasite manipulation

As for *C. volutator* parasitized by nematodes (Chapter 3), my prediction of the 'manipulation' hypothesis was that female *C. volutator* that were collected while crawling on the surface would have a higher prevalence of encysted infective trematodes than females that remained burrowed within the substrate. Crawling on the surface dramatically increases an amphipod's susceptibility to predation by final host sandpipers, which often feed visually by pecking the surface of the mud (Robert and McNeil 1989; Manseau and Ferron 1991). If parasite manipulation occurred, I also predicted that prevalence of unencysted pre-infective trematodes would not differ between crawling and burrowed amphipods. This argument presupposes that unencysted trematodes cannot be successfully transmitted to sandpiper hosts and that parasite control can be turned on rather abruptly. I further predicted that neither encysted or unencysted trematodes would be associated with increased crawling activity of female *C. volutator* at night. At night, sandpipers forage as much as during the day (Chapter 2), but increased crawling activity of amphipods does not result in increased likelihood of being ingested (at night, sandpipers feed non-visually by probing the mud; Robert and McNeil 1989; Manseau and Ferron 1991). Therefore, if manipulating amphipod behaviour is costly for trematodes (e.g., by producing a short-acting

neurochemical, as suggested for other parasites of amphipods: Helluy and Holmes 1990; Thompson and Kavaliers 1994), then parasites are not expected to cause female *C. volutator* to crawl at night.

Predictions for adaptive host response

I next tested predictions of whether trematodes were associated with reductions in survival of amphipods in the absence of predation by sandpipers, since this point is critical in testing other predictions outlined below. In brief, if parasites are associated with direct mortality costs through energy limitation by growing parasites (or indirectly through increases in probability of ingestion), then there should be selection on young females to invest more in current reproduction (Minchella 1985) or to hasten their onset of becoming receptive to males. Increased early reproduction in response to parasitism has previously been found in female snails *Biomphalaria glabrata* (Say) parasitized by the trematode *Schistosoma mansoni* (Minchella and LoVerde 1981; Thornhill et al. 1986), but not in another snail (*Biomphalaria pfeifferi* Krauss) infected by the same parasite (Meuleman 1972).

I did not expect that female *C. volutator* infected by *G. adunca* could directly increase their likelihood of securing a mating because these amphipods show a male-controlled mating system. Sex ratios are extremely female-biased at 5-15 females/male (Schneider et al. 1994) and males express mate choice by preferring to co-habit with females that are close to becoming receptive (Forbes

et al. 1996). Thus, I predicted that if host life history responses were possible and interpretable in the context of hosts acting to minimize the effects of parasites on their fitness, then young non-ovigerous females would respond to parasitism by becoming receptive to mating sooner. I used moult as an index of receptivity because in *C. volutator*, as in other amphipods, females are physiologically able to mate for only a few days each month, immediately following a **parturial** moult (Chapter 1).

After examining survivorship, I addressed a final issue, i.e., responses of old females to new infections. Here, I chose females that had over-wintered and mated prior to infection. I noted that older ovigerous females would not respond to parasitism by increasing their **reproductive effort** as they had committed to current reproduction already. In general, over-wintered females do not reproduce after releasing their spring brood (Matthews et al. 1992); in fact, these females die soon after releasing broods (pers. obs.). Thus, the only response possible was for old females to reduce their brood size. If there is a cost to reproduction, which is further exaggerated once parasitized, brood reduction might enable old females to live long enough to release some progeny. Unencysted trematodes in new infections have not reached their infective stage so there should be little alteration of host behaviour to increase predation. However, if reproduction is costly for amphipods, then by aborting their broods, older females may survive longer. This would increase the chances of trematodes reaching their infective stages, and being transmitted.

2. Methods

To compare naturally-infected and uninfected females with respect to their likelihood of crawling, I made seven visits to the mudflat at Starrs Point, Nova Scotia (Appendix C) between August 1997 and August 1998 (five trips during the day and two at night; Table 4-1). I made all collections within two 20 x 20 m plots located 1.9 and 2.4 km from the high tide mark, where prevalence of *G. adunca* in *I. obsoleta* is high (33%, $n=126$; McCurdy et al. in press) and predation pressure on *C. volutator* by foraging sandpipers is intense (Figure 2-2). On each trip, I collected crawling female amphipods and then removed 1-2 females of the same or similar size (within 2 mm, rostrum-telson length) from their burrows within 10 cm of the crawling female. In the laboratory, I dissected each amphipod and recorded whether the female was uninfected or infected and, if infected, the number of encysted and unencysted metacercariae. I also recorded the presence of any nematode parasites in amphipods and excluded these infected amphipods from subsequent analyses because nematodes have been shown to affect surface activity of *C. volutator* (Chapter 3). I used χ^2 tests for 2 x 2 comparisons where all of the expected frequencies were ≥ 6 and Fisher exact tests where one or more of the expected frequencies was < 6 (Zar 1996; Chapter 3). I performed power analysis to ensure adequate statistical power for all non-significant χ^2 comparisons (Cohen 1988).

To infect *C. volutator* with *G. adunca*, I collected adult *I. obsoleta* (Say) (>14 mm; Scheltema 1964) and adult female *C. volutator* (>5 mm; Schneider et

al. 1994) from Starrs Point. In the laboratory, I housed each amphipod in a separate 20 x 150 mm culture tube (methods described in Chapter 1). To obtain cercariae of *G. adunca*, I housed each snail in a plastic cup with 100 ml of seawater. Following 24 h, I combined the water from all cups containing cercariae and pipetted 10 ml of the solution into each culture tube containing a female amphipod that was to be infected. I subjected control amphipods to the same procedure, except that I used water from cups containing snails that did not shed cercariae. Following 24 h, I transferred all females to new culture tubes.

To assess the effects of parasites on the likelihood that females would mate, I housed 400 non-ovigerous, recently mature (5-7 mm) females in separate culture tubes (200 exposed, 200 unexposed). Every 12 h, I checked each tube and placed a male (previously housed in a separate culture tube) with any female that had moulted. I then observed paired females every 12 h to determine if they mated (became ovigerous). Following 3 days, I removed each pair and dissected them to determine intensities of metacercariae. I removed females from my analysis if they were housed with an infected male ($n=33$) to avoid potentially confounding responses of infected males. In all experiments, I also removed naturally-infected females that were not exposed to parasites from further analyses ($n=20$) as these females had older parasites, which would confound results.

To examine the effects of parasites on the timing of female moult and on survivorship, I housed 150 recently mature, non-ovigerous females in separate

culture tubes (100 exposed, 50 controls). Most females moulted just prior to treatment (exposure/non-exposure to parasites) or immediately after. I then assessed the time between successive moults (intermoult period) for females that first moulted 1-3 days post-infection/post non-infection. I checked each tube daily and recorded the presence of exuvia as well as any mortality. Following 22 days, or when females died, I dissected them to record intensities of metacercariae (as above). To compare survivorship between infected and uninfected females, I applied Cox's *F*-test (Cox 1964) and I used Cox's Proportional Hazard Regression (Cox 1972) to compare survivorship of infected females in relation to **intensity** of parasite infection (rationale discussed in Chapter 1).

To assess the effects of parasites on ovigerous females, I housed 400 females in separate culture tubes that had over-wintered and that had recently fertilized eggs or 'Stage A' embryos (Peer et al. 1986). These females were large (6-8 mm) and were either exposed (200 females) or not exposed (200 females) to trematodes. I observed each female daily and recorded the presence of any aborted embryos (those released or extruded prematurely from the brood pouch). I removed females and dissected them to determine their parasite loads, either after they had released fully-developed young, after they died, or after they were observed to become non-ovigerous. To compare brood sizes between infected and uninfected females, I used a Mann-Whitney *U* test because the data were not normally distributed (assessed using Shapiro Wilk's test; Zar 1996). Prior to

regressing brood sizes against intensities of infection, I $\ln(x+1)$ transformed brood sizes to correct for a lack of homogeneity of variance in the residuals (Zar 1996). I also included a term for female body size in the model as this has been shown to relate positively to brood size in *C. volutator* (Peer et al. 1986). I used Statistica (Statsoft Inc. 1993) for all analyses.

3. Results

Evidence for parasite manipulation

Overall, prevalence of trematodes in female *C. volutator* at Starrs Point was 6.6% ($n=790$; range=0-ca. 10% per collection) and intensities of infection were low (median $\pm$ lower, upper quartiles = $1 \pm 1, 2$; range=1-10 parasites). During the day, prevalence of encysted trematodes was higher in crawling females (6.6%) than in burrowed females (2.5%; $\chi^2_{[1]}=4.48$, $n=442$, $P<0.05$; Table 4-1). However, at night there was no difference in prevalence of encysted trematodes between females found crawling or in burrows (10.4 versus 9.0%, respectively; $\chi^2_{[1]}=0.18$, $n=348$, NS). Prevalence of unencysted trematodes did not differ between females found crawling or in burrows during the day (3.0 versus 4.1%; $\chi^2_{[1]}=0.36$, $n=442$, NS) or at night (2.6 versus 3.0%; Fisher exact $P_{[1]}=1$, $n=348$). Power (1-B) to detect a significant effect with a 'moderate' effect size ($w=0.3$; Cohen (1988)) exceeded 0.95 in all cases.

Evidence for adaptive host response (young amphipods)

In the laboratory, infected females were no more likely to mate with a male than uninfected females, both before (1-15 days post-infection: $\chi^2_{[1]}=0.01$, $n=87$, NS) and after (15+ days post-infection: Fisher exact $P_{[1]}=0.52$, $n=20$, NS) parasites formed cysts. However, mean intermoult period was shorter for infected females than for uninfected females ($t_{[49]}=2.14$, $P<0.05$; Figure 4-1), although there was a positive relation between intermoult period and intensity of metacercarial

infection for infected females ($r=0.37$, $n=27$, $P=0.05$). Survivorship of infected amphipods ($\bar{x}\pm\text{S.E.}=16.2\pm1.7$ days) was lower than uninfected amphipods ($\bar{x}\pm\text{S.E.}=20.3$ days; $F_{[12,80]}=5.33$, $P<0.001$), but for infected females, those with higher intensities of metacercariae lived longer (i.e., there was a negative relationship between hazard rate and metacercarial intensity; $\chi^2_{[1]}=7.29$, $n=88$, $P<0.01$; Figure 4-2).

Evidence for adaptive host response (old amphipods)

The number of progeny that older, ovigerous females released from their brood pouches was negatively related to the number of metacercariae they were infected with ($r=-0.39$, $n=395$, $p<0.001$; Figure 4-3). Statistically removing the effect of female body size did not affect this result ($\text{beta}=-0.36$, $t=1.96$, $P<0.001$). Furthermore, infected *C. volutator* were less likely to release a brood than uninfected females ($\chi^2_{[1]}=8.66$, $n=395$, $P<0.005$) and of all females that released broods, infected females released fewer progeny than uninfected females ($U=10278.50$, $n=395$, $P<0.001$). Infected females also were more likely to abort embryos (63% of the total) and infected females that were observed to abort embryos did so earlier than uninfected females (infected: median date=2 days post-infection; uninfected: median date=4 days post non-infection; $U=74.50$, $n=38$, $P<0.05$).

Table 4-1. The number of female *Corophium volutator* infected by encysted trematode metacercaria(e) versus the number examined for crawling and burrowed samples collected during the day and at night at the Starrs Point mudflat, Nova Scotia.

Date collected	Crawling no. infected (examined)	Burrowed no. infected (examined)
Day		
21/08/97	3 (12)	1 (28)
13/06/98	0 (53)	1 (52)
23/06/98	0 (48)	0 (52)
22/07/98	6 (48)	0 (41)
17/08/98	4 (37)	4 (71)
Total	13 (198)	6 (244)
Night		
24/08/97	1 (9)	1 (24)
19/08/98	11 (106)	20 (209)
Total	12 (115)	21 (233)

Figure 4-1. Mean ($\pm$ one standard error) numbers of days between successive moults for female *Corophium volutator* experimentally infected versus not infected by the trematode *Gynaecotyla adunca*.

