DISSERTATION

# ALTERED BEHAVIOR AND COST OF MANIPULATION: THE ACANTHOCEPHALAN *LEPTORHYNCOIDES THECATUS* IN ITS AMPHIPOD HOST *HYALELLA AZTECA*

Submitted by

Charles F. Stone

Department of Biology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2014

Doctoral Committee:

Advisor: Janice Moore

Boris Kondratieff
Cameron Ghalambor
Dhruba Naug

ABSTRACT

ALTERED BEHAVIOR AND COST OF MANIPULATION: THE ACANTHOCEPHALAN *LEPTORHYNCOIDES THECATUS* IN ITS AMPHIPOD HOST *HYALELLA AZTECA*

Behavioral manipulation occurs when a parasite causes changes in its host's behavior to the parasite's benefit. The parasite benefits from these behavioral changes by increased survival or transmission. It has been hypothesized that such manipulation carries a cost for the parasite because energy allocated to manipulation does not contribute to growth or reproduction. The acanthocephalan parasite *Leptorhynchoides thecatus* provides a system in which to test this concept. This parasite uses the amphipod *Hyalella azteca* as an intermediate host and fish as definitive hosts; it has not been previously shown to alter host behavior. This system is advantageous for testing costs of manipulation: the size of the larval cystacanth stage in the intermediate host provides an easily quantified measure of fitness. Larger cystacanths establish in the fish host more frequently than smaller cystacanths. If manipulation is costly, I predict that there should be a negative relationship between the strength of behavioral change and fitness measures (larval size).

I compared geotaxis, phototaxis, photophilia, and activity responses of infected and uninfected *H. azteca* to determine whether *L. thecatus* modified behavior. I also measured the responses of infected and uninfected amphipods to alarm pheromones and predator kairomones. I then investigated whether these behavioral changes were correlated with larval size.

I found that *L. thecatus* does indeed alter host behavior. Compared to uninfected amphipods, infected amphipods were found higher in the water column, spent greater time in lighted areas, and were more active. There was no difference in phototaxis; both groups of

amphipods swam away from a direct light source. Infected amphipods also reduced anti-predator responses to alarm pheromones and predator kairomones.

This is the first example of altered alarm pheromones behavior in parasitized amphipods. These findings strongly suggest that *L. thecatus* increases encounters between its intermediate host and definitive host predators and that the parasite increases its transmission rate through behavioral manipulation. None of these behavioral changes were correlated with a decrease in larval size as predicted by the manipulation cost hypothesis. In fact, larger cystacanths altered geotaxis and photophilia more than smaller cystacanths did.

Finally, I compare *L. thecatus* host use data collected from Atkinson Reservoir, Nebraska, between 2008 and 2011 to published data from 1979-1980. Both data sets show that the Green Sunfish (*Lepomis cyanellus*) and Pumpkinseed Sunfish (*Lepomis gibbosus*) are the highest quality hosts for this population. However, the current data suggest a possible shift in secondary hosts from Largemouth Bass (*Micropterus salmoides*) to Bluegill Sunfish (*Lepomis macrochirus*).

Understanding the cost associated with any trait sheds light on the evolution and maintenance of that trait. This dissertation uses a unique population of *L. thecatus* to add this parasite to the growing list of those that behaviorally manipulate their hosts, and to demonstrate that, contrary to predictions from the theoretical literature, behavioral manipulation is not necessarily costly.

ACKNOWLEDGEMENTS

I would like to thank Janice Moore for her guidance, patience, and mentoring in this doctorate process. I am particularly thankful for her influence as an instructor of biology, and her support of my passion and goals. A number of graduate student colleagues have assisted me in this dissertation process. Thank you to all of my wonderful lab mates, Broox Boze, Sarah Bevins, Paige Shilling, and Skyler Bretton. Other graduate students helped with input on my manuscripts and in the field. Thank you Helen Sofaer and Chris Mayack.

A long list of undergraduates helped me in field collections and in laboratory work. Xandra Carroll, Kevin Schauer, Jeremy Gendelman, and Lauren Weid were essential to sampling with a 35' seine, and got first hand parasitology experience with fish guts. Jenny Skinner, Amanda Hines, Kristen Hennig, Andy Sutton, Alexander Jury, Joe Henderer, Hannah Alt, and Torri Kling were all instrumental in culturing the organisms and collecting data.

I wish to thank my family, my wife Sarah Waterson and daughter Isadora Stone. They have supported me in my passion, gotten dirty on collecting trips, and given me drive to complete the work that I am presenting here. Thank you for all of your love and patience.

Lastly, I would like to thank Charlene Paris for her excitement about science happening in Atkinson, the information she was able provide about weather conditions that saved collecting trips from being disasters, and her willingness to put on a pair of waders and get wet to assist a graduate student. Her enthusiasm made collecting trips the highlights of my research. I might have been able to complete this work without her, but it would have been at greater cost and not nearly as fun.

TABLE OF CONTENTS

## CHAPTER 1: Introduction and Study System

### Introduction

Parasitic worms are foreigners that colonize other animals. They live with very different requirements for success and different tools for survival than their hosts and free-living cousins. In many ways these worms appear simplistic; they often lack eyes and ears, complex nervous systems, and even mouths and guts. But this simplicity is deceptive; parasites are incredibly successful at what they do and possess highly effective adaptations to succeed. Lacking eyes and ears, they navigate chemical gradients to specific sites of infection. They deploy an arsenal of hooks, suckers, and chemical cocktails to resist efforts of their hosts to dislodge and eliminate them. Parasites face two primary hurdles: surviving destruction by their host's defenses and getting to new hosts before their current host dies (Combes 2001). This second hurdle can be quite high, because the number of parasites that survive transmission to new hosts is usually vanishingly small. Perhaps the most famous adaptation to overcome this barrier is the mind-bogglingly large number of offspring that many parasitic worms produce. In addition, many worms behave so that they are more likely to encounter their new hosts (e.g. at night for nocturnal hosts), and others employ an impressive strategy: altering their host's behavior to deliver the parasite's most desirable outcome.

