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Factors influencing parasite-related suppression of mating behavior in the isopod Caecidotea intermedius

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Factors influencing parasite-related suppression of mating behavior in the isopod *Caecidotea intermedius*

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Abstract

Parasites with indirect life cycles often facilitate changes in their intermediate hosts in ways that increase the likelihood of transmission to their definitive hosts. Acanthocephalan infections typically correlate with altered pigmentation, antipredatory behavior, and changes in mating behavior in arthropod intermediate hosts that increase risks of predation by definitive vertebrate hosts. Additionally, these changes have been shown to associate with the developmental stage of the parasite which facilitates the likelihood of survival in the final host. These changes have been proposed to due to direct manipulation by the parasite, host counteradaptation to minimize the costs of infection, or are an indirect byproduct of pathology.

The acanthocephalan parasite, *Acanthocephalus dirus*, infects the stream-dwelling isopod *Caecidotea intermedius* as an intermediate host and one of several freshwater fishes as a definitive host. Inside the isopod, *A. dirus* develops from the early non-infective acanthor and acanthella (immature) stages to the late infective cystacanth stage (mature, capable of transmission to the final host). Developmental stage of *A. dirus* also correlates with changes in isopod color, antipredatory behavior, and mating dynamics. *C. intermedius* infected with late-stage parasites have been shown to have reduced pairing success in nature. Additionally, it has been shown that male mating responsiveness (e.g. willingness to mate) is reversible (from no mating attempts to positive mating attempts). However, little is known about the potential ultimate and proximate mechanisms underlying these relationships. Additionally, the potential role of host counteradaptation (compensation) during early stages of infection has not been examined.

To examine isopod mating behavior in early-stage infections, I used field-based experiments to assess if host compensation was occurring in male *C. intermedius*. I found that infected isopods did not increase their mating effort compared to uninfected males. Thus, I concluded that male isopods do not compensate for a future reproductive loss.

To assess factors that influence male mating responsiveness in late stages of parasite development, I used a combination of field and lab-based experiments. Since chemical cues have been shown to be important in aquatic environments and because predation is necessary for completion of the parasite life cycle, I examined if predator cues could influence male mating responsiveness using a lab-based experiment. I found that predator cues alone do not appear to be influencing mating response. However, I did find that reversibility of mating response can be maintained in a laboratory-setting. I also examined if mating responsiveness is flexible and reversible in nature using a field-based experiment. I found that male mating responsiveness is flexible in nature towards the end of *C. intermedius* life cycle. I also found that reversibility of mating response occurs within 200 minutes of removal from a natural setting. Thus, it is unlikely that mating responsiveness could be due to an indirect effect of pathology.

The ultimate mechanisms I have studied indicate that parasite manipulation is the most likely cause of mating behavior in *C. intermedius*. Early-stage parasites can not survive transmission to the definitive host. Thus, manipulation of male mating behavior is not beneficial to the parasite at this life stage. Additionally, since male mating behavior is flexible and reversible in nature, it is plausible that parasites can manipulate

this behavior to conserve energy (absence of predators or towards end of the breeding season) and increase the likelihood of survival into the definitive host.

I examined if neuromodulation could be a proximate mechanism controlling mating behavior. Dopamine and serotonin levels were assayed for infected and uninfected isopods with suppressed mate guarding behavior. I found no difference between infection status and either dopamine or serotonin levels. Thus, these neurohormone levels did not appear to be influencing mating behavior in male *C. intermedius*.

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Introduction

Parasites can be defined as organisms that require a host to either live in or on so that they can undergo development and reproduction (Moore 2002). All organisms are susceptible to parasitic infection at some point during their life cycle. Often, parasites induce changes in host behavior and physiology, which can have potentially life threatening consequences to their hosts (Dobson 1988). In some cases, parasites induce changes in the mating dynamics of their hosts, which can lead to changes in the individuals' reproductive success and as a consequence variation in population dynamics (Bollache et al. 2001; Dunn 2005; Hamilton and Zuk 1982; Moore 2002; Zohar and Holmes 1998; Zuk 1992).

Parasites can also drive evolutionary change and ecological dynamics in some cases (Freeman and Herron 2001; Poulin 1995a; Thomas et al. 2005). For example, parasite-host interactions are thought to be driving the evolution of secondary sex characteristics as well as host mate choice (Hamilton and Zuk 1982). Individually, parasites can alter their hosts' growth (Thompson 1986) and on a population level, parasitic infection can dramatically influence mating interactions and reproductive rates (Dobson 1988; e.g. crickets, Adamo et al. 1995a). Community dynamics can also be regulated by altering predator-prey interactions (Lafferty 1999; e.g. killifish, Lafferty and Morris 1996). Parasites can also influence ecosystem dynamics by altering energy flow (Thomas et al. 1999).

Transmission between hosts occurs either actively or passively (Moore 2002). Active transport is most commonly found in parasites that use arthropods as intermediate hosts and involves parasite-related changes in the phenotype of the arthropod that increases the probability of consumption by the definitive host. Passive transport is most

commonly found in bacteria, viruses, and protists and involves transmission between hosts via vectors (water, food, blood).

Parasites can complete their life cycle in two ways, either directly (no intermediate host), or indirectly (having at least one intermediate host) (Crompton and Nickol 1985; Kennedy 2006; Moore 2002). For both life cycles, active and passive transport can occur (Moore 2002). For example, *Giardia intestinalis* has a direct life cycle and uses passive transport in which it is shed in the stools of their host (mammal) and then consumed by a new host (mammal). Active transport in direct life cycles occurs in parasite-host relationships such as the parasitic isopod, *Cymothoa exigua* which uses environmental cues to find snappers and replace their tongue (Brusca and Gilligan 1983). Passive transport in indirect life cycles occurs in systems such as *Dipylidium caninum* (tapeworm), which requires a larval flea as an intermediate host and is consumed by the definitive host, a dog or cat. Active transport in indirect life cycles is common in acanthocephalans, such as *Pomphorhynchus tereticollis,* which induces behavioral changes in intermediate hosts (amphipod) to increase the likelihood of consumption by the definitive host (bullhead, Perrot-Minnot et al. 2007).

Parasites that have indirect transmission rely on a predation event to complete their life cycle (Dobson 1988; Moore 2002). Since predation is a necessary component of this lifecycle, parasites that have developed strategies that increase the probability that predation occurs have been favored by selection (Adamo 2002; Moore 2002; Thomas et al. 2005). Although it is known that parasitic infection of intermediate hosts can cause changes in behavior and physiology, the mechanisms driving these changes remain unclear (Hurd 1990a; Poulin 1995b). To understand the mechanisms underlying these

host behavioral changes, it is important to examine both proximate and ultimate causes (Poulin 2002).

Host modification could be due to manipulation by the parasite since it's the parasite that ultimately benefits by increased transmission to the final host (Adamo 2002; Moore 2002; Moore and Gottelli 1990; Poulin 1995b; Poulin and Thomas 1999; Thomas et al. 2005). However, it is also possible that these changes are a host counteradaptation that allows the host to accumulate energy which can be used to either outlive the parasite or mount an immune response against the parasite (Adamo 2002; Forbes 1993, 1996; Minchella 1985; Moore 2002; Thomas et al. 2005). A third possibility is that the changes are due to pathological effects associated with parasite infection (Adamo 2002; Holmes and Zohar 1990; Moore 2002; Moore and Gotelli 1990; Thomas et al.2005)

The behavioral and physiological changes seen in infected hosts are often directly correlated with the developmental stage of the parasite because only the final stage can survive transmission to the definitive host (Bethel and Holmes 1974; Moore 2002; Poulin 1995b; Poulin and Thomas 1999). For example, the nematode parasite, *Skrjabinoclava morrisoni*, alters amphipod behavior by increasing surface activity only when it has developed into its infective stage for the final host, the sandpiper (McCurdy et al. 1999). Late development stages have also been shown to correlate with reduced host fecundity (Hurd 1990b). Male amphipods (*Corophium volutator*) infected with a trematode parasite (*Gynaecotyla adunca*) had a decreased mating effort once the parasite had reached the infective stage (capable of transmission to the final host, sandpiper) (McCurdy et al. 2000).

Hosts that are infected with later developmental stages may be utilizing different mechanisms to counteract parasitic infections. A decreased mating behavior could be seen in infected intermediate hosts if the parasite is controlling this behavior; 1) since mating responsiveness is energetically costly to the host, parasites may be using this energy for their own purpose in order to modify the host or for their own growth or 2) if decreasing the mating responsiveness increases exposure to definitive hosts (Poulin 1994, 2002; Thomas et al. 2005). However, if controlled by the host, the decreased mating response seen could be a counteradapatation to parasitic infection (Minchella 1985; Forbes 1993; Poulin 1994). Infected hosts may be allocating energy normally reserved for mating either to fight the parasite, and minimize costs of infection, or to allow for the host to outlive the parasite and thus expend its reproductive effort later in life. At this time, host counteradaptation in regards to mating suppression is theoretical, however other studies have shown that host counteradaptation to parasitic infection for non-mating behaviors does exist in nature (Moore 2002). Specifically, bumblebees infected by a conopid fly disperse to cooler temperatures (behavioral chills) that retard parasitic development which is not seen in their uninfected counterparts (Müller and Schmid-Hempel 1993). Thus, infected bumblebees have a counteradapatation in order to minimize the effects of parasitic infection.