Figure 4-2. Cumulative survivorship functions for non-ovigerous female *Corophium volutator* infected by: 1 metacercaria of *Gynaecotyla adunca* (minimum intensity observed; A), 47 metacercariae (mean intensity of infection; B), and 138 metacercariae (maximum intensity of infection observed; C). Amphipods were collected from Starrs Point, Nova Scotia and housed in the laboratory for 22 days.

Cum. prop. female amphipods surviving

Figure 4-3. The $\ln(\text{number of trematode metacercariae}+1)$ removed from ovigerous female *Corophium volutator* versus the $\ln(\text{number of progeny}+1)$ released from the brood pouch. Amphipods were collected from Starrs Point, Nova Scotia and housed in the laboratory.

4. Discussion

Evidence for parasite manipulation

I found evidence that *G. adunca* manipulate the behaviour of female *C. volutator* to increase their likelihood of transmission to final host sandpipers. Specifically, only female amphipods infected by encysted (infective) metacercariae were more likely to be found crawling on the surface of a mudflat than in their burrows, and only when this would result in increased risk of predation (during the day). Thus, for female *C. volutator*, parasite manipulation can be viewed as a major cost of parasitism in addition to energy lost to parasites during their development. I expected parasite manipulation in my system because parasite manipulation has been observed by *G. adunca* for snails (Curtis 1987, 1990; McCurdy et al. in press) and for *C. volutator* parasitized by nematodes (Chapter 3). The question of interest is the extent to which this manipulation precludes life history responses by hosts.

Evidence for adaptive host response

Considering young amphipods, I observed no difference between infected and uninfected female *C. volutator* in their likelihood of mating with a male. This was expected because while male *C. volutator* exhibit mate choice, it is probably based on reproductive gains per unit time rather than just reproductive gains per possible mating. Evidence for this comes from male *C. volutator* in nature, who choose females more on the basis of time to receptivity than on female body size

(Forbes et al. 1996), which does relate to fecundity (Peer et al. 1986). By mating with infected, but receptive females, males may still be provided with a reproductive gain similar to mating with small females because infected females can release a partial brood (below).

As predicted, I found that young female *C. volutator* decreased their time-to-moult, thereby decreasing their time to receptivity following infection by *G. adunca*, despite the fact that moulting is energetically costly for crustaceans (Shuster 1991b). For females, moulting early poses an additional cost in that they will reproduce at a smaller body size, which results in reduced brood size (Peer et al. 1986). However, trematodes appear to render female *C. volutator* far more susceptible to predation once reaching their infective stage; additionally, metacercariae increase in size by more than 2.5x while developing (Rankin 1940; Appendix G). Thus, females that fail to respond to parasitism by hastening moult would likely suffer an even greater decrease in reproductive success (potentially resulting in no fitness), rather than ensuring output of at least a small brood over their lifetime. The logic of this argument is explored mathematically in Chapter 6.

It is unlikely that hastening onset of moult is due to parasite manipulation by *G. adunca* because moult would decrease the energy available for parasite growth. Furthermore, moulting has been shown to increase an amphipod's susceptibility to cannibalism or predation by other amphipods (e.g., Dick 1995) which are inappropriate final hosts for *G. adunca* (Rankin 1940). Even if susceptibility of amphipods to final host shorebirds is increased by moulting, my

results indicate that this would occur at an inappropriate time (i.e., prior to the parasite's development to the infective stage). My data further suggest a dose-dependency in host control over moulting. Females infected with high intensities of infection appear increasingly unable to respond to parasites by decreasing their intermoult period. These results have bearing on interpreting survivorship data.

My finding that survivorship was lower in infected females than uninfected females indicates that parasites have a negative effect on host survival. However for infected females, I found that survivorship was higher for females with increasingly higher intensities of infection, suggesting that a simple, direct effect of parasitism alone cannot explain higher mortality of infected amphipods. One possibility is that female *C. volutator* infected by low levels of parasitism are able to trade off future survivorship for an early moult, but females with high intensities of infection (who are unable to decrease their intermoult period) simply live longer in the absence of the moulting trade off. Decreases in survivorship have been observed as a result of increased reproductive effort in female fruit flies, *Drosophila melanogaster* L. (Fowler and Partridge 1989). In a recent study, Polak and Starmer (1998) observed that male *Drosophila nigrospiracula* Patterson and Wheeler parasitized by ectoparasitic mites increased courtship, but died sooner.

As indicated above, I found that parasites were associated with reductions in the final brood sizes of old, previously-mated females. This result was unexpected if hosts control investment because these amphipods, even when

uninfected, have a low expectation of future reproduction. It is unlikely that females were simply removing non-viable progeny because parasites did not attack the developing embryos. I have observed that aborted embryos disappear within 1-2 days following their release and on 4 occasions I observed females consuming embryos they had just aborted. Cannibalism of progeny may be observed in animals which are under nutritional stress (Elgar and Crespi 1992). However, because over-wintered female *C. volutator* can only reproduce once in the spring, I was likely observing their last reproductive bouts. Resorbing part of the energy previously allocated to a brood only would be beneficial for females if it increased their likelihood of successfully releasing the remainder of their broods.

A second explanation for why infected females partially or wholly aborted their broods, and specifically, why many aborted their entire broods, is that the brood reductions were a result of parasite manipulation of host behaviour. Trematode parasites often castrate their hosts, including *G. adunca*, which castrates *I. obsoleta* (Curtis and Hurd 1983). By altering a host's energy budget, parasites may increase host growth rates and/or longevity, allowing increased proliferation (Baudoin 1975). In females of the amphipod *Gammarus zaddachi* Sexton, increased growth has been observed following castration by the cestode *Diplocotyle* sp. (Stark 1965). For *C. volutator*, parasites may cause females to abort and consume their broods to extend their opportunity for transmission to

final hosts (probably through increased survivorship of hosts and/or energy available for manipulation).

CHAPTER 5

MATING EFFORT OF MALE *COROPHIUM VOLUTATOR* IN RELATION TO BEHAVIOURAL MANIPULATION BY THE TREMATODE *GYNAECOTYLA ADUNCA*

1. Introduction

In Chapter 4, I tested predictions using female *Corophium volutator* infected by the trematode *Gynaecotyla adunca* and argued that host response need not be mutually exclusive of parasite manipulation, if host- and parasite-mediated control of host behaviour are exerted at different times during parasite development. In this chapter, I tested for behavioural manipulation by *G. adunca* and life history responses by male *C. volutator* using studies in the field and in the laboratory. As outlined in the General Introduction, conducting separate tests of hypotheses related to parasite manipulation and adaptive host response in each sex provides an opportunity to test fine-tuned predictions, in that parasites may manipulate behaviours that are sex-specific in uninfected hosts (Chapter 3) and adaptive responses to parasitism may differ because of differences in selection pressures on males versus females (Zuk 1990). Importantly, unparasitized male amphipods crawl in search of mates, with which they co-habit (Boates 1980). However immediately after arrival of migrant shorebirds (mostly semipalmated sandpipers), >95% of uninfected *C. volutator* stop crawling during the day, dramatically reducing their probability of ingestion (Boates et al. 1995;

Chapter 3).

I first outline my tests of predictions of parasite manipulation by *G. adunca* and then detail my tests of assumptions and predictions of adaptive life history responses by male *C. volutator*. Concerning manipulation, my predictions were the same as for male and female amphipods parasitized by the nematode *Skrjabinoclava morrisoni* (Chapter 3) and for female *C. volutator* parasitized by *G. adunca* (Chapter 4). I predicted that parasitized males would be more likely to crawl than remain burrowed within the substrate during the day. Such crawling would increase susceptibility to predation by final host sandpipers (Chapter 2). Increased crawling should only occur for males infected by *G. adunca* that have reached their encysted (infective) stage. This is because early, pre-infective transmission would most likely result in the parasite's death, similar to pre-infective nematode parasites of *C. volutator* (Wong and Anderson 1988). For laboratory-housed amphipods, I predicted that males with encysted trematodes would be more likely to be on the surface than burrowed as compared to uninfected males, at least during daylight hours.

Trematodes have been implicated in manipulating amphipod behaviour (e.g., Mouritsen and Jensen 1997; Thomas et al. 1997; Chapter 4). However, it is important to demonstrate that changes in host behaviour cannot be explained by host-mediated responses (Poulin et al. 1994). As mentioned above, males do crawl in search of mates and increased crawling could reflect increased investment in current reproduction (Forbes 1993; Chapter 3). To test this idea, I

compared prevalence of trematodes in crawling versus burrowed *C. volutator* at night. As discussed previously (Chapters 3,4), sandpipers forage at night (Chapter 2), but use tactile cues (Robert and McNeil 1989; Mouritsen 1994). As a result, differential predation on crawling versus burrowed amphipods, which occurs during the day (Boates et al. 1995), is unlikely to occur at night. If infected males invest more in current reproduction, I expected that infected males would crawl even more at night, when the costs of crawling also are low. If increased crawling during the day is a parasite-mediated response, then I did not expect increased crawling at night. In fact, others have suggested that manipulation is costly for parasites (e.g., based on evidence for neurochemical modulation in another amphipod; Helluy and Holmes 1990). As such, parasites could benefit from 'turning off' crawling responses when the likelihood of ingestion due to crawling is low.

So far, I have discussed only predictions related to amphipods infected by encysted trematodes. If parasites have a greater negative effect on energy budgets of hosts over time, then hosts should respond to parasitism when initially infected (Forbes 1993) and eventually concede control to parasites (if they manipulate behaviour). Amphipods and snails infected by *G. adunca* do not lose infections and the trematodes continue to grow as they develop (Rankin 1940; Appendix G). In fact, if subsequent manipulation does occur, then this should place a premium on hosts to increase reproductive effort, prior to manipulation (Poulin et al. 1994; Chapter 4).

If males respond to parasitism by increasing their early reproductive effort when first infected, this requires that individual matings be costly (i.e., males do not fertilize all receptive females at maximal levels). In many systems, individual matings are generally not thought to be energetically costly for males (e.g., sperm production is often viewed as having little or no cost; Dewsbury 1982). However, the mating system in *C. volutator* is different in that sex ratios of *C. volutator* are female-biased (Schneider et al. 1994); males mate frequently and appear to have mate choice, preferring to co-habit with females that are close to becoming receptive (Forbes et al. 1996). Males also may mate with several females over a short time (Forbes et al. 1996). I tested the assumption that individual matings are costly for male *C. volutator* by comparing reproductive success of females that mated with (unparasitized) males that obtained a different number of potential mates (1 versus 5 females) and in relation to the number of females mated. If males fertilize females at sub-maximal levels, I expected that females housed with males that obtained many matings would produce smaller broods than females housed with males that mated fewer times.

To test for adaptive responses of male *C. volutator* to parasitism by *G. adunca*, I compared recently-parasitized and unparasitized males with respect to two measures of reproductive effort. First, I predicted that males would be more likely to mate with a receptive female immediately after becoming parasitized. Increased probability of mating should result in greater fitness for parasitized males than waiting to reproduce (Minchella 1985; Forbes 1993; Poulin et al.

1994). I also predicted that parasitized males might allocate more sperm to an individual mating, resulting in a larger brood, if I found evidence that unparasitized males fertilize individual females at sub-maximal levels (above).

Finally, I returned to examining males with encysted trematodes to assess directly whether males might concede control to parasites. If males increase reproductive effort when initially infected, then males with old infections should be less likely than unparasitized males to mate with receptive females. I expected this result, particularly for parasitized males, if increased reproductive effort resulted in a mating (which is also presumed to be costly). Related to this objective, I compared survivorship of parasitized and unparasitized *C. volutator* in the absence of sandpiper predators (in the laboratory) to determine if parasites have a direct mortality cost on amphipods. A direct mortality cost could result in increased commitment to early reproduction in the absence of parasite manipulation, or combine with parasite manipulation to increase expression of early reproduction.