Parasitized hosts frequently behave differently than uninfected ones (Moore 2002). Some behavioral changes help the host rid itself of the parasite, e.g. behavioral fever, while others increase the chances that the parasite succeeds in reaching a new host in reproducing (Moore 2002). These types of behavioral changes can be quite dramatic and specific. The famous horsehair worm causes its cricket host to jump into water; the parasite then escapes the host and swims away to find a mate (Blunk 1924, Thorne 1940, Thomas *et al.* 2002). Some trematodes

cause an ant to climb to the top of a blade of grass and latch on so that they are more likely to be consumed by a sheep (Carney 1969). And thorny-headed worms have been shown to cause aquatic crustaceans to swim away from the safety of the lake bottom to the surface where they come into more frequent contact with a surface-feeding duck (Bethel and Holmes 1973, 1977). While the examples of parasite induced behavioral change are many, there is still much that we do not understand, including how do these manipulations evolve and are maintained.

This study explores the evolutionary cost of manipulation. We know that these manipulations are advantageous because they increase transmission or survival, but at what cost? Conceptual studies have proposed that the cost of manipulation is significant (e.g. Poulin 1994, Vickery and Poulin 2010) and this cost is generally assumed to be a facet of behavioral manipulation, but empirical tests of these costs are lacking. This may be due to the fact that relatively few study systems are suitable for testing such questions. I propose that the acanthocephalan parasite *Leptorhynchoides thecatus* offers an ideal system to answer these questions. In this first chapter I will describe this study system, review the aspects of the particular population used in the following studies, and address why this system lends itself to testing questions of cost.

**Classification and Description**

*Leptorhynchoides thecatus* (Kostylev) is placed in the phylum Acanthocephala. This phylum is commonly known as the "thorny-headed worms" because of the prominent proboscis on the anterior end, which is covered with a large number of hooks. This phylum of parasitic worms infects arthropod intermediate hosts and vertebrate definitive hosts. In the intermediate host, acanthocephalans grow through of three developmental stages. First, when an appropriate

arthropod ingests the egg, the motile acanthor stage leaves its egg casing and penetrates the host's gut (Roberts and Janovy Roberts 2009). The worm settles in the host's hemocoel, becomes relatively immobile, and begins to grow in the acanthella stage. The acanthella is not infective to final hosts and at this point resembles an adult worm with a spine-covered proboscis and single body region. The final larval stage is the cystacanth, in which the proboscis withdraws into the body, a cyst wall forms around the parasite, and the parasite is infective to its definitive host (Roberts and Janovy 2009).

All acanthocephalans live in the vertebrate intestinal tract as dioecious sexually reproducing adults. They lack an internal digestive tract and absorb all nutrients through the body wall. The body cavity of the female is used almost exclusively for egg production. Each female produces large numbers of eggs, which pass with the host's feces into the environment to continue the lifecycle, when the egg is ingested by an appropriate arthropod.

*Leptorhynchoides thecatus* is in the class Palaeacanthophala, a group in which adult worms infect fish, amphibians, reptiles, birds, and mammals. This acanthocephalan class is characterized by a double walled proboscis receptacle and fragmented cement glands and hypodermal nuclei (Amin 1987). *Leptorhynchoides thecatus* is placed the order Echinorhynchida and family Rhadinorhynchidae. The genus name *Leptorhynchoides* comes from the Greek roots for *lepto*, "thin," and *rhynch*, "nose," and *oides*, "like." The specific epithet, *thecatus*, "cased (Gr)" refers to the theca, or casing, around the base of each of the spines projecting from the proboscis.

Linton (1891) first described *L. thecatus* and placed it in the genus *Echinorhynchus* (Lincicome and Van Cleave 1949a). The species was moved to the genus *Leptorhynchoides* along with a similar European species by Kostylev (1924) and placed in the family

Rhadinorhynchidae (Lincicome and Van Cleave 1949a). The natural geographical range of *L. thecatus* stretches across North America, from northern Canada to the Caribbean and Atlantic seaboard in the East to generally the Mississippi river in the West (Steinauer *et al.* 2006), but has been introduced in other areas (see below). This parasite encompasses a great deal of morphological variation across the range. Lincicome and Van Cleave (1949a) distinguished between populations in Canada and the United States by the number of spines on the proboscis, a trait that also differs between male and female worms. In the United States, male worms are 3-12mm in length and the proboscis bears 12, or rarely 14, rows of hooks with 11-15 (average 13) hooks each. Female worms are larger, between 6-26mm long, and 12 rows of hooks with 12-16 (average 13) hooks in each row (Lincicome and Van Cleave 1949a).

The only known intermediate host for *L. thecatus* is the freshwater crustacean *Hyalella azteca* (Saussure, Amphipoda). DeGuisti (1949) used *H. azteca* and rock bass (*Ambloplites rupestris* Rafinesque) to demonstrate the life cycle of the parasite and the details of its development within the amphipod. While *H. azteca* is the only known intermediate host for this parasite, this host specificity is unlikely a limit the parasite's range because *H. azteca* inhabits a great variety of freshwater habitats across North America, from northern Canada to Central America and from Pacific to Atlantic coasts (Smith 2001). Infections take approximately 30 days to mature from the time the acanthor penetrates the amphipod's gut to infective cystacanth (DeGuisti 1949). However, this process is temperature dependent and colder temperatures retard the development of the parasite. Infections in *H. azteca* cultured at 13°C took twice as long to mature as those cultured at 20-25°C (DeGuisti 1949). Cystacanths in the amphipod are soft white in color and the parasite does not sequester carotonoids as some other acanthocephalans do, so dissection is necessary to reliably tell whether an amphipod is infected by a cystacanth. Uznanski

and Nickol (1980) tested *L. thecatus* for lethal and sublethal effects on *H. azteca* and found no significant difference in survival between infected and uninfected amphipods in the first 24hrs or one month of infection. To my knowledge, there are no studies on the effects of *L. thecatus* infections on the reproductive capacity in female *H. azteca*. While other parasites do interfere with oogenesis in amphipods, the results of Uznanski and Nickol (1980) suggest that this is not the case for this host-parasite system.