In some cases host counteradaptation can occur in early stage parasite development (Minchella 1985; Forbes 1993, 1996). For example, exposure to the trematode parasite, *Schistosoma mansoni*, causes increased reproductive effort early in the life of the snail, *Biomphalaria glabrata* (Minchella and Loverde 1981). Thus, the reproductive compensation can be considered an adaptive change in an effort for the snail

to maximize their reproductive success. Shifting to early reproduction at the onset of parasite infection maximizes the infected host's fecundity. This shift to early reproduction has also been seen with the crustacean, *Daphnia magna* when it is parasitized by *Glugoides intestinalis* (microsporidian) (Chadwick and Little 2005). This shift in reproductive efforts also correlates with a decrease in later clutch sizes. Thus, it appears that *D. magna* is compensating earlier in life for a later reduced reproductive output (Chadwick and Little 2005).

In terms of proximate mechanisms, several recent studies have proposed that parasite-related changes in behavior may be mediated through neuromodulation (Adamo 2002, Helluy and Holmes 1990; Maynard et al. 1996; Poulin et al. 2003; Rojas and Ojeda 2005; Tain et al. 2006). In the invertebrate CNS, neuromodulation can occur through neuropeptides, such as hormones or small molecule neurotransmitters, which can influence behavior (Birmingham and Tauck 2003; Thompson and Kavaliers 1994; Truman 2002). Monoamines such as dopamine, serotonin, and octopamine (analogous to norepinephrine in vertebrates; Adamo et al. 1995b) are neurotransmitters that have been shown to play a role in movement, aggression, and the flight or fight response (Purves et al. 2004). For example, octopamine levels correlate positively with aggression and flying behavior in crickets (Adamo et al. 1995b) and dopamine regulates crawling behavior in leeches (Puhl and Mesce 2008). In addition, elevated levels of serotonin have been linked to a decrease in mating in the sphinx moth (Kloppenburg and Heinbockel 2000). Thus, since these neurotransmitters have been shown to regulate movement and behavior in invertebrates, parasites or hosts may manipulate these hormones when infection is established.

Acanthocephalan parasites (Phylum: Acanthocephala) are obligate, bilaterally symmetrical endoparasites commonly referred to as either thorny-headed or spiny-headed worms. Acanthocephalans are characterized by a retractile and invaginable proboscis which the parasite uses to attach to the intestine of its final host (Bullock 1969; Crompton and Nickol 1985; Kennedy 2006; Morris and Crompton 1982; Nickol 2006). Historically, the phylum Acanthocephala has been considered to contain three classes; Eoacanthocephala, Archiacanthocephala, and Palaeacanthocephala (Crompton and Nickol 1985; Kennedy 2006). However, recently a fourth small class, Polyacanthocephala comprising of four species has been recognized (Nickol 2006; Kennedy 2006).

Acanthocephalans are one of only two known phyla (the other is Nematomorpha) that are exclusively parasitic and undergo no development outside of a host (Crompton and Nickol 1985; Kennedy 2006). A relatively small monophyletic phylum, the distribution of Acanthocephala is widespread, with species occurring in almost every terrestrial, marine, and aquatic environment (Crompton and Nickol 1985; Kennedy 2006; Nickol 2006). All species of acanthocephalans (~1100 species) have indirect life cycles with active transmission and require an arthropod as an intermediate host in which development from the egg to the acanthellae (immature) and the cystacanth (mature) stage occurs. All species then require a vertebrate as a definitive host in which reproduction occurs (Crompton and Nickol 1985; Kennedy 2006; Morris and Crompton 1982). Acanthocephalan species exhibit little variation in internal anatomy and in stages of development, however they are considered successful based on their wide geographic distribution (Kennedy 2006). These parasites generally have devastating impacts by

altering behavior of their intermediate hosts which results in death by predation (Crompton and Nickol 1985; Kennedy 2006, Moore 2002).

Acanthocephalan-related changes in the host include habitat shifts, color alterations, and changes in antipredatory behavior (Crompton and Nickol 1985; Kennedy 2006; Moore 2002). Specifically, the infected invertebrate often undergoes alterations in its pigmentation that increases the conspicuousness to the definitive host by causing a contrast with backgrounds and altering host behavior (Crompton and Nickol 1985; Camp and Huizinga 1979; Kennedy 2006; Moore 2002; Poulin and Thomas 1999). For example, parasitized *Asellus aquaticus* (isopod) are darker in pigmentation then their respective uninfected counterparts (Munro 1953). In contrast, *Caecidotea intermedius* (isopod) appear to have a loss of pigmentation and look lighter in color when infected with the cystacanth parasite *Acanthocephalus dirus* (Camp and Huizinga 1979; Seidenberg 1973; Sparkes et al. 2004). However, this "loss of pigmentation" is actually the loss of the infected isopods ability to develop color and is more accurately described as pigmentation dystrophy (Oetinger and Nickol 1981). In terms of behavior, Bethel and Holmes (1977) found that gammarids infected with *Polymorphus paradoxus* have an increased risk of predation by mallards. Uninfected *P. paradoxus* have a negative phototaxis response (away from light) and are therefore generally out of range of the surface-feeding mallards. However, infected *P. paradoxus* have a positive phototaxis response (towards light) and thus increases the likelihood of consumption by the definitive hosts (Bethel and Holmes 1977).

Acanthocephalans have also been shown to negatively influence the mating dynamics of their intermediate hosts (Bollache et al. 2001; Dezfuli et al.1999; Oetinger

1987; Sparkes et al. 2006; Zohar and Holmes 1998). Numerous studies have shown that infected invertebrates are rarely found in mating pairs in nature (Bollache et al. 2001; Oetinger 1987; Zohar and Holmes 1998). In some cases these changes seem to be due to a decrease in male competitive ability (Zohar and Holmes 1998) or a decrease in both male competitive ability and male responsiveness to females (Bollache et al. 2001).

Previous studies on acanthocephalan-host relationships indicate that neuromodulation could be a potential mechanism underlying host modification (Helluy and Holmes 1990; Maynard et al.1996; Poulin et al. 2003; Rojas and Ojeda 2005; Tain et al. 2006). Acanthocephalans have a nervous system that consists of a cerebral ganglion that resides in the proboscis receptacle and nerves that branch away from it running the length of the body (Nickol 2006). Recent studies have shown that acanthocephalan infection correlates with a manipulation of their hosts anatomy and behavior. For example, *Polymorphus paradoxus* infection in amphipods (*Gammarus lacustris*) influences serotonin content in cell bodies (Maynard et al.1996) which is suggestive that changes could be a result of serotonin manipulation. Injection of serotonin has also been shown alter the phototactism seen in infected *Gammarus pulex* (by acanthocephalan parasites, *Pomphoruhynchus laevis* and *P. tereticollis*). However, the habitat shift (swimming closer to the surface) seen in infected *G. pulex* was not seen in uninfected amphipods injected with serotonin (Tain et al. 2006). Thus, increased serotonin appears to be driving some, but not all of the altered host behavior seen in *G. pulex* as a direct result of parasitic infection. Dopamine has also been shown to be increased in crabs infected with *Profilicollis antarcticus* (acanthocephala) which exhibit increased activity levels and metabolic rates (Rojas and Ojeda 2005).

I examined the relationship between the freshwater isopod, *Caecidotea intermedius* (Forbes; Figure 1) and the parasite, *Acanthocephalus dirus* (Van Cleave; Figure 2). *A. dirus* is a member of the genus, Acanthocephalus (Family: Echinorhynchidae; Class: Palaeacanthocephala) which contains 47 species and subspecies (Crompton and Nickol 1985). Within the class Palaecanthocephala, most are aquatic in origin and account for 57% of the known species (Kennedy 2006). While the distribution of Acanthocephalus is widespread, only three species (*A. dirus, A. tahlequahensis, and A. alabamensis*) are found in North America (Amin 1985). *A. dirus* is found in thirteen states, mostly along the Mississippi River and also has the greatest definitive host distribution of the North American species (65 species of freshwater fish; Amin 1985). Infected *C. intermedius* have been shown to have altered antipredatory behavior in which they spend more time in the open exposed to predators (Camp and Huizinga 1979; Hechtel et al. 1993). Additionally, infected *C. intermedius* undergo pigment dystrophy which makes them more conspicuous to visually hunting predators than uninfected isopods (Camp and Huizinga 1979; Oetinger and Nickol 1981).

Figure 1: Infected and uninfected *C. intermedius*. The isopod on the left is infected with *A. dirus* and the isopod on the right is uninfected.

Figure 2: *A. dirus* inside *C. intermedius*. Shown are two *A. dirus* parasites.