2. Methods

To assess crawling activity of male *C. volutator* in relation to parasite development and time (daytime versus nighttime) in a natural population of hosts and parasites, I made trips to Starrs Point, Nova Scotia (Appendix C) during the day and at night between August 1997 and August 1998 to record prevalence of *G. adunca* in amphipods (Table 5-1). As in Chapter 4, I made collections from two 20 x 20 m plots located 1.9 and 2.4 km from the high tide mark (where *G. adunca* is common in *I. obsoleta*; McCurdy et al. in press). On each trip, I followed the ebbing tide and removed a sample of males that were crawling on the surface using live-specimen forceps. I then removed 1-2 burrowed males of a similar size within 10 cm of each crawling male. In the laboratory, I measured the body length of all males (rostrum-telson) and then dissected them to determine if they were infected by unencysted (recent) or encysted (infective) metacercariae.

To test for manipulation of male *C. volutator* by trematodes using an experimental approach, I collected and housed 70 adult males (≥ 5 mm; Schneider et al. 1995) in the laboratory. Each amphipod was housed in a separate culture tube with seawater, mud, and antibiotics (see Chapter 1 for a detailed description of housing methods). I then exposed 35 males to cercariae of *G. adunca* by collecting adult *I. obsoleta* (>14 mm; Scheltema 1964) from Starrs Point on 18 July 1998 and obtained cercariae that were shed by snails at 24-h post-infection (unexposed controls=35; see protocols in Chapter 4). After 24 h of exposure/non-exposure to cercariae, I transferred each male amphipod to a

new culture tube that contained an adult female. I did this to give males the option of being burrowed or on the surface (adult male *C. volutator* are unable to construct burrows and must co-habit with females, who construct them; Meadows 1964). Females were never observed to evict males from their burrows and in many cases they constructed several burrows (when the male occupied the original burrow for >2 days). Once paired with a female, I recorded the location (surface/burrowed) of each male at 3 days post-infection/post non-infection (prior to parasites developing to their infective stage) and at 17 days post-infection (following development of the metacercariae to their infective stage; Appendix G). I then dissected all males to determine whether or not they had become infected by trematodes.

When comparing prevalence of trematodes between crawling and burrowed males (either from collections or in the laboratory), I excluded amphipods that were parasitized by the nematode *Skrjabinoclava morrisoni* from my analysis ($n=21$) because this nematode has been shown to influence crawling activity of male *C. volutator* (Chapter 3). I used Cohen's (1988) method of power analysis to assess statistical power for non-significant χ^2 tests. A logistic regression (Zar 1996) was used to determine if body length related to the likelihood that a collected male was parasitized.

To test the assumption that matings are energetically costly for (unparasitized) males (specifically, that males may fertilize females to sub-maximal levels), I collected 102 adult male and 600 non-ovigerous, adult female amphipods

from Starrs Point on 26 May 1999. I collected males that were crawling on the surface of the mudflat, while females were collected by removing them from their burrows within the substrate. In the laboratory, I housed each amphipod in a culture tube (conditions as described in Chapter 1). After each female moulted (within 24 h), she was placed in a 300-ml plastic cup lined with mud and containing 150 ml of seawater treated with antibiotics. I retained only recently-moulted females for use in this experiment because I observed (Chapter 1) that female *C. volutator* are only able to mate for ca. 4 days following their moults, over which time their receptivity to mating decreases (as in other crustaceans, female *C. volutator* are only receptive to mating for a few days following their parturial moult).

I housed females in cups at densities of 1 and 5 females per cup. I then added a male to each cup and housed him with the female(s) for 3 days. The body length of each male was then measured (tip of rostrum to end of telson). Females were left in their cups for an additional 3 days to allow any eggs to develop to the point where they could be identified as fertilized. I then measured the body length of each female and counted the number of fertilized eggs (if any) in each female's brood pouch. I could be certain that all females that mated did so with the experimental male because female amphipods, including *C. volutator*, cannot store sperm across their moults (Sexton 1935; Chapter 1). I excluded three males that were observed to have older (natural) infections of *G. adunca* (these were visible because the parasites were larger and encysted). I also dissected all females and confirmed that none were found to be infected by metacercariae. I *In*-transformed

mean brood size to correct for a lack of homogeneity of variance in the residuals (Zar 1996) to compare mean brood size of mated females (housed with unparasitized males) in relation to the number of matings that males performed (1-4),

To assess the impact of parasites on mating effort of male *C. volutator*, I collected and housed 800 non-ovigerous, adult female amphipods in the laboratory (collection and housing methods as described previously). Every 12 h, I checked each female to see if she had moulted to be certain that females were receptive. Once a female moulted, I paired her (within 12 h) with a male that had recently been infected or not infected by metacercariae (methods of infection as described above). After 3 days, these females were removed, scored for ovigery, and the numbers of embryos (if any) in their brood pouches were counted. Females were then measured (rostrum-telson length) and dissected to look for parasites. In total, I housed 222 males (half infected by parasites and each with a different female) over the first 8 days of the experiment (before metacercariae began to reach their infective stage). Following a first pairing, most males ($n=178$) were housed alone in a new culture tube until they died, or until the end of the experiment (24.5 days post-infection/post non-infection). To determine the probability of mating of males with old infections (harbouring encysted metacercariae), I housed each of 44 males (described above) with a different, second receptive female (procedure as described above) between 14 and 21 days post-infection/post non-infection (after encysted metacercariae were

observed in dissected males). After receiving a second female, males were measured and dissected when they died, or at the end of the experiment and females housed with them were scored for ovigery (as above).

In the mating experiment, I removed all males that were housed with parasitized females from my analysis ($n=10$) because parasitism may have affected female reproduction (Chapter 4). I also excluded males that were not exposed to cercariae, but were parasitized when assessed at the end of the experiment ($n=22$) because the age of these 'natural' infections could not be scored accurately (all males exposed to cercariae became infected by parasites). To compare survivorship between parasitized and unparasitized males and between males that mated and those that did not, I used Cox's F -test (Cox 1964; Chapter 1). To assess survivorship in relation to the number of metacercariae found in parasitized males, I used Cox's Proportional Hazard Regression (Cox 1972; Chapter 1). As in previous chapters, I used Fisher exact tests for 2×2 contingency tables where one or more of the expected frequencies was <6 (Zar 1996). All analyses were conducted using the Statistica software package (StatSoft Inc. 1993).

3. Results

Evidence for parasite manipulation

From my collections, I observed that encysted (infective) metacercariae of *G. adunca* were more likely to be found in crawling than in burrowed male amphipods during the day (Fisher exact $P_{[1]} < 0.01$, $n=285$; Table 5-1). At night, however, there was no difference in prevalence of encysted metacercariae between crawling and burrowed males ($\chi^2_{[1]} = 0.57$, $n=340$, NS). For males infected by unencysted (recent) metacercariae, prevalence of *G. adunca* did not differ between crawling and burrowed males during the day (Fisher exact $P_{[1]} = 0.50$, $n=285$) or at night (Fisher exact $P_{[1]} = 0.72$, $n=321$). The mean body length of crawling males ($\bar{x} \pm \text{S.E.} = 6.98 \pm 0.03$) did not differ from that of burrowed males ($\bar{x} \pm \text{S.E.} = 6.99 \pm 0.03$; $t_{[620]} = 0.19$, NS) and there was no relationship between male body size and likelihood of infection ($\chi^2_{[1]} = 0.03$, $n=622$, NS). In the laboratory, parasitized males were no more likely to be on the surface as unparasitized males when checked after 3 days post-infection/post non-infection ($\chi^2_{[1]} = 0.14$, $n=69$, NS). However, at 17 days post-infection/post non-infection, parasitized males were more likely to be observed on the surface than unparasitized males ($\chi^2_{[1]} = 4.71$, $n=57$, $P < 0.05$; Figure 5-1). Power (1-B) to detect a significant difference for a 'moderate' effect size ($w=0.3$; Cohen 1988) exceeded 0.99 for all non-significant χ^2 tests.

Assumption of adaptive host response

Twenty two of the 52 unparasitized males housed with 1 female mated, while 32 of the 47 males housed with 5 females mated at least once (10 mated with 1 female each, 9 mated with 2 females, 12 mated with 3 females, and 1 mated with 4 females; for a total of 68 females). Mean brood sizes of mated females decreased as the number of females mated by males increased ($r=-0.65$, $n=32$, $P<0.0001$; Figure 5-2) and the maximum brood sizes of females that mated also decreased as the number of females mated by males increased ($r=-0.47$, $n=32$, $P<0.005$). For males that mated, total reproductive success (number of eggs summed across all mated females) did not relate to the number of females that males mated with ($r=0.12$, $n=32$, NS).

Evidence for adaptive host response

Over the first 8 days of my laboratory experiment (when all males were paired with their first female), females paired with parasitized males were no more likely to become ovigerous (59%) than females paired with unparasitized males (54%; $\chi^2_{[1]}=0.54$, $n=222$, NS). However, parasitized males (61%) were more likely than unparasitized males (43%) to mate with females on the first day of observation ($\chi^2_{[1]}=3.89$, $n=113$, $P<0.05$). Over the remaining 7 days of observation, there was no difference in the likelihood that parasitized and unparasitized males would mate (low sample sizes precluded separate analyses by day, but there was no significant difference when pooling days 2-7; $\chi^2_{[1]}=0.86$, $n=109$, NS). The

likelihood that a female amphipod would become ovigerous when paired with a parasitized male between 1 day and 8 days post-infection was not related to the number of metacercariae infecting her mate ($\chi^2_{[1]}=0.33$, $n=202$, NS) nor to male body size ($\chi^2_{[1]}=0.22$, $n=202$, NS). However, larger females were more likely to mate than smaller females ($\chi^2_{[1]}=9.82$, $n=203$, $P<0.005$). Females paired with recently-parasitized males had more fertilized eggs than females paired with unparasitized males ($t_{[113]}=2.15$, $P<0.05$; Figure 5-3), a result which also did not change after including covariates for male and female body size ($F_{[1,110]}=5.03$, $P<0.05$).

My results for females paired with males infected by encysted (infective) trematodes differed from those for females housed with recently-infected males. Overall, females paired with males parasitized by encysted trematodes were no more likely to become ovigerous (20%) than females paired with unparasitized males (42%; Fisher exact $P_{[1]}=0.20$, $n=44$). However, the mating history of males had an effect. Specifically, females were less likely to become ovigerous when paired with a parasitized male that had already mated (20%) than with an unparasitized male that had already mated (64%; Fisher exact $P_{[1]}<0.05$, $n=23$). For males that did not mate with their first female, there was no difference between parasitized and unparasitized males in their likelihood of mating with their second female (25 versus 23%, respectively; Fisher exact $P_{[1]}=1$, $n=21$). As with first females, the likelihood of a second female becoming ovigerous when paired with parasitized males did not relate to the number of metacercariae

infecting males ($\chi^2_{[1]}=0.33$, $n=46$, NS). The mean numbers of fertilized eggs produced by second females that mated with parasitized males ($\bar{x}\pm\text{S.E.}=11.2\pm2.3$) versus unparasitized males ($\bar{x}\pm\text{S.E.}=8.6\pm2.6$) did not differ ($t_{[12]}=0.69$, NS), even after adding covariates for male and female body sizes ($F_{[1,10]}=0.31$, NS).

Survivorship of both parasitized and unparasitized males over the experiment was low (<14%), but did not differ between parasitized and unparasitized males ($F_{[164,200]}=1.01$, NS). Survivorship also did not differ between males that mated with a female and those that did not ($F_{[22,138]}=1.26$, NS). However, for parasitized males there was a negative relationship between hazard rate and metacercarial intensity (i.e., survivorship increased with intensity of parasite infection; $\chi^2_{[1]}=9.57$, $n=90$, $P<0.002$; Figure 5-4).