Centrarchid fish are the primary definitive hosts for *L. thecatus* (see below). After an infected amphipod is consumed, the cystacanth activates in the stomach of the fish and establishes in the pyloric caeca at the junction of the stomach and intestine (Uznanski and Nickol 1982). Not all cystacanths go directly to the pyloric caeca, but must migrate within the anterior intestine to the establishment site (Richardson *et al.* 2008). One-week post infection, worms are found exclusively in the pyloric caeca, and a typical Green Sunfish (*L. cyanellus* Rafinesque) provides sufficient resources for establishment of 10-15 worms (Uznanski and Nickol 1982). Adult worms copulate and fertilization occurs 3-4 weeks post infection (DeGiusti 1949, Richardson *et al.* 1997), but adult development can be retarded at lower temperatures (Olson and Nickol 1995). *Leptorhynchoides thecatus* does not form exclusive mating pairs. Males copulate indiscriminately and repeatedly during their lifetime and are known to couple with other males (Richardson et al. 1997). Females produce eggs with a fibrillar outer coat that shreds while exiting the host's digestive tract and in the outer environment. This increases the chances that eggs catch on aquatic vegetation where the egg is more likely to be consumed by *H. azteca* (Barger and Nickol 1998).

*Leptorhynchoides thecatus* is found in an extraordinarily variety of definitive and paratenic hosts. This parasite is documented from a wide variety of vertebrates in North America

including fishes, amphibians, and reptiles, but it is likely that the latter two are at best paratenic hosts (Lincicome and Van Cleave 1949b). The parasite is found in 79 different species of fish; the centrarchid fishes are the primary hosts. Functional hosts may not be so broadly distributed, because the reports often did not determine the reproductive status of the parasites (Ashley and Nickol 1989). Across the parasite's range, smallmouth bass (*Micropterus dolomieu* Lacépède) is considered the most common host, followed by largemouth bass (*Micropterus salmoides* Lacépède) and rock bass (*A. rupestris*) in frequency (Steinauer *et al.* 2006). In addition, many fish may be used as paratenic hosts, where *L. thecatus* cystacanths encysts in the mesenteries and viscera of fish. Visceral cystacanths are particularly common in environments with additional established trophic levels (Steinauer *et al.* 2006).

The large geographic range and geographic differences in host use led Steinauer *et al.* (2007) to investigate the inter-specific variability of this parasite. Molecular techniques, host use, habitat use, and transmission types suggest that the species called *L. thecatus* comprises six highly divergent and independent lineages that should be considered separate species (Steinauer *et al.* 2007). These cryptic species tend to be geographically constrained with little overlap in their ranges (Steinauer *et al.* 2007). These species have not been formally distinguished, but the geographic distinction of lineages is important for my study of this parasite's manipulation of its host. The *L. thecatus* super-species is generally limited to a range east of the Mississippi River, although it is found westward into Louisiana and Texas (Steinauer *et al.* 2006). The greatest concentrations are around the Great Lakes, and as far west as watersheds in Minnesota, and Gulf Coast regions. *Leptorhynchoides thecatus* has spread west of the Mississippi by several instances of human introduction. A small number of established populations are known in Nebraska and Oklahoma, and we have a single account of *L. thecatus* in Spokane, Washington, of larvae only

(Lang and Edson 1976). Morphological and molecular data suggest that the Nebraska population of interest to this study was most likely introduced from a population in Wisconsin (Steinauer *et al.* 2007). No accounts of this parasite are known from Colorado or Wyoming (Pete Walker, Colorado Department of Wildlife - Fish Pathology, personal communication).

A survey of water bodies throughout Nebraska between 1971 and 1974 found *L. thecatus* in seven of seventy-two ponds or dams sampled (Samuel *et al.* 1976). All of these water bodies are in the northern Sandhills region of central Nebraska, and are constrained to two watersheds: the Elkhorn River and Niobrara River. However, the Niobrara yielded *L. thecatus* from only one site at Spencer Dam and only from Largemouth Bass (*M. salmoides*). Atkinson Reservoir, in Atkinson, Holt County, Nebraska, had the highest concentration of *L. thecatus* in nine host fish species, and was also home to three other species of fish acanthocephalans: *Pomphorhynchus bulbocolli* (Linkins in Van Cleave), *Neoechinorhynchus prolixus* (Van Cleave and Timmons), and *Neoechinorhynchus cristatus* (Lynch) (Samuel *et al.* 1976).

### ***Leptorhynchoides thecatus*** **Host Use in Atkinson Reservoir, Nebraska**

The *L. thecatus* population in Atkinson differs from other populations in its fish definitive host use. As noted above, this *L. thecatus* population shares traits with a population in Wisconsin (Steinauer *et al.* 2007), which suggests that Atkinson parasites were introduced from the Wisconsin subspecies. Whereas in the Great Lakes region bass are the major hosts for *L. thecatus* (Steinauer *et al.* 2006), in Atkinson, green sunfish (*L. cyanellus*), pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus), bluegill (*Lepomis macrochirus* Rafinesque), and largemouth bass (*M. salmoides*) are most frequently infected (Ashley and Nickol 1989). Atkinson Lake is the only known population in which *L. cyanellus* is the host species most likely to harbor gravid

female worms; *L. macrochirus* is much less likely to host reproductively mature female worms (Ashley and Nickol 1989). Green sunfish is considered the primary host for this population of *L. thecatus* (Ashley and Nickol 1989).