A. dirus infection also correlates with a decrease in mating success of both male and female isopods (Bierbower and Sparkes 2007; Oetinger 1987; Sparkes et al. 2006). Furthermore, the timing of these behavioral changes correlates with development so that they are most pronounced when the parasite has reached the cystacanth stage (capable of surviving transmission to the definitive host) (Oetinger and Nickol 1982; Sparkes et al. 2004, 2006). *C. intermedius* infected with these late-stage parasites experience decreases in both pairing success and responsiveness to receptive females (Sparkes et al. 2006).

I used a combination of field-based behavioral surveys, lab-based behavioral trials, and neurological assays to investigate mechanisms that could underlie mating suppression in the cystacanth-infected male isopod, *C. intermedius*. Previous research on this parasite-host relationship has shown that mating suppression typically occurs from March to May when the infected males contain cystacanth-stage parasites (9-11 months post-infection). This suppression is not due to pathological effects on either sperm supplies (Bierbower and Sparkes 2007) or energy reserves (Korkofigas 2007) and is not influenced by the presence of rival males (i.e., male-male competition) (Sparkes et al. 2006; Bierbower and Sparkes 2007). It has also been shown that suppression of male mating behavior can be reversed both in the lab and field by removing the infected males from their natural environment (Bierbower 2006; Bierbower and Sparkes 2007). Here, I examined whether this reversal could be explained by variation in predator exposure using a lab-based experiment.

The distribution of predators in a natural environment is often heterogeneous (Begon and Mortimer 1986). If suppression of mating behavior is controlled by the parasite and if this control is energetically costly for the parasite (Brown 1999; Poulin

2002), then the parasite could benefit by limiting suppression of host behavior to periods when predators (definitive hosts) are present. Predator presence and predator-related chemical cues have been shown to increase hiding behavior in many invertebrates including *C. intermedius* (Hechtel et al. 1993; reviewed in Lima and Dill 1990). Recently, it has been shown that amphipods infected with an acanthocephalan parasite (*Pomphorhynchus laevis*) do not avoid olfactory cues given off by a fish predator (Baldauf et al. 2007). Thus, I examined if predator cues may be driving variation in the suppression of male mating behavior in *C. intermedius* in nature.

I also used field-based behavioral trials to examine whether host compensation (increased mating effort during early stages) occurred in males in response to mating suppression during late-stage infections. Given this reproductive cost, males would benefit by increasing mating effort during early stages of infection (Forbes 1993; Minchella and Loverde 1981; Minchella 1985; Møller 1997). Thus, I examined whether infected males were more responsive to females and had higher pairing success than uninfected males during early stages of infection (1-4 months post-infection).

Finally, I examined whether neuromodulation could play a role in parasite-related suppression of mating behavior. Several recent studies have shown that neuromodulation may play a role in acanthocephalan-related changes in host behavior (Helluy and Holmes 1990; Maynard et al. 1996; Poulin et al. 2003; Rojas and Ojeda 2005; Tain et al.2006). Here, I examined whether either serotonin or dopamine levels differed between uninfected and cystacanth-infected male isopods. This study is the first to examine the relationship between neurohormone levels and infection status in *C. intermedius*.

Study Organisms

Isopods and creek chub were collected from Buffalo Creek, a stream located approximately 60 km north of Chicago, IL in 2007 and 2008. The species of isopod that dominate in this stream are *Caecidotea intermedius*, which are the main invertebrate detritivores in the stream ecosystem. Within this population of isopods, there is a high infection rate by the trophically transmitted parasite, *A. dirus* (prevalence = 54%, Sparkes et al. 2006). *A. dirus* has an indirect life cycle that requires two hosts (*C. intermedius* – intermediate host, creek chub – definitive host) to complete its life cycle. Infection of *C. intermedius* typically occurs during the summer months when the isopod consumes detritus along with the eggs of *A. dirus*. Inside the intermediate host, *C. intermedius*, the parasite undergoes development from the egg stage to the final infective cystacanth stage which is capable of surviving transmission to the definitive host (Oetinger and Nickol 1982). The cystacanth stage then dominates infections between September and May of the following year. During May, all of the mature isopods present in the population senesce and the population becomes dominated by the next generation of *C. intermedius*.

The breeding season for *C. intermedius* typically occurs from March to September, which includes two separate cohorts. The isopods mating between March and May are members of the first cohort and are aged between 9 and 12 months. These isopods will either be consumed by predators or will senesce before the end of May. The isopods mating between June and September are members of the second cohort and are the offspring of the first cohort. Infected isopods mating between March and May contain cystacanth-stage *A. dirus*, whereas infected isopods mating between June and September contain acanthor, acanthella and some early cystacanth-stage infections

(Bierbower and Sparkes 2007; Sparkes et al. 2006). Previous research on this system has shown that cystacanth-infected males are less likely to be found in mate guarding pairs in nature and are not responsive to females in comparison with uninfected males (Sparkes et al. 2006). Isopods infected with acanthor- or acanthella-stage parasites are as likely to be found in mating pairs in nature (Sparkes et al. 2006). In contrast with the cystacanth-stage of development, presence of these early does not correlate with a decreased mating response of the male isopods (Sparkes et al. 2006).

Typical mating behavior for *C. intermedius* involves males walking around on the stream bed searching for reproductive females. Once a male encounters a female, a mating contest is initiated in which the male grabs the female and wrestles with her. Although males initiate mating contests, females can prevent males from mating by resisting (coiling) during the contest (Sparkes et al. 2006). When mate guarding does occur, the male carries the female underneath his body for a period of 1-4 days after which the female molts and copulation occurs (Bierbower and Sparkes 2007). However, previous research on the *A. dirus – C. intermedius* relationship has shown that parasite-related suppression of male mating behavior is not due to female behavior because the infected males do not initiate mating contests with females.

Host Compensation during Early Stages of Infection

METHODS

To assess if male isopods were compensating for mating suppression that occurs during late stages of infection by increasing mating attempts and pairing success during early-stage infections, I used a field-based experiment. Infected and uninfected single

isopods were collected in late-July (0-1 month post-infection) and early September (1-3 months post-infection) of 2007. Reproductive females were collected from mate guarding pairs at the same time (July n = 20, Sept n = 20). The behavioral experiment was then run in the field. All trials took place between 8:00 a.m. - 4:30 p.m. For July, I ran 75 trials over three consecutive days (7/23/07 - 7/25/07) and for September I ran 77 trials over two days (9/8/07 – 9/9/07). At this time, infected and uninfected isopods can not be determined visually (there is no obvious pigment dystrophy).

For each trial, one male and one reproductive female were placed in an experimental arena (PVC pipe 6 cm diameter x 7 cm height) submerged in stream water (along the stream-bank) and mating interactions recorded for up to 20 minutes. Each trial was considered completed after one of three events occurred. Trials were concluded if twenty minutes passed from the beginning of the trial, the male had ten physical encounters with the female, or if the male initiated a mating attempt (grabbing and wrestling with the female). A male was scored as 'responsive' (+) if he initiated a mating attempt with a female and 'non-responsive' (-) if either 20 minutes had passed or he had 10 encounters with a receptive female without initiating a mating attempt. These trials were run upon male removal from the stream to quantify male mating responsiveness to females (Time 0).

To measure male pairing success, each male was then left with the female after completion of the trial. Males were then visually assessed at 60 minutes in order to determine pairing success. Male isopods were then preserved in 70% ethanol and transported back to the laboratory. The same females were used on a rotating basis

throughout each day and then sacrificed along with their original paired male (i.e., the male that the female was paired to when she was captured).

In the laboratory, isopods were dissected, body length recorded, and infection status was determined. The parasites were then dissected and both developmental stage and parasite volume ((π x length x (width^2)/6) following Dezfuli et al. 2001) recorded. The developmental stage of each parasite was identified, as either acanthella or cystacanth, using three measures of development (following Schmidt 1985; Hasu et al. 2007). A parasite was recorded as a cystacanth if the reproductive structures were developed (ovaries or testes), invagination of the proboscis had occurred, and if the spines located on the proboscis were fully developed. A parasite was recorded as an acanthella if it lacked any of these developmental measures.

For the behavioral trials, I used heterogeneity χ^2 tests to examine if the relationship between infection status and mating behavior (mating attempts at 0 minutes, pairing success at 60 minutes) differed between samples (Zar 1999). If there was no effect of sample month on mating behavior, I combined the values and ran individual χ^2 tests for each mating component. To determine whether body size differed between uninfected and infected males for the behavioral trials, I used t-tests for each sample. I also used heterogeneity χ^2 tests to determine whether the relationship between infection status and pairing success differed between July and September in males collected from mate guarding pairs in nature.

RESULTS

To determine whether host compensation occurred during early stages of infection, I ran a total of 152 trials (July n = 75, September n = 77). However, five "males" were found to be females for the July trials and seven "males" were found to be females for the September samples. These trials were excluded, which resulted in a total of 140 trials (July n = 70, September n = 70) that were included in the analysis. Upon dissection for the September sample (total infected, n = 57; uninfected, n = 13), 35 of the infected isopods had at least one cystacanth-stage parasite present and 22 were infected with either acanthor- or acanthella-stage parasites. I used a G-test to assess if mating behavior differed between developmental stages. I found no relationship between developmental stage and either mating attempts ($G_1 = 2.09$, $0.20 > p > 0.10$) or pairing success ($G_1 = 0$, $p > 0.98$). Therefore, I grouped all infected isopods for September for further analysis.