Table 5-1. Numbers of crawling versus burrowed male *Corophium volutator*

infected by unencysted (pre-infective) *Gynaecotyla adunca*, encysted infective *G. adunca*, or uninfected by trematodes. Amphipods were collected from the mudflat at Starrs Point, Nova Scotia during the day and at night.

Time/Date	No. crawling amphipods			No. burrowed amphipods		
	Unencysted	Encysted	Uninfected	Unencysted	Encysted	Uninfected
Day						
8/21/97	0	3	5	1	0	30
6/23/98	3	0	49	0	0	46
7/22/98	2	3	44	2	0	41
8/17/98	0	2	18	1	1	43
Total	5	8	116	4	1	160
Night						
8/24/97	1	2	15	2	3	21
8/19/98	1	9	90	4	13	187
Total	2	11	105	6	16	208

Figure 5-1. The proportions ($\pm 95\%$ confidence levels) of parasitized and unparasitized male *Corophium volutator* that were on the surface at 3 days and 17 days post-infection/post non-infection by the trematode *Gynaecotyla adunca*. Amphipods were collected from Starrs Point, Nova Scotia and housed in the laboratory. Sample sizes are shown above the bars.

Figure 5-2. Mean numbers of fertilized eggs produced by single female *Corophium volutator* (A) and groups of 5 females (B) that were housed with an unparasitized male in the laboratory (where at least one female mated. The number of mated females (1-4) that each mean (data point) is based on is indicated on the x-axis. Amphipods were collected from the mudflat at Starrs Point, Nova Scotia.

Ln (mean brood size of mated females)

Figure 5-3. Mean ($\pm$ one standard error) number of fertilized eggs of female *Corophium volutator* that mated with males that were experimentally parasitized versus not parasitized by *Gynaecotyla adunca*.

Figure 5-4. Cumulative survivorship functions for male *Corophium volutator* infected by: 1 metacercaria of *Gynaecotyla adunca* (minimum intensity observed; A), 6 metacercariae (mean intensity of infection; B), and 28 metacercariae (maximum intensity of infection observed; C). Amphipods were collected from Starrs Point, Nova Scotia and housed in the laboratory for 24.5 days.

4. Discussion

Evidence for parasite manipulation

I found evidence in the field and in the laboratory that *G. adunca* manipulate behaviour of male *C. volutator* and cause them to crawl on the surface. By doing so, the trematode may increase the likelihood that its second-intermediate host will be eaten by a final host sandpiper (crawling amphipods are >50x more likely to be eaten by sandpipers than those that remain burrowed; Chapter 6). As predicted, only encysted (infective) trematodes were associated with increased crawling, and only during the day, when this would increase the likelihood that amphipods would be eaten by sandpipers. Similar results have been found in female *C. volutator* infected by *G. adunca* (Chapter 4), and *C. volutator* infected by the trematode *Maritrema subdolum* Jägerskiöld (Mouritsen and Jensen 1997) and the nematode *Skryabinoclava morrisoni* (Chapter 3), where parasite larvae are associated with increased surface activity of amphipods. *Gynaecotyla adunca* also has been shown to manipulate behaviour of its first host by causing mud snails to move to areas where second-intermediate host amphipods are found (Curtis 1987, 1990; McCurdy et al. in press).

My results indicate that cues from sandpipers are not necessary to trigger altered behaviour in amphipods as it was observed before sandpipers arrived (on 23 June, prior to sandpiper arrival in mid-July that year; pers. obs.) and in the laboratory (where cues of predators were absent). Given that *G. adunca* are rare in populations of *C. volutator* before sandpiper arrival (Chapter 4; this chapter),

most parasites likely manipulate amphipod behaviour when sandpipers are present. However, other shorebirds are present in low numbers throughout the year (Boates 1980) and also may serve as appropriate final-hosts for *G. adunca* (Rankin 1940). Thus, trematodes that infect and manipulate behaviour of amphipods prior to, or following, the sandpiper migration may still increase their likelihood of successfully reaching a final host and completing their life cycles.

I found no evidence that increased crawling was due to adaptive responses of amphipod hosts to parasitism. If this was the case, crawling should have occurred in newly-infected males because *G. adunca* likely becomes more costly to its host as it develops (*G. adunca* increases in size from $<100\ \mu\text{m}$ to $>300\ \mu\text{m}$ in diameter in a 1-11 mm-long amphipod as it develops; Appendix G) and because parasitized *C. volutator* may suffer from increased predation-risk or other sub-lethal effects (Chapter 4; this chapter). Furthermore, if increased crawling was a host response, it should have occurred at night because infected amphipods would benefit by crawling at night (similarly to uninfected amphipods), when sandpipers likely capture fewer crawling amphipods than during the day (Chapter 2).

Assumption and evidence for adaptive host response

I found evidence that individual matings are costly for males in that males appeared to flexibly alter their reproductive allocation to each female depending on the number of matings they already obtained. Total reproductive success (in terms of

the number of fertilized eggs) remained constant over 1-4 matings, despite differences in the mating opportunities or the number of matings obtained. Furthermore, the relatively lower numbers of fertilized eggs of individual females in cases where males mated with greater numbers of females cannot be explained by a simple depletion effect (i.e., males do not appear to fertilize successive mates to a decreasing extent). This is because even the largest single broods of females housed with males that mated several times were smaller than the largest broods of females housed with males that mated fewer times. One possibility is that males apportion their investment to individual females based on the number of 'appropriate' receptive females available. This idea is supported by observations of Forbes et al. (1996), who found that mate-searching male *C. volutator* usually do not reside with the first receptive females that they encounter. Forbes et al. (1996) and I have observed that male *C. volutator* defend individual females and patches of females from other males, and that males continue to mate-search even at times when most females are not receptive (Chapter 1).

Nearly all studies on early reproductive effort of hosts in relation to parasitism have focused on female function (e.g., Meuleman 1972; Minchella and LoVerde 1981; Thornhill et al. 1986; Chapter 4). For example, in *Biomphalaria* snails parasitized by *Schistosoma mansoni*, snails increase their egg-laying rates prior to the onset of castration by the parasite (Minchella and LoVerde 1981; Thornhill et al. 1986; but see Meuleman 1972). However, Polak and Starmer (1998) observed that male *Drosophila nigrospiracula* increased courtship activity

following infection by a mite, *Macrocheles subbadius* Berlese. *Corophium volutator* provided a similar opportunity to test predictions of the 'adaptive host response' hypothesis, using males. I found evidence that male *C. volutator* increase their mating effort when first parasitized by *G. adunca*. Although there was no overall difference between parasitized and unparasitized male *C. volutator* in their likelihood of mating with receptive females, parasitized males were more likely to mate when first infected by parasites, as predicted for the 'adaptive host response' hypothesis. I also found that newly-parasitized males fertilized more eggs of females than unparasitized males.

It is unlikely that my observations of increased mating effort in newly-parasitized males were due to responses of females because male *C. volutator* appear to exhibit mate choice (Forbes et al. 1996; Chapter 1). Even if females have mate choice, they only should attempt to increase their reproductive effort when paired with parasitized males if infected males indicate that the female's future expectation of parasitism is high (thereby allowing females to respond prior to infection preemptively). Transmission between the sexes appears unlikely because cercariae resorb their stylets once they have penetrated a second-intermediate hosts (Rankin 1940). I also have followed the development of metacercariae in amphipods and it appears that metacercariae are unable to enter a new amphipod once their stylets are resorbed.

I observed that males parasitized by older, infective metacercariae were less likely to mate with females, but only if males already had mated in the

laboratory. This result likely reflects a loss in the ability of males, particularly males that had mated previously, to deal with increasing energetic costs imposed by parasites (Dewsbury 1982). As mentioned above, similar decreases in long-term reproductive effort following early bouts of reproduction were observed by Minchella and LoVerde (1981) and Thornhill et al. (1986). In my study, the eventual decrease in mating effort of male amphipods may be due to a direct loss of energy to developing metacercariae or to indirect effects of parasites on host behaviour, resulting in greater likelihood of predation.

My results suggest that trematodes pose no direct survivorship cost for male *C. volutator*, at least under my laboratory conditions in the absence of predation. While preliminary, this result was not totally unexpected given that killing the host prior to development to the infective stage would likely result in reduced fitness for *G. adunca*. The higher cumulative survivorship I observed in parasitized amphipods infected by higher numbers of metacercariae was somewhat unexpected and may reflect a change in the trade-off between *total* reproductive effort (reduced in parasitized males) and survivorship. In female *C. volutator* infected by *G. adunca*, I observed evidence for this trade-off between survivorship and reproduction; where females with few parasites respond by hastening onset of reproduction. However, infected females with higher intensities of infection respond less and show higher survivorship (Chapter 4). If a similar trade-off between survivorship and reproductive effort occurs in

parasitized male *C. volutator*, this may be clarified by further study of other costly activities of males, such as mate guarding (Forbes et al. 1996).

CHAPTER 6

AN EMPIRICAL MODEL ON THE OPTIMAL TIMING OF REPRODUCTION FOR FEMALE *COROPHIUM VOLUTATOR* INFECTED BY THE TREMATODE *GYNAECOTYLA ADUNCA*

1. Introduction

Reasoning from life history theory has been used to make predictions of how hosts should respond to parasitism (Pianka 1994; Minchella 1985; Stearns 1992; Forbes 1993). One prediction is that hosts should allocate more energy to reproduction when first infected, if parasites have an increasingly negative effect on hosts over time, through their growth or proliferation. Such hosts that allocate resources to early reproduction should not be as badly affected by parasitism as similarly infected hosts that delay commitment to reproduction (Minchella 1985; Forbes 1993). In Chapter 4, I found general support for this argument in that female amphipods (*Corophium volutator*) infected by a trematode (*Gynaecotyla adunca*) showed an earlier onset of reproduction than uninfected females. There is also evidence from other parasite-host associations suggesting that changes in behaviour and life history of hosts (following infection) are complex and beneficial to hosts, making them unlikely to have arisen by chance (e.g., Minchella and LoVerde 1981; Polak and Starmer 1998; cf. Poulin 1995).

To further demonstrate support for 'adaptive host responses' to parasitism, researchers need to show that infected hosts have higher fitness than they would

realize otherwise, by altering their behaviour or life histories in certain ways (Forbes (1993), Poulin's (1995) fourth criterion). Such evidence is currently lacking, largely because this criterion is difficult to meet. For example, hosts that are infected and show a particular onset of reproduction have to be compared, with respect to lifetime fitness, with hosts that are infected but show some other timing of reproductive onset. The latter category of host may not exist in nature or may not be induced experimentally (Forbes 1993). One way to compare fitness consequences of different responses is to explore them theoretically, using empirically derived measures of effects of parasites on life history traits such as growth, survivorship, and fecundity.

Using an empirically-derived model, I evaluated whether Poulin's fourth criterion might be met by amphipods that reproduce immediately following infection by trematodes. In my model, I examined parasite effects on growth, fecundity (several aspects including developmental time of broods and its effect on sizes of broods released), and on survivorship. I then examined how these parasite effects influence the optimal timing of host reproduction and also fitness gradients around this optimum. In so doing, I first ask whether observed timing of reproduction by infected females accords well with predicted timing and second, I address which parasite-mediated effects (e.g., reduction in growth, increased susceptibility to predation) are necessary and/or sufficient to account for observed responses.

More specifically, I modeled fitness consequences of 'decisions' concerning timing of reproduction by females of the intertidal amphipod *Corophium volutator*, that were either infected or not infected by the trematode *Gynaecotyla adunca*. Female *C. volutator* are ideal for this type of study because much is known about the effects of predators and parasites on their survivorship and fecundity and about their general ecology (Boates 1980; Matthews et al. 1992; Chapters 1,4). These amphipods are second-intermediate host for *G. adunca* (the gastropod *Ilyanassa obsoleta* serves as the first host and shorebirds serve as final hosts of this trematode; McCurdy et al. in press; Appendix G). In Chapter 4, I determined that female *C. volutator* infected by metacercariae of *G. adunca* moulted sooner than females that were not infected. I reasoned that by hastening their onset of moult, females increased their reproductive effort when newly infected (female *C. volutator* are able to mate only immediately following a moult; Chapter 1). I suggested that this might be an adaptive response to trematode parasitism by female *C. volutator*.