*Leptorhynchoides thecatus* has a rather low prevalence among amphipods in Atkinson does. Ashley and Nickol (1989) sampled each month for a year, inspected nearly 4000 amphipods, and determined the total, yearly prevalence of *L. thecatus* cystacanths to be 0.7%, with a peak in the month of May of a 2.4% (N=128 amphipods). In addition, in an entire year of sampling only one amphipod was infected by more than one cystacanth, suggesting that multiple infections are extremely rare in the natural setting.

Centrarchid fishes in Atkinson have a much higher prevalence than in the intermediate host. Approximately sixty-percent of fish collected in 1978-1979 were infected with *L. thecatus*, with an intensity of 1-75 worms, and a mean relative density (Relative density is defined as the mean number of individuals of a particular parasite species per host examined – per Margolis *et al.* 1982) of 3.5 worms per infected fish (Ashley and Nickol 1989).

**Atkinson Reservoir Description**

Atkinson Reservoir is an impoundment on the Elkhorn River at Atkinson, Holt County, Nebraska (42°33'N, 98°58'W, elevation 2,125ft). The dam creating the lake was built in 1967 by the city of Atkinson and state and federal agencies. There is a main channel running from NW to S side, and 3 finger-like coves 50-100 yards in length off the main body of water (See Figure 1). Ashley and Nickol (1989) described the lake as an alkaline, eutrophic reservoir of nearly uniform depth, with a maximum depth of nearly 2m. While sampling for this study between 2007-2011, I did not test eutrophication, but estimated that the lake lies somewhere between mesotrophic and

eutrophic in nature. Water samples yielded a pH of 7.1-7.3 and General Hardness of 161 ppm KH/GH. I did not measure the lake's depth, but I found by wading that the centers of the coves were deep enough to prevent crossing and estimated the depth of the cove between 2-3m. The main channel was associated with sandbars that moved yearly depending on hydrological conditions.

Yearly dynamics of the lake are such that some of the shallow coves freeze to the bottom during winter and lake water levels may drop considerably during summer (Ashley and Nickol 1989). However, in the summers of 2010 and 2011 the Elkhorn River experienced heavy flooding, which inundated the lake; the conditions during these periods were opposite to normal summer depth drops. In June 2010 the river reached record flood levels high enough to effectively overtop the dam (see Figures 1-2).

**Management of Atkinson Lake**

Nebraska Game and Parks Commission (NGPC) managed the reservoir and associated land, including a rudimentary campground, from the 1970's until 2008. During this time NGPC stocked the reservoir with desirable sport fishing species (see Chapter 5). Management of the reservoir was transferred from NGPC to the Municipality of Atkinson in 2008, and no stocking has occurred since. In 2012 and 2013, the Municipality took advantage of low summer depths to significantly deepen and widen the channels and bays of the lake (Charlene Paris, personal communication).

**Previous Studies on *Leptorhynchoides thecatus* from Atkinson Reservoir**

Steinauer and Nickol (2003) used this population of *L. thecatus* to test whether cystacanth size influences the parasite's adult success. They divided cystacanths into different size classes, fed them to *L. cyanellus*, and six weeks later dissected the fish and checked for parasite survival and condition. Larger cystacanths survived the transition from larva to adult and established in the definitive host significantly more frequently than smaller cystacanths. The authors suggest that transition from larva to adult and establishment in the host may be energetically costly, and smaller worms may have a harder time migrating from the intestine to the pyloric caeca. However, many other adult traits did not differ between larger or smaller cystacanths. All adult worms reached similar sizes regardless of their cystacanth size, and females from both cystacanth size classes developed to a gravid state at equal rates. This study provides an important base for my research, because larger cystacanths are more likely to survive and establish than smaller cystacanths, and thus they have a fitness advantage.

Steinauer and Nickol (2003) also statistically analyzed the traits that influence cystacanth size. They found that cystacanth size was significantly influenced by intensity of infection, sex of the cystacanth, amphipod length, and an interaction between amphipod length and cystacanth sex.

***Leptorhynchoides thecatus* and Cost of Manipulation**

This system presents an opportunity to test for manipulation because this population of *L. thecatus* has a measure of fitness in the larval stage. We know that cystacanth size is a determinant of survival, which is a primary component of fitness and we have an idea of the factors that influence cystacanth size.

Before presenting my study of behavioral modification and cystacanth size in this system, I would like to emphasize six pertinent aspects of this study system, summarized in Table 1. First, acanthocephalans inhabit the hemocoel of their arthropod host. This means that the proximate mechanism of behavioral change is one carried out at distance from the central nervous system (CNS) and other tissues. Whereas other parasites that infect the CNS can alter host behavior by physically harming the nervous tissue, *L. thecatus* must cause behavioral changes remotely. Remote mechanisms likely involve parasite-produced chemicals that are assumed to be costly for the parasite to produce. Second, acanthocephalans generally have low infrapopulation sizes (number of parasites per host) in their intermediate hosts - particularly amphipods. This means that a single parasite must incur the cost of producing behavioral change and the cost cannot be spread between many parasites (concept reviewed in Chapter 2). *Leptorhynchoides thecatus* has a very low rate of multiple infections (Ashley and Nickol 1989). Third, this parasite has a measure of fitness in cystacanth size because larger cystacanths have higher survival success. This provides a potential measurement of fitness that can be correlated with behavioral change.

Fourth, altered behavior and behavioral manipulation have been documented in most, but not all, acanthocephalans (Moore 2002). Prior to this study, *L. thecatus* was not known to alter host behavior, but its similarities to other acanthocephala-amphipoda systems make it likely to exhibit behavioral change (see Moore 2002 for full list). Fifth, *L. thecatus* infections in the amphipod are cryptic; we cannot reliably tell whether an amphipod is infected without killing it and dissecting it. The first implication of cryptic infection is that behavioral experiments are by necessity blind. An experimenter may know whether the amphipod has been exposed to infection, but there is no way to know if the individual amphipod being observed is infected.