Results obtained for mating behavior are shown in Figure 3. In terms of mating behavior, there was no effect of sample month on the relationship between infection status and either mating attempts ($\chi^2_1 = 0.17$, $p > 0.20$) or pairing success ($\chi^2_1 = 0.28$, $p > 0.20$). Therefore, I combined values from July and September and ran individual χ^2-tests for each mating component. I found that there was no relationship between infection status and either mating attempts ($\chi^2_1 = 1.39$, $p > 0.20$) or pairing success ($\chi^2_1 = 0.28$, $p > 0.20$). Therefore, early-stage infected males and uninfected males had comparable levels of mating responsiveness and pairing success. Body size of infected and uninfected males did not differ in the July sample ($t_{68} = 0.52$, $p = 0.60$). I found that infected males were larger in the September sample ($t_{68} = 2.53$, $p = 0.01$).

In terms of pairing success in nature, there was no effect of sample month (χ^2_1 = 2.30, $p > 0.10$). Therefore, I combined the samples and ran an overall χ^2 test. I found no difference between the level of infected males found in mate guarding pairs and the level of infected isopods found in nature ($\chi^2_1 = 2.34$, $p > 0.10$). Thus, infection status did not influence pairing success in nature.

Factors Influencing Mating Suppression during Late Stages of Infection

Mating Suppression and Reversibility

METHODS

Previous research has shown that parasite-related mating suppression can be reversed over a ten-hour time period (Bierbower 2006). I ran field-based behavioral trials within this ten-hour time period to identify potential mechanisms underlying this relationship.

Over three non-consecutive days in 2007 (March 22, 26, and 27th), I ran 120 behavioral trials (n = 60 cystacanth-infected, n = 60 uninfected) to examine this relationship. Infected and uninfected male isopods were collected from the stream and randomly assigned to one of four groups. In two groups (infected, n = 30; uninfected, n = 30), males were held on the stream bank in mesh containers (16 cm x 13cm x14 cm) submerged in stream water. In the other two groups, (infected, n = 30; uninfected, n = 30) males were held in mesh containers (16 cm x 13cm x14 cm) submerged in the stream (Figure 4). Trials were run in groups of four (containing one of each treatment group). For each trial, five reproductive females were placed in the experimental arena (17.2 cm x 9.2 cm x 3.7 cm) containing approximately 2 cm of water and a single male was then

added. The same four groups of females (5 per group) were used throughout each trial day. Following each trial, males were placed into their assigned holding conditions. This approach was then repeated for all males at 200, 400, and 600 minutes. All trials were run between 7:00 A.M. and 7:30 P.M. daily with the exception of the third day. On the third day, hazardous weather conditions limited access to the field site so that I was only able to run the first two time-points (0 minutes, 200 minutes).

Mating responsiveness of males to females was recorded following the same approach as the previous experiment (see previous section). Upon completion of the trials, isopods were preserved in 70% ethanol and transported back to the laboratory where they were dissected and isopod length was recorded and infection status was confirmed. Parasites were also dissected with developmental stage, parasite intensity (number per isopod), and parasite volume ($(\pi \times \text{length} \times (\text{width}^2)/6)$) recorded.

To determine if male body size differed between groups for this experiment, I first tested for normality and ran a two-way ANOVA (Systat 10). To determine whether the relationship between infection status and mating responsiveness changed over time I used a heterogeneity χ^2 test in which I compared values obtained for infected males at all time-periods to uninfected males at time 0. Individual chi-square tests were then used to determine if there was a treatment effect at each time-period.

RESULTS

All isopods used for this experiment were infected with cystacanth-stage parasites. Two-way ANOVA analysis revealed a significant interaction between trial day and treatment group ($F_{3,106} = 5.4$, $p = 0.002$). Thus, I used separate one-way ANOVAs to

examine body size for each trial day. I found that there was no difference in male body size across treatment groups for day one ($F_{3,30} = 1.6$, $p = 0.20$). Body size did differ across treatment groups for days two and three (day 2: $F_{3,36} = 4.4$, $p = 0.009$; day 3: $F_{3,36} = 7.2$, $p = 0.001$). Multiple comparisons for day two reveled that this difference was due to a size difference between two of the four groups (uninfected isopods held in the stream were smaller than the infected isopods held in the stream). However, for day three infected males were larger than uninfected males in general.

The relationship between mating responsiveness and time is shown in Figure 5a. Heterogeneity χ^2 analysis showed that the relationship between infection status and mating responsiveness differed between time-points ($\chi^2_6 = 13.23$, $p < 0.02$). Individual χ^2 analysis then revealed infection status influenced mating responsiveness at time 0 ($\chi^2_2 = 13.30$, $p < 0.005$), but not at time 200, 400, or 600 (200: $\chi^2_2 = 0.03$, $p > 0.20$; 400: $\chi^2_2 = 1.87$, $p > 0.10$; 600: $\chi^2_2 = 0.11$, $p > 0.20$). Thus, reversal of mating suppression was complete within 200 minutes.

I also examined the relationship between holding conditions and infection status using a heterogeneity χ^2 tests. I found no effect of time on the relationship between infection status and holding conditions for either uninfected or cystacanth-infected isopods (infected: $\chi^2_3 = 1.31$, $p > 0.20$; uninfected: $\chi^2_3 = 1.25$, $p > 0.20$). This allowed for the data to be combined based on infection status. This analysis revealed there was no effect of holding conditions on mating response for either infected or uninfected males (infected: $\chi^2_1 = 0.28$, $p > 0.20$, uninfected: $\chi^2_1 = 0.28$, $p > 0.20$) (Figure 5b).

Mating Suppression and Flexibility

METHODS

Suppression of mating response is known to be reversible under experimental conditions. However, it is not known if this reversibility occurs in nature. Reversibility of mating suppression could potentially benefit both the parasite (e.g. avoiding energy costs of manipulation) and the host (e.g. host counteradaptation to achieve mating at the end of the season). Hence, I examined whether reversal of suppression of cystacanth-infected isopods occurred in nature by running field-based mating trials over four days (March 22, 26, and 27th, April 29th, 2007). For this analysis, data from the previous experiment on reversibility (time 0 from previous section) was combined with data collected for one additional day (April 29th). The same procedures were used for day four as outlined above for days one through three.

A heterogeneity χ^2 test was used to determine if there was a difference in the relationship between infection status and mating response over the four trial-days. This difference would be expected if reversal of mating suppression occurred naturally. To evaluate if there was a difference in male body size of cystacanth-infected isopods and uninfected males used in this experiment I used a 2-way ANOVA (main effect: body size, sample date). I then used logistic regression analysis to examine whether mating responsiveness was size-dependent.

To evaluate if cystacanth-infected isopods were mate guarding in nature, I took random samples of mating pairs over four months in the Spring and Summer of 2007 (March 22, 26, 27; April 14, 21; May 10, 31; and June 22). Due to unusually small sample sizes obtained within each month, I combined samples within months and used a

heterogeneity χ^2 test to examine if there was a difference in mate-guarding behavior between months. Random samples of single males were also collected on the last three sample days (May 10, May 31, June 22) to assess whether infection status influenced pairing success during the final stages of breeding season.

RESULTS

Data on variation of male mating response was collected on four days resulting in 192 trials ('infected' = 108; 'uninfected' = 84). However, several of these trials were either removed from the analysis ('males' were females, individuals died during the experiment) or regrouped ('infected' were uninfected and vice versa). Therefore of the initial 192 trials, I ran analysis on 188 (day 1: infected = 17, uninfected = 22; day 2: infected = 22, uninfected = 18; day 3: infected = 21, uninfected = 19; day 4: infected = 44, uninfected 27).

The results regarding variation in male mating response in nature are shown in Figure 6a. There was a significant effect of trial day on male mating response ($\chi^2_3 = 17.31$, $p < 0.001$) indicating that there was significant variation in this behavior occurring naturally. I then examined the relationship between infection status and mating response for each trial-day. I found that suppression of mating response was present for three of the trial-days (Day 1: $\chi^2_1 = 11.84$, $p < 0.001$; Day 2: $\chi^2_1 = 6.86$, $p < 0.01$; Day 3: $\chi^2_1 = 8.84$, $p < 0.01$) and absent on the last trial-day ($\chi^2_1 = 0.58$, $p > 0.20$).

To assess if there was an effect of infection status and trial day on male body size I used a 2-way ANOVA (following log transformation). I found that there was a significant interaction between trial day and infection status. Thus, I ran separate t-tests

for each trial day (following F-tests). I found a significant difference between body size of cystacanth-infected and uninfected males for three of the four trial days with cystacanth-infected males being larger than uninfected males for three days (Day 2: t_{36} = 3.71, p = 0.001; Day 3: t_{32} = 4.73, p < 0.001); Day 4: t_{66} = 6.72, p < 0.001) (Figure 6b).