I now examine whether the reproductive potential of infected females that moult and reproduce early is reduced less than the reproductive potential of infected females reproducing several days following infection by *G. adunca*. The potential benefits of waiting to reproduce include increases in growth and/or fecundity over time (brood sizes generally increase with body size in this species; Fish and Mills 1979; Peer et al. 1986). As such, I included growth and size-specific fecundity in my model. I also addressed other factors such as developmental time

of broods that could differ between infected and uninfected amphipods and that could affect maximum **realized fecundity** (after mating, all females hold their clutch in a brood pouch until it hatches; Fish and Mills 1979). There is some loss of broods during development, which affects brood size at release. This in turn might influence optimal timing for reproduction.

The major cost of delaying reproduction, however, is thought to be mortality. I assessed mortality either due to shorebird predation or due to factors other than shorebird predation. As parasites grow, they may become increasingly in control of host resources and actually 'manipulate' them to increase the parasite's likelihood of successfully reaching its final host. In previous studies, I found that infected male and female *C. volutator* increased crawling activity above controls (uninfected females rarely crawled; Chapters 3-5). Semipalmated sandpipers (*Calidris pusilla*), which are final hosts for *G. adunca*, prefer to feed on crawling *C. volutator* (Hicklin and Smith 1984; Boates and Smith 1989). In this chapter, I quantified a female amphipod's risk of predation using published data on foraging behaviour of sandpipers and data on crawling activity by infected and uninfected amphipods. In doing so, I determined if increased crawling (whether due to manipulation by parasites or not) may pose a sufficient cost to lead to increased reproductive effort in recently-infected amphipods (as observed in Chapter 4). More specific parameters included in my model are detailed in the next section.

2. Methods

Where possible, I set the parameters for my model using data collected from a natural population of *C. volutator* that was syntopic with sandpipers and trematodes (Starrs Point, Bay of Fundy, Nova Scotia; Appendix C). Starrs Point supports high densities of *Calidris pusilla*, *C. volutator* and *G. adunca* (Chapter 2, McCurdy et al. in press). Additionally, there is good information on the ecology and trophic interactions among these species at this site (e.g., Boates 1980; Hicklin and Smith 1984; Matthews et al. 1992; Boates et al. 1995; Chapters 1-5). I also used data from a nearby mudflat at Blomidon, Nova Scotia (Appendix C) because sandpiper predators and parasites of *C. volutator* were not present there (Matthews et al. 1992; Chapter 5). These data allowed me to estimate parameters (e.g., mortality) caused by factors other than predation by sandpipers, as detailed below (e.g., predation by fish; Imrie and Daborn 1981). Finally, I used data from laboratory experiments on breeding *C. volutator* where effects of infection by *G. adunca* were investigated (Chapter 4). Here, I considered 2 levels of infection by trematodes: low intensities of infection (1-5 parasites; these parasitemias represent >95% of all parasitemias at Starrs Point) and high intensities of infection (6-10 parasites; this covers the remainder of intensities observed in female *C. volutator* at Starrs Point; Chapter 4). In my model, I considered only adult amphipods (5-11 mm; Watkin 1940).

Growth

To determine daily length-specific growth (g) for uninfected *C. volutator*, I used shifts in modes of length-frequency histograms (following methods in Peer et al. 1986) for non-ovigerous female amphipods from the summer generation at Blomidon (data from Matthews et al. 1992). Daily length-specific growth was calculated as (Equation 1):

$$g = 1 / d \cdot \ln (l_2 / l_1) \quad (1)$$

where d is the number of days between measurements, and l_1 and l_2 are the rostrum-telson lengths of female *C. volutator* on the first and last dates, respectively. To determine if *G. adunca* affected growth of *C. volutator*, I compared data on rostrum-telson lengths of 145 non-ovigerous female *C. volutator* that were infected, or not infected by cercariae of *G. adunca*, and housed in the laboratory for 23 days with abundant food (Chapter 4).

Fecundity

I assessed the effects of the following 3 factors on female fecundity (number of offspring per brood): body size, reductions in brood size during development, and parasitism. To measure the impacts of the first 2 factors, I collected uninfected female amphipods that were carrying either recently-fertilized eggs (1-2 days old) or late-stage embryos that were close to being released (ca. 12-14 days old;

stages following criterion in Peer et al. 1986). These females were collected from Blomidon on 21 July, 1997 (locations and sampling methods described in Matthews et al. 1992). I then measured the rostrum-telson length of each female using a stage micrometer and determined if brood loss rates differed for females of different body sizes, using ANCOVA (Zar 1996). To measure any additional impacts of low and high intensities of trematodes on brood sizes at release, I used data from a laboratory experiment where newly-ovigerous females from Starrs Point were experimentally infected or not infected by *G. adunca* (Chapter 4). I determined if infections of different ages affect brood sizes differently by comparing brood sizes between ovigerous females with recent versus old infections (metacercariae unencysted versus encysted). Finally, I calculated time-specific fecundity (m_x) for females that mated 1-9 days (x) post-infection by taking the product of the number of fertilized eggs produced by females of a given brood size at mating and the proportion of eggs retained until release (14 days post-mating; Fish and Mills 1979).

Survivorship

To address predation risk of infected and uninfected amphipods caused by sandpipers, I modeled an area of Starrs Point where densities of *C. volutator* and *C. pusilla* were high and where *G. adunca* has been found infecting amphipods (plots 'E-H' in Chapter 2). When sandpipers were present, all *C. volutator* in this area are at risk of predation (Boates 1980). I first calculated a daily survivorship

constant for *C. volutator* while they were burrowed within the substrate, but still exposed to sandpiper predators (P_b ; Equation 2):

$$P_b = 1 - (t_1 \cdot f \cdot s_1 / c) \quad (2)$$

Term t_1 is the average feeding time of sandpipers per day, including nighttime foraging (Table 6-1). Term f is the number of burrowed *C. volutator* captured by each sandpiper, s_1 is the density of sandpipers, and c is the density of burrowed *C. volutator*. Term c was held constant because over the migration season of sandpipers recruitment of adult amphipods balances mortality (Wilson 1989).

Term P_b applies to all *C. volutator* because even amphipods that crawl (below) spend >98% of their time burrowed while sandpipers forage (Boates et al. 1995).

Sandpipers also feed on *C. volutator* that crawl for a few minutes during the day and at night, soon after the tide ebbs over their burrows (Boates and Smith 1989; Chapter 5). I considered crawling amphipods to be more susceptible than burrowed amphipods only during the day because sandpipers use non-visual cues while feeding at night (Robert and McNeil 1989; Mouritsen 1994). I calculated a daytime survivorship constant (P_c) for crawling amphipods as (Equation 3):

$$P_c = 1 - (t_2 \cdot s_2 \cdot b \cdot i) \quad (3)$$

Term t_2 is the time each day that amphipods crawl, whereas s_2 is the foraging density of sandpipers during the 'crawling' period (Table 6-1). Capture rates of crawling *C. volutator* by foraging sandpipers have not been measured directly, unlike rates of capture for burrowed amphipods. Thus, I created a parameter, b , which represents the area that each sandpiper searches (per minute) while foraging on crawling *C. volutator* (assessed using of sandpipers feeding on *C. volutator*, Boates 1980; Metcalfe 1985). I assumed that all amphipods were captured by birds searching within this area, following observations by Boates (1980) and Metcalfe (1985). The final term, i , is the probability that an amphipod will crawl each day. As mentioned above, <1% of uninfected females and females with unencysted, pre-infective trematodes crawl during the day (Chapters 4,5). I set $i=0$ for these females. For females infected by late-stage parasites, I assessed the probability that these females crawl using data collected at Starrs Point (Chapter 4).

I assessed other sources of amphipod mortality using data collected for uninfected, non-ovigerous females at Blomidon (where sandpipers do not visit; Matthews et al. 1992). Using modal densities, I calculated a daily survivorship constant, P_0 , which I applied to both infected and uninfected amphipods. I had no reason to expect that non-avian predators forage differentially on infected versus uninfected amphipods. To determine any additional impacts of low and high intensities of trematodes on survivorship, I used data for amphipods that were experimentally infected/not infected by trematodes (P_p ; Chapter 4). Finally, I took

the product of the *cumulative* values of the 4 survivorship constants (P_b , P_c , P_o , P_p) to calculate time-specific survivorship (l_x ; from day 1 following infection of infected amphipods until brood release) for females with low versus high intensities of *G. adunca*, and for uninfected females.

Model execution

To calculate the reproductive potential of females that mated at different times following infection/non-infection by trematodes (r_x ; hereafter referred to as realized fecundity; Pianka 1994), I multiplied the cumulative survivorship of each female (l_x) by the time-specific fecundity (m_x) of the female (on the day she mated following infection/non-infection). Female *C. volutator* of the summer generation at Starrs Point likely reproduce only once during their lives (Matthews et al. 1992), so their first brood represents their lifetime reproductive success.

Table 6-1. Definitions and values for terms used to calculate growth of female *Corophium volutator* and rates of sandpiper predation on burrowed and crawling amphipods at the Starrs Point mudflat, Nova Scotia.

Term	Definition	Value used	Source*
Growth			
d	time between measurements	21 days	1
l_1, l_2	modal length (mm) at start and end times	3 mm, 6 mm	1
Mortality by sandpipers (burrowed)P_b			
t_1	total time that sandpipers forage	424.6 min/day	2,3,4
f	number of amphipods captured	25 bird ⁻¹ ·min ⁻¹	2
s_1	density of sandpipers at low tide	0.016 /m ²	2
c	density of amphipods	15000 /m ²	2,4
Mortality by sandpipers (crawling)P_c			
t_2	total time that amphipods crawl	6.7 min/day	3,5
s_2	density of sandpipers that forage on ebb tides	0.045 /m ²	2
b	search area of a sandpiper	2.5 m ² ·bird ⁻¹ ·min ⁻¹	2,6
i	prop. uninfected amphipods that crawl	0	7
	prop. infected amphipods (1-15 days p.i.) that crawl	0	7
	prop. infected amphipods (15+ days p.i.) that crawl	0.68	7

* 1) Matthews et al. 1992. 2) Boates 1980. 3) Chapter 2. 4) Canadian Hydrographic Service 1998.

5) Boates and Smith 1989. 6) Metcalfe 1985. 7) Chapter 4.

3. Results

Parameter values

Daily growth (g) for non-ovigerous, uninfected females at Blomidon was 0.033 mm / day. I found that body lengths of infected and uninfected females (body lengths of these groups did not differ when measured 23 days post-infection/post non-infection (infected: $\bar{x} \pm \text{S.E.} = 6.38 \pm 0.07$; uninfected: $\bar{x} \pm \text{S.E.} = 6.35 \pm 0.08$; $t_{[97]} = 0.29$, NS). Therefore, effects of *G. adunca* on growth of *C. volutator* were not considered further.

For uninfected females, larger individuals produced larger broods of 1-2 day old embryos ($r = 0.88$, $n = 93$, $P < 0.0001$) and 12-14 day old embryos ($r = 0.49$, $n = 20$, $P < 0.05$; Figure 6-1). Overall, uninfected females lost 39% of their fertilized eggs during embryonic development, after correcting for effects of female size on initial and final brood sizes ($F_{[1,110]} = 118.33$, $P < 0.0001$). Magnitude of brood loss did not depend on female size ($F_{[1,109]} = 1.37$, NS). Females with low and high intensities of infection by *G. adunca* lost an additional 43% and 56% of their embryos over uninfected females (respectively). When combining this information with daily growth (above), fecundity increased over time (post-infection/post non-infection; m_x) for both infected and uninfected females, but less rapidly for females with more parasites (Figure 6-2).