Prevalence among exposed amphipods was at no time above 35% for my studies. The second implication is that it is difficult to conduct predation studies to determine whether infected amphipods are consumed at higher rates. Without reliable infection status, we cannot set up experiments with particular numbers, or ratios, of infected and uninfected amphipods and test whether a sunfish consumes infected amphipods at higher rates. Because of this, it is difficult to demonstrate an increase in transmission rates for this parasite compared to other parasite systems. Sixth and lastly, there has extensive study of amphipod behavior and how it relates to predation. We can show that behaviors that have been demonstrated to alter predation in amphipods are changed in this system and infected amphipods behave in more risky ways.

Despite this final aspect, the ability to investigate the connection between a measure of fitness and behavioral modification offers an opportunity not present in other parasites. It allows us to test the assumptions of the cost of manipulation, to provide important experimental evidence to inform theoretical models, and advance our understanding of the complex phenomenon of behavioral manipulation.

Table 1.1. Summary of the *Leptorhynchoides thecatus* study system.

| | Aspect of study system | Implication |
|---|---|---|
| 1 | Acanthocephalans infect hemocoel of arthropod host | Physical damage to central nervous system tissue by parasite not possible.<br>Behavioral change caused remotely.<br>Mechanisms are hypothesized to be more costly for remote proximate mechanism |
| 2 | Very low chance of multiple infection in amphipod | Parasite must manipulate host alone and cannot share costs |
| 3 | Larger cystacanths have higher establishment success and survival (fitness) than small cystacanths | Cystacanth size may be used as a measure of fitness |
| 4 | Most acanthocephalans alter or manipulate host behavior | *L. thecatus* is likely to cause some sort of behavioral change |
| 5 | Infections in *H. azteca* are cryptic and host must be dissected to determine infection status | Behavioral experimental protocols are blind<br>Predation studies are very difficult to conduct |
| 6 | Uninfected amphipod behavior is well studied | We know what behaviors correlate with increased predation |

Figure 1.1. Atkinson Lake Recreation Area on July 26, 2013. This photo demonstrates normal conditions during summer. The view is from south (downstream of the dam) looking north toward the lake where sampling occurred. Photo courtesy of Charlene Paris. Atkinson, Nebraska

Figure 1.2. Atkinson Lake Recreation Area on 14 June 2010. This photo was taken during the high flood stage. Photographer is standing on or near the bridge that crosses the Elkhorn River on a raised road south of the dam. Photo courtesy of Charlene Paris, Atkinson, Nebraska.

**REFERENCES**


Amin, O.M. 1987. Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polycanthorhynchida). Journal of Parasitology. 73: 1216-1219.

Ashley, D.C., and B.B. Nickol. 1989. Dynamics of the *Leptorhynchoides thecatus* (Acanthocephala) suprapopulation in the Great Plains reservoir. Journal of Parasitology. 75: 46-54.

Barger, M.A., and B.B. Nickol. 1998. Effects of coinfection with *Pomphorhynchus bulbocolli* on development of *Leptorhynchoides thecatus* (Acanthocephala) in amphipods (Hyalella azteca). Journal of Parasitology. 85: 60-63.

Bethel, W.M., and J.C. Holmes. 1973. Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. Journal of Parasitology, 59: 945–956.

Bethel, W.M., and J.C. Holmes. 1977. Increased vulnerability of amphipods to predation owing to altered behavior induced by larval acanthocephalans. Canadian Journal of Zoology. 55: 110-115.

Blunk, H. 1922. Die Lebensgeschichte der im Gelbrand schmarozenden Saitenwurmer. *Zoologische Anzeiger.* 54: 111–132 and 145–162.

Carney, W.P. 1969. Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. American Midland Naturalist. 82: 605-611.

Combes, C. 2001. Parasitism: The Ecology and Evolution of Intimate Interactions. University of Chicago Press, Ltd. London.

DeGuisti, D.L. 1949. The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. Journal of Parasitology. 35: 437-460.

Kostylev, N.N. 1924. Le genre *Leptorhynchoides*, nouveau genre d'acanthocephales parasite des poisons. Annales de Parasitologie Humaine et Comparee. 2: 214-223.

Lang, B.Z., and S.A. Edson. 1976. Parasites of the western speckled dace from eastern Washington. Journal of Parasitology. 62: 93.

Lincicome, D.R., and H.J. Van Cleave. 1949a. Distribution of *Leptorhynchoides thecatus*, a common acanthocephalan parasitic in fishes. American Midland Naturalist. 41: 421-431.

Lincicome, D.R., and H.J. Van Cleave. 1949b. Review and redescription of the acanthocephalan species, *Leptorhynchoides thecatus*. Transactions of the American Microscopical Society. 68:304-313.

Linton, E. 1891. Notes on Entozoa of marine fishes with descriptions of new species. Part III. Acanthocephala. Report. U.S. Committee on Fish and Fisheries for 1888: 523-542.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris, and G.A. Schad. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology. 68: 131-133.

Moore, J. 2002. Parasites and the Behavior of Animals. New York, NY: Oxford University Press.

Olson, P. D., and B. B. Nickol. 1995. Effects of low temperature on the development of *Leptorhynchoides thecatus* (Acanthocephala) in *Lepomis cyanellus* (Centrarchidae). Journal of the Helminthological Society of Washington. 62: 44–47.

Poulin, R. 1994. The evolution of parasite manipulation of host behaviour: a theoretical analysis. Parasitology. 109: 109–118.

Richardson, D.J., J.K. Martens, and B.B. Nickol. 1997. Copulation and Sexual Congress of *Leptorhynchoides thecatus*. Journal of Parasitology. 83: 542-543.

Richardson, K.E., D.J. Richardson, and B.B. Nickol. 2008. Emigration of *Leptorhynchoides thecatus* (Acanthocephala) in Green Sunfish (*Lepomis cyanellus*). Comparative Physiology. 75: 49-51.