Logistic regression analysis was used to assess whether mating attempts were influenced by body size, trial day and infection status. I found that the final model indicated that length, infection status, and trial-day contributed to variation in male mating responsiveness (final model = length, infection status, trial day x infection status; G_3 = 37.51, p < 0.001). Given that there was a significant effect of time, I ran individual logistic regressions for each trial-day to examine the relationship between body size, infection status, and mating responsiveness. I found that the saturated model (length, infection status, length x infection status) was significant for the first three trial days (Day 1: χ^2_3 = 17.3, p = 0.001; Day 2: χ^2_3 = 21.5, p = 0.007, Day 3: χ^2_3 = 13.8, p = 0.003; Day 4: χ^2_3 = 2.9, p = 0.4). However the individual analysis revealed that there was no consistent final model for these days (Day 1: infection status, G_1 = 15.8, p < 0.001; Day 2: body size, infection status, G_2 = 11.3, p < 0.004; Day 3: body size, body size x infection status, G_2 = 10.0, p < 0.007).

To examine whether the effect of parasite infection on pairing success is also reversible in nature, I examined the relationship between infection status and mate guarding behavior towards the end of the breeding season (May, June). There was a significant difference in infection status between single and paired males (χ^2_3 = 14.01, p < 0.001). Thus, the effect of parasite infection on pairing success was not reversible late in the breeding season.

Mating Suppression and Predator Cues

METHODS

To determine if the presence of predator cues (creek chub) was driving the suppression of mating behavior in cystacanth-infected isopods, I used a laboratory based experiment. Creek chub, single infected and uninfected male isopods, and reproductive females were collected from Buffalo Creek in the spring of 2007. Reproductive females were collected from mated pairs and continually collected as needed throughout the experiment. The experiment took place over two weeks in April.

Creek chub were housed in ten-gallon aerated tanks (fed isopods daily) and isopods were suspended over a mesh guard in each tank in individual containers with leaves replaced as needed. A total of 25 male isopods were collected for each treatment group; uninfected with predator, infected with predator, uninfected without predator, and infected without predator. For the first week, isopods were housed in fish breeders (16 cm x 13 cm x 14 cm) suspended over the sides of each tank (n = 40). However, this system led to several escapes and the methodology was changed for the second week. For the second week, isopods (n = 60) were housed individually is PVC pipes (6 cm diameter x 7cm height) with mesh secured to the bottoms (Figure 7).

Isopods were collected on Saturday's and kept in the lab for 24 hours to allow for temperature acclimation (from stream temperature to room temperature). This time-period also allows for the infected males to undergo reversal of mating suppression (see previous study). Trials were then run from 9:45 a.m. to 1:00 p.m. daily for six days to determine if isopods were initiating a mating attempt with the reproductive females on each day. Five females were used for each trial and placed in an experimental arena

(17.2 cm x 9.2 cm x 3.7 cm) with approximately 2 cm of water from the tank that housed the experimental male. Male isopods were then placed individually in the experimental arena and male type alternated based on infection status to account for a potential time effect. Trials were considered complete and scored based on the three criteria described in the compensation study.

To evaluate if the relationship between infection status and mating attempts varied during the six trial days, I used a heterogeneity χ^2 test. To examine if body size differed between cystacanth-infected isopods and uninfected isopods I used an ANOVA following tests for normality.

RESULTS

The results of the predation experiment are shown in Figure 8. I ran a total of 100 trials over a two-week time period (25 for each group). Upon dissection, one 'infected' male was uninfected and one 'uninfected' male was infected. These isopods were regrouped according. Additionally, five males either died or escaped during the laboratory trials and were excluded from analysis. Therefore, I ran analysis on a total of 95 trials. All infected isopods used for these trials contained cystacanth-stage parasites. For these trials, male body size of infected and uninfected isopods did not differ between treatment groups ($F_{1,60} = 1.60$, $p = 0.195$).

To assess if predator cues could influencing the mating suppression of cystacanth-infected isopods I compared mating responsiveness over the six trial-days. There was no effect of trial-day on mating responsiveness ($\chi^2_{15} = 11.79$, $p > 0.10$). This allowed for the overall χ^2 test to be run and it showed that there was no difference between treatment

groups ($\chi^2_3 = 5.75$, $0.20 > p > 0.10$). Thus, predator cues alone do not appear to be driving male mating responsiveness.

Neuromodulation and Mating Behavior

METHODS

To assess if neurohormone levels could be a potential mechanism driving mating suppression, I quantified serotonin and dopamine levels in infected and uninfected male isopods. Mating pairs and single males (infected and uninfected) were collected from Buffalo Creek (March 2008, approximately 500 individuals). The isopods were then transported in aerated containers to the University of Kentucky in Lexington, Kentucky. Mating trials were run to select for only infected and uninfected males that initiated a mating attempt with a reproductive female. Behavioral trials were run at a 1:2 ratio (males: females) to verify whether males were responsive to females. Each isopod that initiated a mating attempt was then microdissected and the neural cord and brain tissue removed and flash frozen using dry ice (Figure 9).

For the dissections, isopods were bathed in physiological saline (1.0 CaCl2 2H2O, 20 MgCl2, 70 NaCl, 5 KCl, 10NaHCO3, 5 trehalose, 115 sucrose, 5 BES (N,N-bis[2-hydroxy-ethyl]-2-aminoethanesulfonic acid) and adjusted to a pH of 7.2) at room temperature to minimize tissue degradation (Stewart et al. 1994). Dissections were run in synchronous blocks of three (i.e., three people were dissecting at once) with each examiner rotating infection status between blocks. Ten individual male isopods (brain and nerve cord combined) represented one replicate. All dissecting was done between 12:00 p.m. – 9 p.m. (March 16 infected = 30 individuals, uninfected = 30 individuals,

March 17 infected = 30 individuals, uninfected = 30 individuals), which yielded a total of six replicates per group.

Isocratic high performance liquid chromatography (HPLC) system (ESA Biosciences Inc., Chelmsford, MA) joined with a dual-channel electrochemical array detector (model 5300A, ESA, Inc., Chelmsford, MA) was then used to quantify serotonin (5HT) and dopamine levels for each replicate (following Hall et al. 1989). To assess if there was a relationship between these catecholamines for infected and uninfected isopods, I ran two-sample t-tests (following F tests for variance).

RESULTS

The results obtained for dopamine and serotonin levels are shown in Figure 10. There was no effect of infection status on either serotonin (5HT) levels ($t_{10} = 0.40$, $p = 0.70$) or dopamine levels ($t_{10} = 0.40$, $p = 0.70$).

DISCUSSION

Acanthocephalan parasites often influence the mating behavior of their intermediate hosts (e.g. Bollache et al. 2001; Dezfuli et al. 1999; Zohar and Holmes 1998). Consistent with this relationship, development of the acanthocephalan parasite, *A. dirus* into the cystacanth stage correlates with suppression of male mating behavior in its intermediate host, *C. intermedius* (Oetinger 1987; Sparkes et al. 2006). Here, I examined the relative importance of both parasitic manipulation and host counteradaptation to variation in male mating behavior during both early and late stages of parasite

development. During early stages of development, I found that there was no relationship between infection status and mating success indicating that there was no evidence of host compensation. During late stages of infection, I found that male mating responsiveness to females was flexible and reversible both in the field and in the lab. Additionally, I found that this positive mating response was not associated with predator cues. Below, I discuss the relevance of these findings in the context of both parasitic manipulation and host counteradaptation.

Host Compensation During Early Stages of Infection

Forbes (1993, 1996) proposed that changes in the reproductive success of hosts can correlate with parasite infection both positively (increased reproduction, compensation) and negatively (decreased reproduction). Consistent with the latter prediction, previous studies have shown that suppression of mating behavior occurs during late stages of parasite development for *C. intermedius* (Bierbower and Sparkes 2007; Sparkes et al. 2006). Thus, given this reproductive cost *C. intermedius* would benefit by increasing mating effort during early stages of infection (see Forbes 1993; Minchella 1985; Møller 1997, for discussion). I examined variation in the mating behavior of *C. intermedius* infected with early-stage parasites to assess if increased mating responsiveness occurred as an indicator of host compensation. Contrary to this prediction, I found that infection status did not correlate with either mating responsiveness or pairing success of males (during July and September). These results are consistent with a previous study on this system which found that host compensation did not occur in males collected in August (Sparkes et al. 2006). Thus, compensation in

regards to male mating effort does not occur during the first three months of infection in *C. intermedius*.