Daily survivorship was high for female *C. volutator* exposed to sandpiper predators while burrowed ($P_b = 0.99$; i.e., there was a 99% chance that a female would not be eaten by a sandpiper during the time she remained burrowed). For

females that crawled (infected females infected by metacercariae for 15+ days), daily survivorship was low ($P_c=0.49$). Daily survivorship of female *C. volutator* over the summer at Blomidon (predatory sandpipers and parasites absent) was very high ($P_o=0.99$). Parasites also directly decreased survivorship of female *C. volutator* in the laboratory, particularly when intensities of infection were high (Figure 6-3). When combining the survivorship constants together, I found that overall survivorship (l_x ; from 1 day post-infection/post non-infection until females released their broods) was high in females without parasites, and much lower among infected females (Figure 6-4). Removing the sandpiper components of predation on females caused a moderate increase on survivorship of uninfected females and those with low intensities of infection. However, survivorship of females with high intensities of infection remained low (<0.1% overall).

Model execution

When sandpiper predators were included, there was a linear increase in realized fecundity (r_x) over time for females that were not infected by *G. adunca* (Figure 6-5) until body length reached its maximum (11 mm), after which r_x declined gradually over time. For females with both low or high intensities of infection, maximum realized fecundity was low and never exceeded 25% that of uninfected females (in most cases it was more than 500x lower than among uninfected amphipods). Realized fecundity of infected amphipods decreased sharply across all size classes as the time between infection by metacercariae and mating (x)

increased. In fact, by 6 days post-infection r_x almost always reached 0 (these females did not release a brood).

Removing the effects of sandpiper predation on survivorship of amphipods altered the magnitude of all relationships between r_x and time post-infection/post non-infection, but not the direction of most relationships. Realized fecundity of uninfected females increased over time until females reached their maximum size, although more rapidly than when sandpipers were present (Figure 6-5). For females infected by metacercariae, r_x remained much lower than for uninfected amphipods and still decreased over time for females infected at most sizes, although more gradually than when sandpiper predation was included (particularly for females with low intensities of infection). Exceptions to these declines occurred in females that became infected at the smallest adult size (5 mm), where r_x increased slightly over time, but remained close to 0.

Figure 6-1. Numbers of fertilized eggs in brood pouches of uninfected female *Corophium volutator* in relation to body lengths of females. Data and lines-of-best-fit are shown for females with recently-fertilized embryos (1-2 days old; O) and females with embryos near release (ca. 12-14 days old; □). Amphipods were collected at Blomidon, Nova Scotia.

Figure 6-2. Time-specific fecundity (size at brood release; m_x) for female *Corophium volutator* that mated 1-9 days post-infection/post non-infection by the trematode *Gynaecotyla adunca*. Sets of curves are shown for amphipods infected with low intensities of metacercariae (1-5 cysts), high intensities of metacercariae (6-10 cysts), or not infected by trematodes. Each curve represents a female that became infected/not infected at a different body length (5-11 mm from bottom-top, at 1 mm intervals).

Figure 6-3. Survivorship of female *Corophium volutator* in the laboratory (from 1 day post-infection until brood release; P_p) that were infected by low intensities (1-5 cysts; L) and high intensities of trematode metacercariae (6-10 cysts; H) and mated 1-9 days post-infection. Values shown for each day have been corrected for 'background' levels of survivorship by removing mortality observed in a group of uninfected amphipods.

Figure 6-4. Overall survivorship of female *Corophium volutator* that mated 1-9 days post-infection/post non-infection by the trematode *Gynaecotyla adunca* (measured from 1 days post-infection/post non-infection until brood release; I_x). Sets of curves are shown for amphipods infected with low intensities of metacercariae (1-5 cysts; L), high intensities of metacercariae (6-10 cysts; H), or not infected by trematodes (0).

Figure 6-5. Realized fecundity (r_x) for female *Corophium volutator* that were exposed versus not exposed to sandpiper predators and mated 1-9 days post-infection/post non-infection by the trematode *Gynaecotyla adunca*. Sets of curves are shown for amphipods that were not infected by trematodes (top), amphipods harboring low intensities of metacercariae (1-5 cysts; middle), or high intensities of metacercariae (6-10 cysts; bottom). Within each graph, individual curves represent females that became infected/not infected at a different body lengths (5-11 mm from bottom-top, at 1 mm intervals).

4. Discussion

My results indicate that the reproductive potential (measured as realized fecundity) of uninfected adult female *C. volutator* increases over time, whereas the reproductive potential of infected females decreases steadily following infection by trematodes. The rapid decrease in realized fecundity for infected females following its maximum level indicates the already high costs associated with parasitism increase rapidly for females that mate even a few days after becoming infected by trematodes; far more quickly than for uninfected females, whose realized fecundity remains high for several days prior to the optimal time for mating. Despite the high cost of parasitism on reproductive success of females (at best, hosts infected with high intensities of parasites are unlikely to release even a single egg), selection should still act on females to reproduce at an optimal time. However, once an infection is established, there is likely a trade-off between energy spent in reproduction versus that used to respond directly to parasitism.

Even in the absence of shorebird predators, which also had a strong effect on realized fecundity of infected females, the optimal time for infected females to mate did not change (i.e., it remained stable at 1 day post-infection). The only group of females for whom the optimal time to mate was not 1 day post-infection were those that became infected at the smallest body length (5 mm). In my model (based on data from Blomidon), females at 5 mm had a potential fecundity of 0. Thus, the increase in reproductive potential of these females over the first

few days post-infection was the result of an increase in time-specific fecundity due to onset of reproduction.

Constraints in natural populations

Although I determined that female *C. volutator* should mate as soon as they become infected by trematodes to maximize their lifetime reproductive success, there are several constraints on females in natural populations that may delay or prevent them from doing so. First, there is a physiological constraint imposed by the requirement that a female must moult before she is able to mate (Chapter 1). Second, although females are able to decrease their time between moults when parasitized, their intermoult period is still ca. 14 days (Chapter 4). Thus, if a female becomes infected soon after her moult, but after her 'window of receptivity' is past, it is unlikely she will survive long enough to mate and release a brood.

A third constraint on females in natural populations is that they do not exhibit mate-choice. Female *C. volutator* reside in burrows, while males crawl in search of mates (Boates et al. 1995). When sandpiper predators are present (and most infections by *G. adunca*; Chapter 4), males reduce their mate-searching activity, likely to avoid predation (Boates et al. 1995). As a result, opportunities to receive a mate decrease for all females. This constraint is further compounded by the fact that females greatly outnumber males in populations of *C. volutator* in the Bay of Fundy (sex ratios are often biased between 5-15-fold

toward females; Schneider et al. 1994). Although males do prefer to reside and mate with receptive females (Forbes et al. 1996) and they do not appear to discriminate against parasitized females when choosing mates (Chapter 4), males may prefer to mate with larger females (at least in the laboratory; Chapter 5). As a result, females may not be able to obtain a mating (particularly if they are recently-mature), even if they are receptive when first infected by trematodes. Finally, the observation in a previous study that uninfected females moult (and mate) with a high degree of synchrony in natural populations (on alternate spring tides), suggests that there may be an additional cost(s) for females that breed at different times from other females, possibly limiting dispersal of adults or their offspring (Chapter 1). Given that realized fecundity of females decreases sharply over time following infection, my results indicate that early onset of parturial moult in *C. volutator* (as observed in Chapter 4) is an adaptive host response to parasitism.

SUMMARY AND PROSPECTUS

It is clear that researchers have only begun to understand the complex interactions between parasites and their hosts. In Chapters 1-6, I investigated ecological and evolutionary hypotheses in individual hosts (*Corophium volutator*) infected by one of two parasites (*Skrjabinoclava morrisoni* or *Gynaecotyla adunca*). Below, I summarize these results and discuss their implications for future studies of *Corophium volutator* and other parasite-host systems. I begin by discussing a unique aspect of the reproductive biology of *C. volutator* (female-biased sex ratios, Chapters 1,3-5) and indicate how this species could be used to test hypotheses related to the evolution of biased sex ratios. I then suggest directions for future studies of parasite manipulation of host behaviour (tested in Chapters 3-5) and life history responses of hosts to parasitism (tested in Chapters 4,5). Following this, I discuss how results from studies of *C. volutator* at the individual-level apply to the populations of this 'keystone' species.

1. Female-biased sex ratios

In Chapters 1,4, and 5, I found evidence that *C. volutator* has a male-control mating system. The rationale for this is that males are the limiting sex due to a bias in sex ratio toward females in populations of *C. volutator* in the Bay of Fundy (often >10 females/ male; Schneider et al. 1994). Future studies should investigate the proximate and ultimate causes for these biased sex ratios, which

are usually unstable through evolutionary time (Fisher 1930). *Corophium volutator* could be used as a model organism to test hypotheses related to the evolution of biased sex ratios (e.g., 'local mate competition': Hamilton 1967; Taylor and Crespi 1994; 'local resource competition': Komdeur et al. 1997) by investigating dispersal patterns and genetic relatedness of amphipods at small versus large spatial scales (e.g., Wilson et al. 1997). The presence of female-biased sex ratios in hosts has consequences for parasites of *C. volutator* because of recent evidence for sex biases in parasitism arising from differences in immunological responses and/or exposure of male versus female hosts (McCurdy et al. 1998). Sex-ratio biases in hosts also may affect parasites that manipulate sex-specific behaviours of these hosts (e.g., crawling in *C. volutator*, Chapter 3).

In addition to investigating the possibility that sex-ratio biases increase fitness of individual *C. volutator*, studies on the mechanism(s) behind such biases should be conducted. This is because several studies have found that parasites may be responsible for causing such biases through mechanisms other than sex-biased mortality. Rickettsial bacteria of the genus *Wolbachia* have been shown to feminize isopods by transforming genotypic males in phenotypic females (Godfray and Werren 1996). Because *Wolbachia* is transovarially inherited (it can only be passed to offspring via a female's eggs, but not through a male's sperm) males that become infected are unable to pass the parasite to their offspring. Thus, by altering the sex of males, *Wolbachia* increases its

transmission rate (Hurst et al. 1993). In preliminary investigations using antibiotics known to eliminate *Wolbachia* as well as genetic testing, I have not found any evidence that *Wolbachia* is present in female *C. volutator* that produce female-biased broods. However, microsporidian parasites, which also are transovarially-inherited, have recently been implicated in causing female-biased sex ratios in the amphipod *Gammarus duebeni* Lilljeborg (Dunn et al. 1993; Terry et al. 1998). Future studies should consider the possibility that such parasites cause biased sex ratios in *C. volutator*.

2. Manipulation of host behaviour by parasites

Based on my results in Chapters 3-5, I suggest several approaches that researchers should consider when conducting future tests of the 'manipulation' hypothesis. First, researchers must consider the possibility that behavioural changes in hosts are not always adaptive for parasites. Clearly, non-adaptive side-effects of infection and host responses to parasitism also may result in changes in host behaviour (Chapters 4,5). Second, future tests of the 'manipulation' hypothesis should include a close examination of assumptions (as in Chapter 6) or experiments designed to demonstrate that increased transmission rates to final hosts directly result from altered behaviour. In *C. volutator*, an observational approach could involve capturing foraging sandpipers and measuring prevalence of *S. morrisoni* and *G. adunca* in *C. volutator* found in the digestive tracts of birds. If increased crawling in amphipods is an adaptation

to increase the likelihood of predation by sandpipers, prevalence of parasites should be higher in *C. volutator* in guts of sandpipers than in *C. volutator* that remain burrowed within the substrate (but similar to prevalence in crawling amphipods).