Roberts, L.S. and J. J. Janovy. 2009. Foundations of Parasitology. McGraw-Hill Higher Education. Boston, Massachusetts. 8th Edition.

Samuel, N., B.B. Nickol, and M.A. Mayes. 1976. Acanthocephala of Nebraska Fishes. American Midland Naturalist. 96: 391-406.

Smith, D.G. 2001. Pennak's freshwater invertebrates of the United States: Porifera to Crustacea. Wiley. New York. 4th Edition.

Steinauer, M.L., and B.B. Nickol. 2003. Effect of cystacanth body size on adult success. Journal of Parasitology. 89: 251-254.

Steinauer M.L., J.E. Parham, and B.B. Nickol. 2006. Geographic analysis of host use, development, and habitat use of an acanthocephalan species, *Leptorhynchoides thecatus*. Journal of Parasitology. 92: 464-472.

Steinauer M.L., B.B. Nickol, and G. Orti. 2007. Cryptic speciation and patterns of phenotypic variation of a highly variable acanthocephalan parasite. Molecular Ecology. 16: 4097-4109.

Thomas, F., A. Schmidt-Rhaesa, G. Martin, C. Manu, P. Durand, and F. Renaud. 2002. Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? Journal of Evolutionary Biology 15: 356-361.

Thorne, G. 1940. The hairworm *Gordius robustus* Leidy, as a parasite of the Mormon cricket, *Anabrus simplex* Haldeman. Journal of the Washington Academy of Sciences. 30: 219–231.

Uznanski, R. L., and B.B. Nickol 1980. Parasite population regulation – lethal and sublethal effects of Leptorhynchoides-thecatus (Acanthocephala, Rhadinorhynchidae) on Hyalella-azteca (Amphipoda). Journal of Parasitology 66: 122-126.

Uznanski, R.L., and B.B. Nickol. 1982. Site selection, growth, and survival of *Leptorhynchoides thecatus* (Acanthocephala) during the prepatent period in *Lepomis cyanellus*. Journal of Parasitology. 68: 686-690.

Vickery, W.L., and R. Poulin. 2010. The evolution of host manipulation by parasites: a game theory analysis. Evolutionary Ecology. 24: 773-788.

## CHAPTER 2: Cost of Behavioral Manipulation: Review and Concepts

### Introduction

It may seem intuitive that adaptations come with associated costs. Resources are required to build and maintain such traits, and resources used for one trait may not be available for another (Stearns 1992). Beyond such costs, adaptations may reduce certain aspects of fitness. What may benefit the organism in certain environments may harm its fitness in another. While these ideas are easily conceived, defining the currency by which costs are measured and showing that these cost exist is a challenging endeavor. This task is made no easier by the question about cost I wish to answer: the cost of parasitic behavioral manipulation.

Parasitic manipulation occurs when a parasite alters its host's behavior or phenotype to increase the likelihood of transmission (Thomas *et al.* 2005). Parasitic manipulation describes an extremely broad phenomenon and has been documented in nearly every parasitic taxon (see review in Moore 2002, also Poulin 1994a). Because this phenomenon has been documented in such a diverse array of organisms, Poulin (2010) suggests that it may have evolved as many as 20 separate times. Despite the prevalence of behavioral manipulation, we have a limited understanding of the proximate mechanisms that parasites use to alter host behaviors.

This chapter will address the cost of manipulation in two parts. First, I will provide a review of how manipulative costs have been approached in theoretical analyses and how demonstrations of mechanistic pathways have influenced how we think about costs. Second, I will categorize the types of potential costs parasites might incur; I will address how the few demonstrations of cost that we do have fit into the larger picture, and how we may best demonstrate these types of costs in the future.

*Definitions*

Before reviewing studies on the costs of manipulation, a brief review of the history of "parasitic manipulation" is in order, along with how the term has changed over time. Parasitic manipulation is currently, and most frequently, defined as an alteration in behavior or phenotype that benefits the parasite, usually by increasing its transmission rate (Thomas *et al.* 2005). Early studies did not use this term, but rather documented "altered" or "modified" behaviors (Bethel and Holmes 1973, Bethel and Holmes 1977, Moore 1983). The term "manipulation" became popular around 1990 (Moore and Gotelli 1990), and likely took hold because of its simplicity, ease in comprehension, and powerful psychological impact. While the early studies did not use the term manipulation, today we recognize them as having the attributes of manipulative host-parasite systems.

After the introduction of the manipulation hypothesis, the term was used in a variety of ways. Poulin (1994b) used the term manipulation in his theoretical approach to the cost of manipulation. However, his definition of manipulation included "only changes in host behavior that follow specific actions by the parasite" (Poulin 1994b, pg. S110). I have contemplated Poulin's definition and its implications for understanding of cost of manipulation is a good starting point. In the twenty years since that publication, we do not have clear demonstrations of such specific actions. Other authors have adopted terms that limit the scope of manipulation in order to more easily understand the phenomenon. While there is merit in such careful definition of manipulation, to do so when addressing the cost of manipulation leaves us with too few studies within a given category and even more opportunities for speculation. For clarity I will use the definition of manipulation proposed by Thomas *et al.* (2005; see above).

**Theoretical Approaches to Cost of Manipulation**

Although our theoretical understanding of the cost of manipulation is rudimentary, it has been addressed in models of manipulation. I will address three central theoretical approaches. The first, put forth by Poulin (1994b), incorporates cost to explain patterns of manipulation. The basis for his analysis is the assumption that energy used for manipulation is not available for reproduction, growth, or fighting the host's immune system. This idea fits the definition of a physiological trade-off as described by Stearns (1992). Poulin (1994b) narrows his focus by defining "active manipulation" as manipulation requiring an active involvement of the parasite at the cost of decreased growth or reproduction. Building from this assumption, costs can be optimized to the greatest increase in transmission with the least manipulation effort (ME; see Figure 1 in Poulin 1994b). If the manipulative effort can be optimized by the parasite, a set of 6 predictions follow that address how certain parasite population characteristics would affect ME* in each case. For example, a small parasite infrapopulation size (parasites per individual host) is predicted to have a higher ME* because single, or few, parasites must invest all of the energy required for manipulation; parasites with large infrapopulations could possibly share the costs of manipulation (Poulin 1994b). To my knowledge, the predictions presented in this paper have not been revisited (but see Poulin et al. 2005) and we are as yet unable to assess the accuracy of these theoretical concepts. Regardless, Poulin's paper is significant in that it was the first to propose costs of manipulation.