The results obtained here are not consistent with life-history theory which predicts that parasitized animals should increase reproductive effort during early stages of infection to compensate for future reproductive loss (Forbes 1993, 1996). This type of relationship has been observed in other studies. For example, the snail (*Biomphalaria glabrata*) increases reproductive output early as part of a host counteradaptation strategy when infected with a trematode parasite (*Schistosoma mansoni*; Minchella and Loverde 1981). Similarly, male amphipods (*Corophium volutator*) increase mating effort during early stages of infection by the trematode *Gynaecotyla adunca* (McCurdy et al. 2000). Thus, it is currently unclear given the significant fitness costs why *C. intermedius* does not compensate for a future reproductive loss. However, Forbes (1993) suggested that the increase in reproduction during early stages of infection predicted by life history theory may not apply to acanthocephalans for two reasons. First, acanthocephalans have been proposed to gain control of host resources to favor their own development (Baudoin 1975). Thus, since host modification appears to be regulated by the parasite developmental stage, it is possible that manipulating mating behavior does not benefit the parasite at this early stage. Second, acanthocephalans have been proposed to gain control of their hosts to increase transmission rates (Dobson 1988). Since single males are more likely to be found in the open searching for females and because early stage *A. dirus* can not survive transmission to the final host these ideas are plausible.

Alternatively, it is possible that host compensation is not occurring in *C. intermedius* because they have not had enough evolutionary time to develop the trait.

Coevolutionary relationships require significant time in close association and the rate of evolution will be dependent on the rate of favorable mutations (Futuyma 1998). Thus, it is possible that *C. intermedius* has not evolved a trait for host compensation that would increase reproductive effort during early stage infections.

Factors Influencing Mating Suppression During Late Stages of Infection

Mating suppression of *C. intermedius* during late stages of infection could benefit the parasite if it is part of its strategy to increase transmission to the final host. Mate guarding pairs are rarely found in the open, thus they are typically not conspicuous to predators (Korkofigas 2007). Therefore, decreasing pairing success in infected isopods increases the likelihood that infected isopods are located in the open and at risk for consumption by definitive hosts. Furthermore, since mate guarding is energetically costly to male isopods (Sparkes et al. 1996), decreasing the time spent guarding females could minimize the energy expended by the isopod and allow the parasite to redirect mating-related energy towards itself (Baudoin 1975). Another possibility is that the host suppresses his own reproductive output to conserve energy to mount an immune defense against the parasite (Minchella 1985; Moore 2002; Poulin 1995b; Poulin and Thomas 1999; Thomas et al. 2005).

It has also been proposed that manipulating host behavior and physiology should be costly to the parasite (Brown 1999; Poulin 1994). In this scenario, it would be advantageous for the parasite to manipulate host behavior only when it would increase the likelihood of transmission to the final host. In this way, energy costs associated with manipulation can be minimized. Therefore, it would be beneficial for parasites to be able

to manipulate host behavior only when successful transmission to the final host can be achieved (e.g. predator presence; Brown 1999; Poulin 1994). This type of relationship may be favored if the predators are distributed heterogeneously in the environment. Thus, I examined if suppression of host mating behavior was influenced by predator exposure.

I hypothesized that in the absence of predators, mating behavior of male *C. intermedius* would not be suppressed. Male isopods were brought into the lab and held for 24 hours in the absence of predator cues to allow time for reversal of the mating response to occur (Bierbower 2006). If predator cues are driving mating suppression, exposure to predator cues in the lab after reversal has occurred should induce a re-suppression of male mating behavior. Contrary to this prediction, I found no evidence that predator exposure influenced suppression of mating behavior.

A previous study in a different stream system has shown that *A. dirus* infected *C. intermedius* are attracted to predators but do not show a flexible response to the presence of predators (Hechtel et al. 1993), which is somewhat consistent with the results presented here. In the previous study, antipredatory behavior differed between infected and uninfected *C. intermedius*, but was not affected by exposure to predators (Hechtel et al. 1993). Similarly, I found that mating behavior differed between infected and uninfected male *C. intermedius* and was not affected by exposure to predators.

Two potential parasite-based mechanisms could explain why host mating responsiveness is variable and reversible in nature. First if infected hosts are at the end of their life, they may be conserving energy to mount a late reproductive effort. This proposed host counteradaptation would be consistent with findings that the sex ratio (female: male) is skewed towards females as the breeding season progresses (February:

sex ratio = 0.8, n = 309; April 0.7, n = 225; May 1.3, n = 184; Sparkes TC, unpublished data). However, consistent with other studies (Bierbower 2006; Sparkes et al. 2006), I found that infected males senesce by the end of May and that pairing success of infected males did not increase at the end of the breeding season. Thus, there appears to be no support for the host counteradaptation hypothesis in this system.

Second, if the parasite perceives that the host has been consumed by a definitive host, the parasite may reallocate the energy it uses to manipulate host mating towards preparing itself for successful transmission to the definitive host. Consistent with this hypothesis, it has been shown that parasites undergo numerous biochemical changes as they make the transition from intermediate host to definitive host and that these changes can require at least 30% of the parasite's energy reserves (Crompton and Nickol 1985; Taraschewski 2000).

This type of parasite-regulated mechanism could also explain the different outcomes seen in study on reversibility of mating responsiveness presented here relative to a previous study of reversibility on the same system (Bierbower 2006). For the two experiments, both holding conditions and behavioral trials were comparable. However, unlike the study presented here, in which behavioral trials were run at four time-points (0, 200, 400 and 600 minutes), behavioral trials were run at only two time-points (0 and 600 minutes) in the other study. In the previous experiment, infected males held in the stream had a suppressed mating response at both time-points (Bierbower 2006) whereas in the study presented here, suppression of the mating response occurred only at time-point 0 for infected males held in the stream (Figure 5a). Thus, the fundamental difference between these experiments was that the study presented here involved more handling of

the focal organisms. If it is the case that handling is perceived as a predation event, then the parasite may be undergoing biochemical changes in preparation for transmission to the definitive host that includes cessation of the modification of host mating response.

Previous research has shown that mating behavior of *C. intermedius* follows a predictable sequence of events in which males must mate guard a female before she molts to fertilize her successfully (Sparkes et al. 2006). However, a recent lab-based study has shown that while cystacanth-infected isopods do not achieve pairing success, they were capable of mating successfully with females (Korkofigas 2007). Consistent with this study, it has been shown that that infected isopods produce viable sperm during the breeding season (Bierbower and Sparkes 2007) and I found that infected males may be more responsive to females as the breeding season progressed. Since the sex ratio is skewed towards females late in the breeding season, a reversal of mating suppression at this time could potentially result in successful mating opportunities for infected isopods. However, very few infected males were still alive at this time (approximately 9% of males present in nature in late May).

Based on the results presented here, I propose that variation in the mating responsiveness of cystacanth-infected males is unlikely to be explained by host counteradaptation for two reasons. First, although infected males increased their responsiveness to females late in the breeding season, very few of these males were still alive at this time. Second, infected males do not undergo a reversal of mate guarding behavior either in the field (this study) or in the lab (Korkofigas 2007).

Alternatively, I have proposed that variation the mating responsiveness may be explained by factors that influence the strategy of manipulation adopted by the parasite.

Under this scenario, suppression of mating responsiveness is expected to be reversed whenever the parasite stops manipulating the host. For example, if the parasite perceives that the intermediate host has been consumed or if the parasite is running low on energy reserves late in the breeding season. Thus, it appears that mating responsiveness in *C. intermedius* is most likely mediated by parasite manipulation.

The results obtained here indicate that parasite manipulation is the most likely predictor of mating responsiveness. However, I have not shown definitively that reversal of mating suppression is beneficial to the parasites' establishment in the definitive host. Alternatively, I have proposed that the flexible mating response seen in cystacanth-infected male isopods may be an indirect result of parasite manipulation that does not benefit the parasite. Therefore, I propose that a future study on establishment success in the definitive host be performed in order to delineate why mating responsiveness is reversible in nature.

Neuromodulation and Mating Behavior

My results are consistent with the interpretation that parasites control suppression of male mating responsiveness. Similarly, a previous study on this parasite-host relationship has proposed that parasites also control suppression of mate guarding behavior (Korkofigas 2007). However, suppression on the male mating response is reversible, whereas suppression of mate guarding behavior is not reversible under the same conditions in the laboratory. This difference indicates that these behaviors may be regulated by different proximate mechanisms, which is proposed to be the case in another acanthocephalan-host relationship (Tain et al. 2006).

One mechanism that has been proposed as a way that parasites could control host behavior is through neuromodulation (Adamo 2002; Helluy and Holmes 1990; Holmes and Zohar 1990; Kloppenburg and Heinbockel 2000; Maynard et al. 1996; Moore 2002; Poulin et al. 2003; Rojas and Ojeda 2005; Tain et al. 2006). For example, injecting serotonin and octopamine have been shown to increases agonistic posturing in lobsters (Antonsen and Paul 1997). Although little information is available on neuromodulation in acanthocephalan-host relationships, a few studies have examined this potential mechanism of host modification. For example, crabs show variation in dopamine and serotonin levels when infected with the acanthocephalan parasite, *Profilicollis antarcticus* (Poulin et al. 2003; Rojas and Ojeda 2005). Furthermore, amphipods (*Gammarus* spp.) have altered serotonin levels (clinging behavior, Helluy and Holmes 1990; altered phototactism, Tain et al. 2006) and octopamine levels (clinging behavior, Helluy and Holmes 1990) which alters behavior when infected by acanthocephalans (*Polymorphus* spp.)