My third recommendation for future tests of the 'manipulation' hypothesis is that they be conducted in systems where the phylogenies of the parasite and host are known (e.g., Moore and Gotelli 1996). This approach will enable researchers to determine the extent to which specific manipulations are constrained through evolutionary time. Fourth, I recommend that researchers conduct investigations into the physiological mechanisms and costs involved in manipulating host behaviour. Identifying costs of manipulation would represent one of the first quantifiable costs of reproduction in parasites (where few costs are known; Rea and Irwin 1994) and would allow researchers to investigate life-history trade-offs within parasites. Finally, future studies of parasite manipulation should be conducted in natural populations of hosts and parasites (as in Chapters 3-5) and involve different stages in the life cycles of parasites (e.g., final-host vertebrates). Although parasites are much smaller relative to their vertebrate hosts compared to their invertebrate hosts, Poulin (1994b) found evidence that parasites are often associated with changes in behaviours of vertebrates. The adaptive significance, if any, of such changes in vertebrate behaviour is not known.

3. Adaptive life history responses of hosts to parasitism

In Chapters 4 and 5, I found evidence that amphipod hosts respond to parasitism through variation in their life histories. Specifically, male and female *C. volutator* increased their allocation to reproduction when first parasitized; prior to the onset of high costs of parasitism. To demonstrate that behavioural changes in infected hosts are truly adaptive for hosts, additional studies using this type of experimental approach are required. Such studies could take several forms. One possibility is to experiment with systems where a single species of host is infected by several species of parasites, where each parasite is known to have different effects on current versus future reproduction of the host (e.g., parasites which are lost after infection versus those which are not). Predictions related to host response (increases versus decreases in current versus future reproduction, respectively) could then be tested. Similarly, systems where different species of hosts are infected by a common parasite also could be used to test for adaptive host response, particularly if the timing of reproduction differs with host species. A third approach would be to study parasitism in relation to host age and/or sex. Because selection should act differently on individuals of different ages or sexes to maximize their reproductive success (Zuk 1990; McCurdy et al. 1998), different groups of hosts may be expected to differ in their allocation to current versus future reproduction (Forbes 1993).

Future tests of the 'adaptive host response' hypothesis should investigate responses of hosts that are not directly related to reproduction (e.g., parental

care, social activity; Forbes 1993). For example, indirect costs of reproduction for male *C. volutator* may include mate-searching and mate-guarding behaviours; these behaviours could be more costly for males than the production of sperm (Forbes et al. 1996). Experiments manipulating competition (densities of males) as well as the 'quality' of potential mates (size, condition, time to receptivity of females) could be used to test for additional responses of males to parasitism by nematodes or trematodes.

In testing for life history responses of hosts to parasitism, experiments must be designed with proper controls. At present, researchers often expose a single group of hosts to parasites and define their treatment group as hosts that were attacked and their control group as hosts that were resistant to attack (e.g., Paige and Whitham 1987; Shykoff and Kaltz 1997). Comparisons of life history parameters (growth, survivorship, reproduction) are then made between the treatment and control groups. The problem with this design is that factors that influence host susceptibility also could influence the outcome of the life history variables. For example, hosts that are susceptible to attack may be the least able to respond through life-history variation, not so much because of costs associated with parasites, but because of pre-existing conditions that influenced susceptibility. To establish proper controls, researchers should instead compare a group of unexposed hosts (control group) with exposed hosts (considering the level of susceptibility as a response within this group; e.g., Chapters 4,5).

To demonstrate that a response to parasitism is truly adaptive, researchers should include separate controls to show that responses to parasitism are costly to host fitness when parasites are absent and that parasites are costlier to hosts when they are unable to detect their attacks. This type of approach has recently been used to examine the costs of induced chemical defenses in plants (Agrawal et al. 1999). By exposing hosts to compounds shown to elicit defensive responses or by exposing hosts to parasites for a short time before removing them (either by physical or chemical means) researchers could induce 'false' life history responses. To assess the cost of attack in the absence of induction cues by parasites, researchers could simulate attacks by wounding hosts. When only induction cues are present, hosts are expected to have higher fitness than when natural or 'simulated' parasites are present, but lower fitness than in uninduced and unattacked hosts.

4. Impacts of parasites on populations of *Corophium volutator*

In previous chapters, I focused on the effects of parasites on behaviour and reproduction of individual amphipods. However, there is evidence that impacts of parasites may transcend higher scales of organization, such as the population and community levels (Scott and Dobson 1989; Minchella and Scott 1991). Thus, future studies of *C. volutator* should consider the possibility that *S. morrisoni* and *G. adunca* have effects on *C. volutator* at the population level. *Corophium volutator* may also be considered as a 'keystone' species in the soft-bottom

intertidal zone because of the numerous effects it has on other species (Appendix H). If this is the case, population-level effects on *C. volutator* caused by parasites could profoundly influence the structure of intertidal ecosystems (Lauckner 1986).

In Chapters 2-6, I found no evidence to indicate that either predation by shorebirds or parasitism by nematodes and trematodes at present levels could significantly impact or regulate populations of *C. volutator*. While intense, shorebird predation may account for only a fraction of total mortality (Chapter 6). Parasites, which caused mortality, appear to be rare in natural populations (overall prevalence was <1% and never exceeded 15%; Chapters 3-5). Despite this, researchers and conservation biologists should continue to monitor parasites in populations of *C. volutator* in the Bay of Fundy. This is because prevalence of trematodes in other populations (in Europe) is also low in most years (<1%), but periodically increases to >99% (Bick 1994; Meißner and Bick 1997). In fact, trematodes are thought to be responsible for recent crashes in populations of *C. volutator* in the Baltic Sea (Jensen and Mouritsen 1992; Meißner and Bick 1999); these crashes have led increased erosion of mudflats (Mouritsen et al. 1998). Unexplained crashes of *C. volutator* also have been reported in the Bay of Fundy (Shepherd et al. 1995; Appendix I) and now that trematodes have been identified in one of these populations, the possibility that parasites are involved in future crashes should be investigated. The additional impact of human activities on mudflats (e.g., baitworm harvesting, which has

been shown to decrease densities of *C. volutator*, Shepherd and Boates 1999), also must be considered in population-level studies of *C. volutator*.

There is reason to believe the impacts of parasites on populations of *C. volutator* may be increasing. This is because recent models predict that ocean temperatures will increase 2-6°C when atmospheric CO₂ levels double (Lehtonen 1998) and prevalence and intensities of infection of *C. volutator* by trematode cercariae have been shown to increase >4-fold with an increase in temperature of <5°C (Mouritsen and Jensen 1997; pers. obs.). Epizootics in *C. volutator* could have severe impacts on migratory shorebirds and juvenile fish; these feed almost exclusively on *C. volutator* (Hicklin and Smith 1979; Imrie and Daborn 1981; Appendix H).

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APPENDIX A

GLOSSARY OF TERMS

Acanthocephalan: An endoparasite of the Phylum Acanthocephala. All members have two hosts in their life cycles and a long proboscis covered with spines (Ruppert and Barnes 1994).

Acuaroid: A nematode of the Family Acuaroidae (Wong and Anderson 1987).

Cercaria: A form of a digenean trematode which hatches from a sporocyst/redium, leaves an intermediate host, and remains free-living (usually propelled by a tail) until it contacts and penetrates an appropriate second-intermediate host (usually by means of a stylet).

Cestode: A flatworm of the Class Cestoda.

Complex life cycle: A parasite life cycle which includes more than one host.

Digenean: A trematode that requires more than one host to complete its life cycle.

Epizootic: An outbreak of parasites in a host population which usually causes high mortality.

Exuvium: A moulted exoskeleton.

Final host: The host of a parasite wherein sexual reproduction occurs (in digeneans, this is most often a vertebrate) (Moore 1981).

Fitness: The proportionate contribution of individuals to future generations of a population (Pianka 1994). For a parasite this is related to its likelihood of completing its life cycle.

Harem-defense polygyny: A reproductive strategy where males defend a group of females in order to mate with them. This strategy occurs when females exist in defensible groups and are receptive to mating at the same time (Just 1988).

Hazard rate: A statistical term that refers to the probability that an individual that has survived to the beginning of a time interval will die in that time interval (Cox 1972).

Intensity: A term used to describe the number of parasites within infected hosts (excluding uninfected hosts) (Bush et al. 1997).

Intermediate host: A term for the host of a parasite (usually an invertebrate) where sexual reproduction does not occur (asexual reproduction may occur, but need not).

'Keystone' species: A species whose presence strongly influences diversity and abundance of other species through various direct and indirect interactions (Pianka 1994).

Metacercaria: A form of a trematode parasite which infects second-intermediate hosts, often forming a cyst within the body of the host.

Microsporidian: A protozoan parasite which has recently been implicated in causing female-biased sex ratios in hosts.

Miracidium: A ciliated, free-living form of a trematode parasite which occurs when a fertilized egg is released by an adult trematode and hatches. This form

enters an intermediate host and reproduces asexually (producing sporocysts).

Ovigerous: A term to describe a female that is carrying fertilized eggs or recently-hatched offspring.

Parturial moult: A moult which is associated with the onset of reproduction (herein associated with receptivity in female *Corophium volutator*).

Prevalence: The percentage of hosts in a population that are infected by one or more parasites (Bush et al. 1997).

Pseudogamy: A reproductive strategy whereby a female must mate with a male in order to reproduce, but the male makes no genetic contribution to the offspring (Beukeboom 1998).

Realized fecundity: The number of offspring that a female of a given age is expected to produce over the remainder of her lifetime when accounting for sources of mortality on the female and her developing offspring.

Receptivity: The ability of a female to mate and become ovigerous (herein defined specifically as the physiological ability of a female to mate).

Reproductive effort: The amount of resources an individual allocates to activities related to reproduction, regardless of the output from this allocation (e.g., obtaining and defending mates, mating, carrying offspring to term).

Reproductive success: The number of reproductively viable offspring produced by an individual. For females this refers to the total number of offspring

that are brought to term and for males this refers to the number of offspring brought to term by mated females.

Reproductive synchrony: The degree to which different females in a single population reproduce after accounting for general factors, such as climate.

Spicule: A paired sex organ found in male nematodes which is used to inseminate females. This shape and size of this organ is often species-specific and is used in identification (Wong and Anderson 1987).

Sporocyst: A form of a trematode parasite that is produced asexually in an intermediate host gastropod and gives rise to several free-living forms of a parasite (cercariae).

Stylet: An arrow-shaped structure that protrudes from the front of a cercarial trematode and is used to penetrate a second-intermediate host.

Trophic transmission: A method of parasite transmission where the parasite is transmitted to a final host when its intermediate host is eaten by a final host.

APPENDIX B

Figure B-1 (A,B). A diagram of the external anatomy of the amphipod *Corophium volutator* (female shown) illustrating components that are discussed in Chapters 1-6 and a micrograph of an adult male and an adult female *C. volutator* illustrating the difference in the length of their second antennae.

Figure B-1 (A). Diagram of the external anatomy of the amphipod *Corophium volutator* (female shown).

A

Figure B-1 (B). Micrograph of an adult male and an adult female *C. volutator*.

B

APPENDIX C

Figure C-1. Map of the Minas Basin, Nova Scotia, Canada showing the locations of the two mudflats used in my studies. The mudflat at Blomidon (45° 13' N; 64° 22' W) was used to collect data on *Corophium volutator* in the absence of both predation by shorebirds and parasitism by nematodes and trematodes (Chapters 1,3,6). The mudflat at Starrs Point (45° 08' N; 64° 22' W) was used to study the effects of predation by sandpipers (Chapter 2), to conduct surveys of parasites in a natural population of *C. volutator*, and as a source for parasites used in laboratory experiments (Chapters 3-6). *Corophium volutator* at Starrs Point are subjected to seasonal predation by semipalmated sandpipers (*Calidris pusilla*) as well as parasitism by *Skrjabinoclava morrisoni* and *Gynaecotyla adunca* (Chapters 2-5).

Figure C-1. Map of the Minas Basin, Nova Scotia.