In the original paper, Poulin (1994b) discusses the fact that we have no information on the physiological costs he hypothesizes. In a 2010 review, Poulin (2010) slightly modified the conceptualization of costs by adding a curve representing the increased probability of dying early because the energy that could have been used by the parasite to support life has been invested in

manipulation instead (see Figure 1 in Poulin 2010). In the same paper, Poulin (2010) notes that the nature of the curve in these figures has not been demonstrated. While Poulin offers a significant starting point for understanding manipulation, it is possible that additional factors may have been overlooked. For example, if a threshold must be reached before manipulation occurs, then parasites may be forced to invest so long as the increased likelihood of transmission remains larger than the fitness costs associated with the change.

The second analysis that illuminates the cost of manipulation was presented by Parker *et al.* (2009). The purpose of this analysis was to predict host manipulation by trophically transmitted helminths in two forms: decreasing host mortality (suppression of predation on the host) and increasing host mortality (enhancement of predation). The model is extensive and includes 40 mathematical parameters - including five representing various aspects of costs for both suppression and enhancement. The authors model cost by assuming that increased manipulation decreases the parasite's reproduction, in terms of egg production.

Parker et al. (2009) note that among other components of mortality, suppression of predation (decreasing predation below that achieved by normal predator-avoidance behavior) is important early in the intermediate host infection, and allows maturation of the larva so that it can be infective to the next host. Manipulation that suppresses predation in this stage increases parasite fitness because all parasites must reach infectivity if they are to be successfully transmitted. Suppression of predation is simpler than enhancement of predation because reducing all predation tends to happen earlier in the parasite's development and does not have to target specific predators.

Enhancement of predation, on the other hand, is more complex and involves two components: the length of survival in the intermediate host and the cost in egg production later in

the definitive host. If a parasite survives for a long time, then enhancement of predation will evolve only if the manipulation is more likely to increase predation by the appropriate host (selective manipulation) rather than predation by inappropriate hosts (non-selective) (Parker *et al.* 2009). Additionally, if the host suffers high mortality due to non-predator-related factors, then enhancement should not evolve. "Predation enhancement manipulation requires the increase in transmission rate, devalued due to the reduction in fecundity through the manipulative effort, to be greater than the average increase in the mortality of its intermediate host." (Parker *et al.* 2009, pg. 451). When parasites do not live long periods in the intermediate host, the benefits of predation enhancement, selective (increasing predation by viable hosts) and non-selective (increasing all predation), are greater because any increase in the chance of suitable host predation is better than a certain demise. Between these two longevity extremes in the intermediate host, natural selection favors predation enhancement that is as selective as possible.

Reduced egg production as a result of manipulation also influences whether predation enhancement evolves. High reduction in egg production prevents enhancement from developing. However, Parker *et al.* (2009) also conclude that small manipulative costs can explain enhancement even when it increases all components of intermediate host mortality. In this case an enhancement mutation may not spread by selective advantage, but could spread by genetic drift if the costs of manipulation are very small (approaching 0) (Parker *et al.* 2009).

Parker *et al.* (2009) model costs in terms of future reproductive success, but also suggest that in the context of mortality-associated costs (Poulin *et al.* 2005) their model can be interpreted in terms of mortality due to manipulation. They do conclude, "High costs may be a key reason why host manipulation is observed in some parasite species, but not in other closely related species."(Parker *et al.* 2009, pg 457) However, comparative studies of manipulation are

lacking in the literature (despite calls by Moore and Gotelli (1990) nearly 20 years ago), and this issue will take some time to resolve.

My final example of theoretical approaches to cost of manipulation is a game theory model of manipulation (Vickery and Poulin 2010). This analysis models a trematode system; trematodes commonly share intermediate hosts with both kin and non-kin conspecifics. These authors incorporate three costs. First, they assert that manipulation requires effort (energy) that reduces growth rate in the intermediate host and potentially decreases survival or reproduction in the definitive host. They cite evidence of behavior-altering chemicals and suggest that these are produced in specialized structures that must be built and maintained in manipulative parasites. Second, manipulation costs parasites when they are exploited by conspecifics that do not expend energy on manipulation. These "hitch-hiking" parasites gain the advantage of manipulation but do not pay the costs (Thomas *et al.* 1998). Vickery and Poulin (2010) incorporate work by Brown (1999) that suggested that parasites could balance individual selective disadvantages with kin-selection advantages when they share hosts with kin. The last type of cost Vickery and Poulin (2010) address in their model is the disadvantage that manipulative parasites experience in competition with non-manipulative parasites that do not invest in manipulation. The logical justification of both the second and third costs assumes that the first energetic cost exists and is significant.

Using this model, Vickery and Poulin (2010) conclude that a lone parasite should invest up to one-half of its fecundity for the benefits of manipulation. However, in the presence of competition from other parasites this investment should decrease. The benefits of manipulation are sufficient to remain profitable even in the presence of other kin, so long as they do not overload the host and kill it (Vickery and Poulin 2010). Relatedness and the passive transmission

rates appear to be key determinates of the investment that parasites put into manipulation for this model.