My study was the first to examine the relationship between neurohormones, behavior, and infection status in *C. intermedius*. To gain insights into possible neuromodulatory mechanisms underlying parasite-related variation in mating behavior, I examined dopamine and serotonin levels in infected and uninfected males. These males did not differ in mating responsiveness to females (i.e. suppression of the mating response was reversed for infected males) but did differ in mate guarding behavior (i.e. mate guarding behavior was suppressed in infected males). I found that there was no difference in either dopamine or serotonin levels between infected and uninfected males.

Thus, parasite-related variation in mate guarding behavior is unlikely to be explained by variation in these neurohormone levels.

One potential limitation of the neuromodulation study presented here is that only the nerve cord and brain were included in the neurohormone assays. In other studies, neurohormone levels present in the haemolymph have been shown to influence behavior (Adamo 2002; Adamo et al. 1995b; Nagao et al. 1991). Thus, dopamine and serotonin can not be ruled out as potential mechanisms associated with suppression of male mating behavior at this time. Additionally, it has been shown that octopamine can influence behavior of invertebrates (Adamo et al. 1995b; Antonsen and Paul 1997; Glanzman and Krasne 1983). Thus, more detailed analysis that incorporates additional neurohormones and sampling sites within the body are required for an accurate assessment of this potential proximate mechanism.

Future Directions

I have proposed that parasite manipulation is the most likely mechanism underlying mating responsiveness in cystacanth-infected *C. intermedius* and that a flexible mating response may occur if the parasite is reallocating energy to survive transmission to the final host. An alternative explanation is that the changes in observed mating response could be due to unrelated, non-beneficial changes in the parasites manipulative response of host behavior following handling. Therefore, I propose that future work should examine establishment success in the definitive host in relation to variation in 'handling' of the isopods.

Two outcomes of this approach could provide insights into the mechanism underlying changes in male mating responsiveness. First, if handling results in a suppression of host mating response as the parasite prepares for transmission to definitive hosts, then handling should correlate positively with establishment success in the definitive host. In contrast, if handling results in suppression of host mating response as an indirect effect of a stress response, then handling should not correlate with establishment success in the definitive host. Thus, I propose that infecting the definitive host with handled (proposed predator event) and non-handled (proposed non-predator event) infected male isopods should provide further insights into this relationship.

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Figure 3: Effect of early-stage infection on mating behavior of *C. intermedius*. Shown is the relationship between infection status (infected: n = 106, uninfected: n = 34) and both positive mating response (0 min) and pairing success (60 min) for the two sample months combined (July, September).

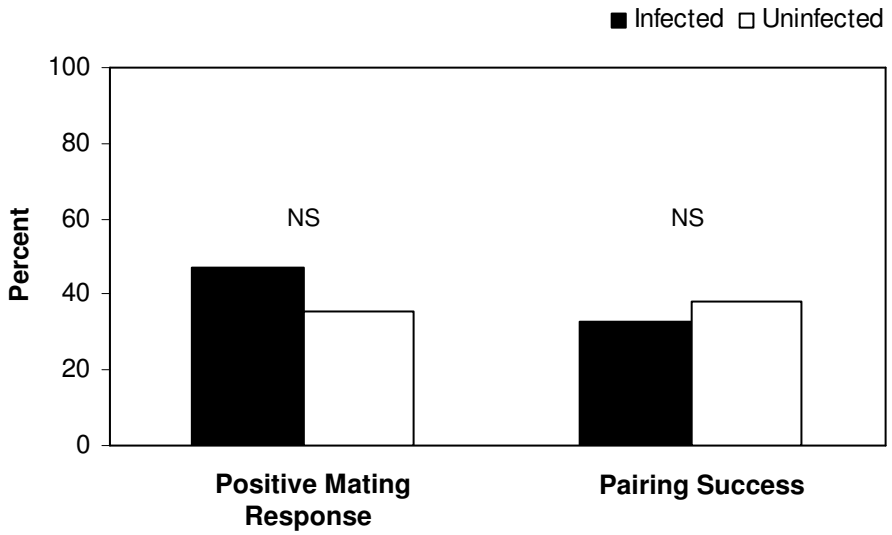

Infected
Uninfected
100
80
60
40
20
0
Percent
NS
NS
Positive Mating Response
Pairing Success

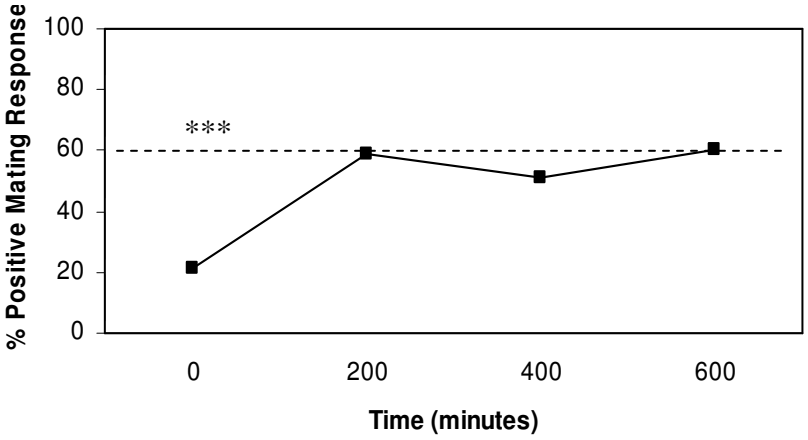
Figure 4: Holding conditions for the reversal experiment. a) Infected and uninfected isopods held in stream individually in fish breeders. b) Infected and uninfected isopods held on the stream-bank individually in fish breeders.

a)

b)

Figure 5: a) Relationship between positive mating response, infection status, and time. The dashed line indicates positive mating response for uninfected males at time 0. The solid line indicates positive mating response for infected males at all time points. *** indicates $p < 0.001$. b) Relationship between positive mating response, infection status, time, and holding conditions. The solid black bars represent uninfected males at time 0. The white bars (II) indicate infected isopods held in the stream between trials; the gray bars (IO) indicate infected isopods held on the stream-bank between trials; and the black bars (U) indicate uninfected isopods. The letters above the bars (a,b) represent significant differences between treatment groups.

a)


% Positive Mating Response
100
80
60
40
20
0
***
0
200
400
600
Time (minutes)

b)

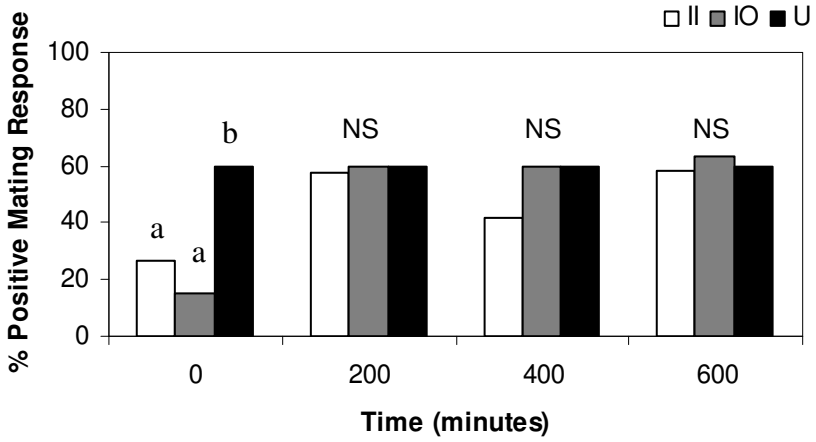
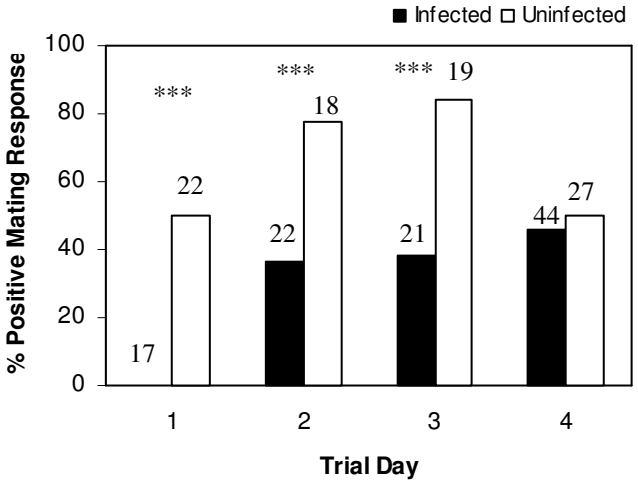

II
IO
U
% Positive Mating Response
100
80
60
40
20
0
a
a
b
NS
NS
NS
0
200
400
600
Time (minutes)

Figure 6: a) Percentage of cystacanth-infected and uninfected males that initiated a positive mating response over the four trial-days. *** represents $p < 0.001$. The numbers above the bars indicate sample sizes. b) Relationship between body size of cystacanth-infected and uninfected males. *** represents $p < 0.001$.

a)