APPENDIX D

In Chapter 1, I used the 'actual tidal method' (Reaka 1976) to determine whether females would have tended to moult during spring tides (those increasing in magnitude) or neap tides (those decreasing in magnitude). To do this, I calculated the lowest mean height of the tides above the geodetic datum for each date at Blomidon (Canadian Hydrographic Service 1998). I then determined if the mean low water for each date was above (neap) or below (spring) the overall mean value calculated for the bimonthly cycle that the given date fell within. I applied a χ^2 test to determine if moult was more common during spring or neap tides.

To examine the relationship between moult and the different phases of the lunar cycle in Chapter 1, I used 'initial lunar analysis' and 'divided lunar analysis' (Reaka 1976). These methods divide the lunar month into four phases, but in different ways. 'Initial lunar analysis' defines each quarter as running from the day of the apex of a phase until the day before the apex of the next phase while 'divided lunar analysis' divides the lunar calendar into segments which center around the apex of each phase (three days prior to the day of, and four days following, the apex). For each method of analysis, I applied χ^2 tests to compare the numbers of moults among the categories.

APPENDIX E

Figure E-1 (A-D). Life cycle of the nematode *Skryabinoclava morrisoni* on intertidal mudflats in the Minas Basin, Nova Scotia. Names of the intermediate and final hosts appear in italics and parasite life stages appear in bold. Also shown are scanning electron micrographs of a male Stage III larva of *S. morrisoni* illustrating the entire body, the structure of the cuticle, and the tail hook.

Figure E-1 (A). Life cycle of the nematode *Skrjabinoclava morrisoni*.

A

Stage I-III

Corophium volutator

Stage III

Eggs

Adults

Calidris pusilla

Figure E-1 (B-D). Scanning electron micrographs of a male Stage III larva of *S. morrisoni* illustrating the entire body (B), the structure of the cuticle (C), and the tail hook (D).

APPENDIX F

In Chapter 3, I compared the complexity of trails of parasitized and unparasitized *C. volutator* using With's (1994) variation of the dividers method (Dicke and Burrough 1988). To do this, I traced the trail of each male (recorded for 5 minute) from a video screen onto a transparency and scanned the image into DesignCAD 2-D (American Small Business Computers 1989). Next, I generated circles of eight fixed diameters (0.4 cm - 3.2 cm in 0.4 cm intervals) and overlaid them on each trail (as in With 1994). I then tallied the number of circles required to completely cover each trail to calculate the total path length of the trail (circle diameter $\cdot$ number of circles used). Finally, I calculated a fractal dimension (D) for each trail by plotting the natural logs of the circle diameters by their corresponding total path lengths and subtracting the resulting line-of-best-fit from one. D values may range between one and two (one represents a perfectly straight line while two describes a line so complex that it fills the entire plane).

APPENDIX G

Figure G-1 (A-E). Life cycle of the trematode *Gynaecotyla adunca* on intertidal mudflats in the Minas Basin, Nova Scotia. Names of first, second-intermediate, and final hosts appear in italics and parasite life stages appear in bold.

Micrographs of *G. adunca* illustrate: sporocysts in *Ilyanassa obsoleta*, a cercarial form, an unencysted metacercariae in *Corophium volutator* (24 h post-infection), and an encysted metacercaria in *C. volutator* (15 days post-infection).

Figure G-1 (A). Life cycle of the trematode *Gynaecotyla adunca*.

A

Figure G-1 (B,C). Micrographs of *Gynaecotyla adunca* illustrating sporocysts in *Ilyanassa obsoleta* (B) and a cercarial form (C).

Figure G-1 (D,E). Micrographs of *Gynaecotyla adunca* illustrating an unencysted metacercariae in *Corophium volutator* (24 h post-infection, one illustrated by an arrow; D), and an encysted metacercaria in *C. volutator* (15 days post-infection; E).

APPENDIX H

Table H-1. Impacts of the amphipod *Corophium volutator* on the physical components (sediment stability and chemistry) and biological components (role as a predator, prey item, and host) of soft-bottom intertidal ecosystems.

Effect	Location	Source
<i>Physical /chemical effects of burrows on sediments</i>		
Net stabilizing effect	Wadden Sea (Denmark)	1
Increase redox potential and carbon content	Laboratory/ Ythan estuary (Scotland)	2
Increase nitrification and denitrification	Laboratory	3
Decrease water content	Rivers Crouch and Blackwater (England)	4
Net stabilizing effect	Laboratory	5
Alter nitrogen transformation	Laboratory	6
Increase shear strength by 50-180%	Clyde estuary (England)	7
Increase or decrease stability	Minas Basin (Canada)	8
Decrease water content	Minas Basin (Canada)	9
Increase stability	Laboratory	11
Increase thermal diffusion	Minas Basin (Canada)	12
Increase stability and decrease water content	Laboratory	12
Increase transfer of nitrogen	Laboratory	13
<i>Competitive effects on other infaunal species</i>		
Increase abundance of free-living nematodes	Laboratory/ Ythan estuary (Scotland)	2
Increase settlement of polychaetes (<i>Marenzelleria viridis</i>)	Laboratory/Darss-Zingst (Germany)	14
Exclude adult polychaetes (<i>Marenzelleria viridis</i>)	Darss-Zingst (Germany)	15
Compete with a polychaete (<i>Nereis diversicolor</i>)	Laboratory	16
Inhibit colonization of a halophyte (<i>Salicornia europaea</i>)	River Crouch (England)	17
Invade brackish environments	Lake Volkerak-Zoommeer (Netherlands)	18
Exclude other amphipods (<i>Corophium arenarium</i>)	Hojer tidal flat (Denmark)	19

Effect	Location	Source
<i>Importance as a host for parasites</i>		
Nematode (<i>Skrjabinoclava morrisoni</i>)	Minas Basin (Canada)	20
Trematode (<i>Gynaecotyla adunca</i>)	Minas Basin (Canada)	21
Trematode (<i>Maritrema subdolum</i>)	Laboratory	22
Trematode (<i>Microphallus claviformis</i>)	Laboratory	23
Trematodes (<i>Levinsoniella brachysoma</i> <i>Maritrema subdolum</i>)	Mecklenburg Bight (Germany)	24
Trematode (<i>Maritrema subdolum</i>)	Laboratory	25
Trematodes (<i>Maritrema subdolum</i> , <i>Microphallus claviformis</i>)	Wadden Sea (Denmark)	26
Trematodes (<i>Levinsoniella brachysoma</i> <i>Maritrema subdolum</i>)	Mecklenburg Bight (Germany)	27
Trematodes (<i>Maritrema subdolum</i> , <i>Microphallus claviformis</i>)	Wadden Sea (Denmark)	28
Nematode (<i>Skrjabinoclava morrisoni</i>)	Laboratory	29
Yeast (<i>Cryptococcus gammari</i>)	Baltic Sea	30
<i>Importance as a prey item</i>		
Sandpipers foraging at night	Minas Basin (Canada)	31
Mud snail (<i>Ilyanassa obsoleta</i>)	Laboratory/Minas Basin (Canada)	32
Sand shrimp (<i>Crangon crangon</i>)	Baltic Sea	33
Dunlin (<i>Calidris alpina</i>) foraging at night	Hojer tidal flat (Denmark)	34
Sand goby (<i>Pomatoschistus minutus</i>)	Aarland (England)	35
Juvenile cod and flounder	Gullmarsvik Bay (Sweden)	36
Grey plover (<i>Pluvialis squatarola</i>)	Wash (England)	37
Isopod (<i>Saduria entomon</i>)	Baltic Sea	38
Semipalmated Sandpipers (<i>Calidris pusilla</i>)	Starrs Point (Canada)	39

Effect	Location	Source
<i>Importance as a prey item cont ...</i>		
Sand goby, <i>Pomatoschistus minutus</i>	Ythan estuary (Scotland)	40
Several species of fish	Ythan estuary (Scotland)	41
Sandpipers and ground fish	Minas Basin (Canada)	42
Polychaete (<i>Nereis diversicolor</i>)	Laboratory/Baltic Sea	43
Numerous epibenthic invertebrate fauna	Gullmarsvik Bay (Sweden)	44
Sandpipers	Minas Basin (Canada)	45
Sandpipers	Chignecto Bay	46
Sand shrimp (<i>Crangon crangon</i>)	Gullmarsvik Bay (Sweden)	47
Atlantic Silverside (<i>Menidia menidia</i>)	Minas Basin (Canada)	48
Juvenile fish	Minas Basin (Canada)	49
Flounder (<i>Platichthys flesus</i>)	Ythan estuary (Scotland)	50
Fly (<i>Hydrophorius oceanus</i>)	Dover Estuary (Wales)	51
Sandpipers	Minas Basin (Canada)	52
Redshank (<i>Tringa totanus</i>)	Ythan estuary (Scotland)	53
Shelduck (<i>Tadoma tadoma</i>)	Ythan estuary (Scotland)	54
Mullets (<i>Mugil labrosus</i> and <i>M. ramada</i>)	various lakes (Tunisia)	55
<i>Importance as a predator</i>		
Remove diatoms	Wadden Sea (Denmark)	1
Remove diatoms	Baltic Sea	56
Remove diatoms	Laboratory	16
Regulate abundance of diatoms	Rivers Crouch and Blackwater (England)	3
Consume 27% of algal production	Pecks Cove (Canada)	57
Remove detritus and diatoms	Evangeline Beach (Canada)	58
Remove diatoms	Cobequid and Chignecto Bays (Canada)	59

Sources: 1) Mouritsen et al. 1998. 2) Limia and Raffaelli 1997. 3) Gerdol and Hughes 1994a. 4) Gerdol and Hughes 1994b. 5) Grant and Daborn 1994. 6) Pelegri and Blackburn 1994. 7) Pender et al. 1994. 8) Daborn et al. 1993. 9) Matthews et al. 1992. 10) Meadows et al. 1990. 11) Piccolo et al. 1993. 12) Meadows and Tait 1989. 13) Henriksen et al. 1983. 14) Zettler 1998. 15) Zettler 1997. 16) Smith et al. 1996. 17) Gerdol and Hughes 1993. 18) van Nes and Smit 1993. 19) Jensen and Kristensen 1990. 20) Chapter 3. 21) Chapters 4,5. 22) Meißner and Bick 1999. 23) Jensen et al. 1998. 24) Meißner and Bick 1997. 25) Mouritsen and Jensen 1997. 26) Mouritsen et al. 1997. 27) Bick 1994. 28) Jensen and Mouritsen 1992. 29) Wong and Anderson 1988. 30) Segerstråle 1937. 31) Chapter 2. 32) per. obs. 33) Norkko 1998. 34) Mouritsen 1994. 35) Aarnio and Bonsdorff 1993. 36) Pihl 1982. 37) le V. Dit Durell and Kelly 1990. 38) Sandberg and Bonsdorff 1990. 39) Boates and Smith 1989. 40) Jaquet and Raffaelli 1989. 41) Matilla and Bonsdorff 1989. 42) Wilson 1989. 43) Roenn et al. 1988. 44) Pihl 1985. 45) Hicklin and Smith 1984. 46) Gratto and Thomas 1984. 47) Pihl and Rosenberg 1984. 48) Gilmurray and Daborn 1981. 49) Imrie and Daborn 1981. 50) Summers 1980. 51) Mills 1980. 52) Hicklin and Smith 1979. 53) Goss-Custard 1977. 54) Buxton 1976. 55) Farrugio 1976. 56) Creach et al. 1997. 57) Hawkins 1985. 58) Stuart et al. 1985. 59) Hargrave et al. 1983.

APPENDIX I

Figure I-1 (A-B). Photographs of two mudflats in the Bay of Fundy taken in June 1997. Starrs Point, Nova Scotia is a mudflat where densities of *Corophium volutator* have remained high over the past 20 years (Shepherd et al. 1995).

Grande Anse, New Brunswick is a mudflat where densities of *C. volutator* have been shown to decline since the 1970s. *C. volutator* were found in high densities throughout Starrs Point, while amphipods were only present (and in low densities) in the raised areas at Grande Anse.

Figure I-1 (A,B). Photographs of Starrs Point, Nova Scotia (A) and Grande Anse, New Brunswick (B).