Infected
Uninfected
% Positive Mating Response
100
80
60
40
20
0
***
***
***
17
22
22
18
21
19
44
27
1
2
3
4
Trial Day

b)

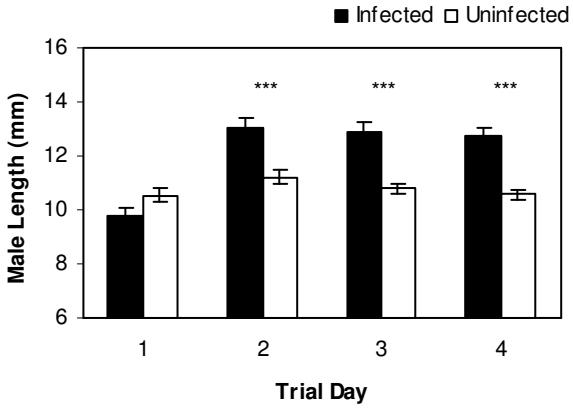

Infected
Uninfected
Male Length (mm)
16
14
12
10
8
6
***
***
***
1
2
3
4
Trial Day

Figure 7: Housing conditions for each week of the predation trials. Single isopods were housed in containers that were suspended from the top of the tank with leaves replaced as needed. a) Week one experimental holding conditions. b) Week two experimental holding conditions.

a)

b)

Figure 8: Relationship between positive mating response, infection status, and predator exposure. Shown are combined values for all six days. The grey bars indicate no predator presence and the white bars indicate predator presence. The numbers above the graph indicate sample sizes. Note that regardless of holding conditions or infection status, a high level of positive mating response was seen and maintained over the six days.

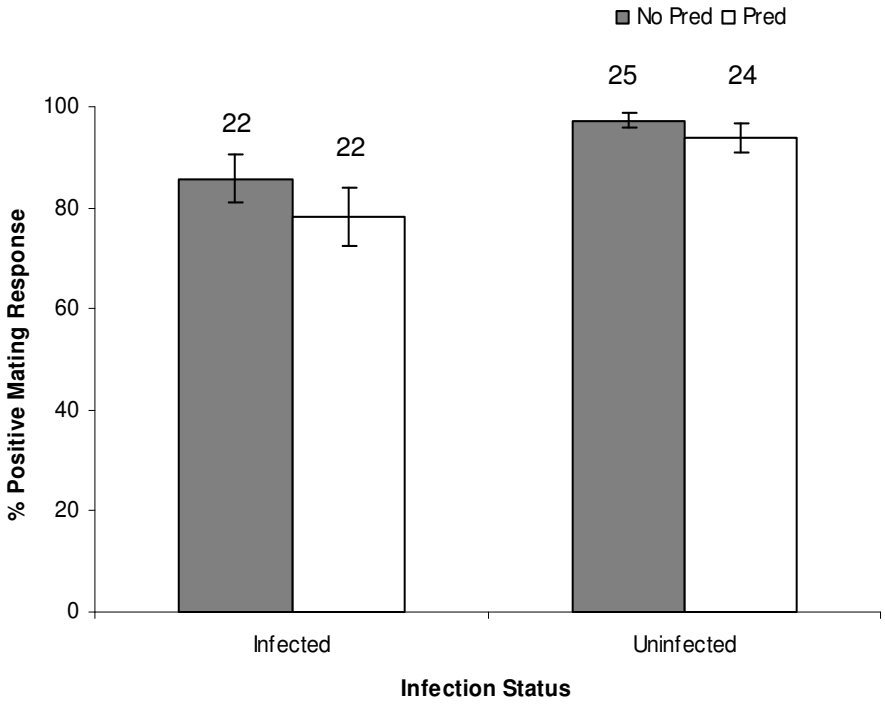
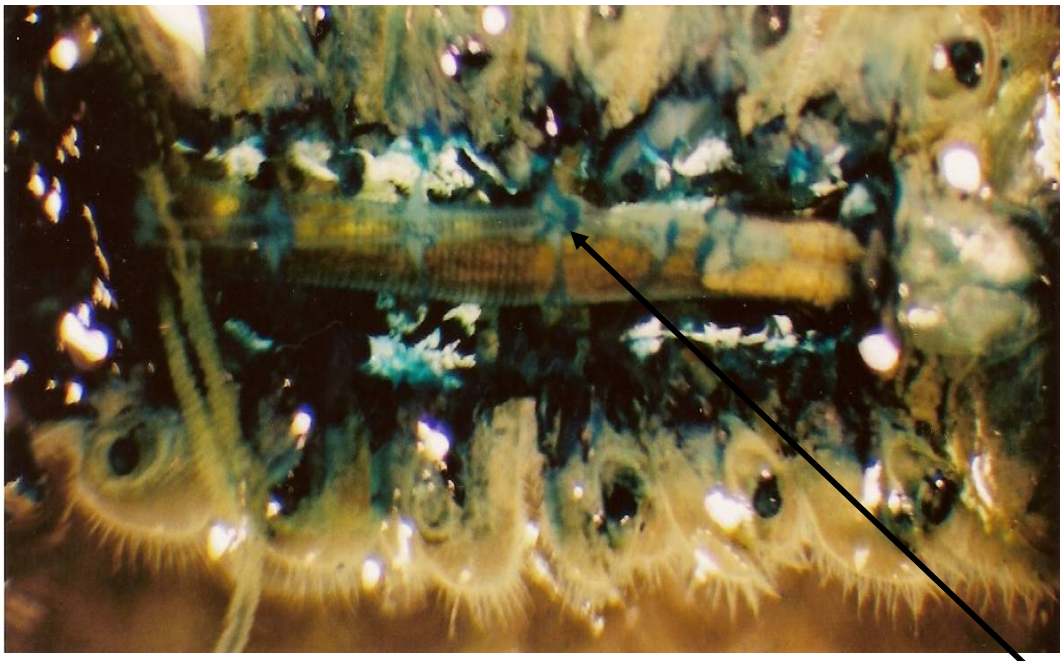

No Pred
Pred
25
24
22
22
100
80
60
40
20
0
% Positive Mating Response
Infected
Uninfected
Infection Status

Figure 9: a) Nerve cord of *C. intermedius* stained with methylene blue. The isopod is lying on its dorsal surface with the cephalothorax to the left. The nerve cord runs laterally along the gut slightly to the left from the brain to the genital tract. b) Close up view of stained ganglia.

a)

10 mm



Nerve cord

Ganglia

b)

1 mm

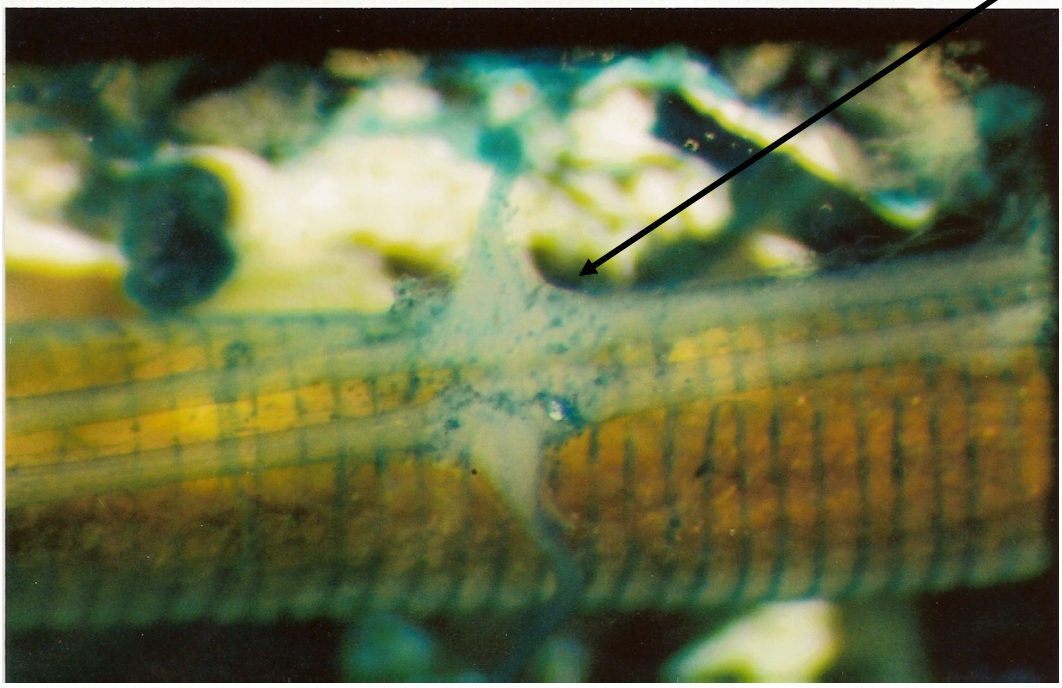


Figure 10: a) Relationship between infection status, serotonin and dopamine levels (infected: n = 6; uninfected: n = 6). Each replicate consisted of the brains and nerve cords of 10 male isopods. Values shown do not account for the amount of CNS tissue recovered, which was not quantified in this study.

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Neurochemical