**Mechanisms and Cost**

Increased understanding of proximate mechanisms of manipulation has influenced how we understand cost and will continue to play a major role in that understanding. Helluy (1983) demonstrated that there are imbalances in serotonin levels in amphipods infected with manipulative parasites. Helluy and Holmes (1990) found octopamine and dopamine imbalances associated with behavioral alterations in amphipods, although dopamine appears to be more important to behavioral change (Tain *et al*. 2006, Tain *et al*. 2007). Dopamine has also been demonstrated in other parasitic behavior manipulations (Prandovszky *et al*. 2011). Norepinephrine (Zuk *et al*. 1990), cytokines (Boucias and Pendland 1998, Friberg *et al*. 2010), phenoloxydases (Cornet *et al.* 2009), and NO (Helluy 2013) are among a growing list of molecules implicated in manipulation (for recent syntheses see Adamo 2013, Helluy 2013, Biron and Loxdale 2013). A comprehensive description of the mechanism is beyond the scope of my focus on cost, but there are a number of helpful sources that do address mechanism (review in Moore 2002, Lefèvre *et al.* 2009, Adamo 2012, Helluy 2013, Lafferty and Shaw 2013, Perrot-Minnot and Cezilly 2013). While it has proven difficult to demonstrate that these neurotransmitters originate from the parasite rather than the host, our understanding of mechanisms suggest that manipulators may pay relatively little energetic cost to induce behavioral changes in some cases.

Adamo (2002) shifted thinking on mechanism by demonstrating that altered behavior can result from the immune response of the host. Thomas *et al.* (2005) incorporated this concept and

proposed two major classes of modification pathways: (1) direct effects, which are chemicals produced by the parasite that affect the host nervous system or muscle, and (2) indirect effects, which comprise affects on non-excitable tissues other than nerves or muscles that result in host-mediated changes in behavior. Adamo (2013) added a third manipulative pathway, suggesting that parasites can induce genomic and/or proteomic activity changes in the brain of the host. Thomas *et al.* (2005) asserted that for indirect mechanisms the cost of manipulation could be surprisingly small, which likely applies to the genomic and proteomic pathway (Adamo 2013).

As researchers begin to untangle the Gordian knot of parasite and host chemical production (and resulting crosstalk), speculation about cost appears to be inevitable. In recent reviews of this subject, Adamo (2013), Lafferty and Shaw (2013), Biron and Loxdale (2013) all mention cost of manipulation, with the prevailing logic being that indirect and genetic/proteomic pathways are cheap and that parasites may avoid more costly direct pathways of manipulation. The involvement of host immune response makes the costs of manipulation much lower, but links manipulation with selection for parasite traits for surviving host immune system attack and pathology (Lefèvre *et al.* 2008, Helluy 2013, Perrot-Minnot and Cezilly 2013). Such links among these factors increase the complexity that must be assessed to understand the evolution of parasitic manipulation and highlights the number of different selective forces parasites balance to maximize fitness (Combes 1997).

**Categories of Cost**

The term cost has applied equally to energy consumption, decreased fecundity, and increased mortality (Vickery and Poulin 2010), and I contend that this generic definition limits our understanding. I believe it useful to categorize the possible costs so that we can be more

specific about the costs we are addressing so that we can facilitate comparisons between studies. I propose three categories: genetic costs, internal costs, and external cost for manipulative parasites, with subcategories within each.

**Genetic Cost of Manipulation**

Before identifying the potential genetic costs of manipulation, I must address the genetic basis of manipulation. Any approach to the evolution of manipulation operates under the assumption that there must be a genetic basis and variation in these traits. There is some preliminary evidence for genetic basis for manipulation (Franceschi *et al.* 2008, Franceschi *et al.* 2010) and intraspecific variation (Benesh *et al.* 2009, Sparkes *et al.* 2004). However, Moore (2013) has pointed out that much work remains to be done in this field because these examples of manipulation genetics are limited in their scope (e.g. study of strains) and confounded by the difficulty of distinguishing between normal variation in host behavior and manipulation. The genetic basis of manipulation is ripe for investigation and the increased ease of genetic analyses suggest that we will see more studies on this subject in the near future.

While we lack comprehensive demonstrations of genetic bases of manipulation, there may be intrinsic genetic costs of manipulation (van Kleunen and Fischer 2005). Genotypes associated with stronger manipulation of the host may have a lower fitness associated with that genotype when compared to genotypes conferring less manipulation (DeWitt *et al.* 1998, van Kleunen and Fischer 2005, Auld *et al*. 2010). The genetic costs may be difficult to distinguish from non-genetic costs (Murren *et al.* 2013). However, as we gain a deeper understanding of the genetic basis of manipulative traits it will behoove researchers to incorporate the techniques of quantitative genetic experimental design and apply the lessons learned by evolutionary ecology to clearly identify the genetic costs of these traits (van Kleunen and Fischer 2005).

**Internal Costs of Manipulation**

In this category I place the production and maintenance costs of manipulative structures, physiological trade-offs, and other costs that occur during development and survival of the individual parasite. Poulin focused on these types of costs (1994b, 2010)

*Metabolic costs – production and maintenance*

First, there may be metabolic costs of production and maintenance of structures required for manipulation. It has been argued that manipulation can be a neutral byproduct of pathology, per Minchella (1985), but as Moore has argued (Moore 2002, Thomas *et al*. 2005, Moore 2013) it is unlikely that any byproduct that routinely increases parasite fitness will remain free of selective pressures for long. There is some evidence that manipulation may be a result of and inseparable from host immune system evasion (Lefèvre *et al.* 2008, Lefèvre *et al.* 2009, Adamo 2013). The metabolic costs of both byproducts and immune system evasion are hypothesized to be small.

It may prove difficult to quantify these production and maintenance costs (DeWitt *et al.* 1998, Auld *et al.* 2010). However, there is some promise in the proteomics investigations into manipulative parasites (Thomas *et al.* 2003, Biron *et al.* 2006, Lefèvre *et al.* 2009, Hughes 2013, Perrot-Minnot and Cezilly 2013). It may be possible to identify what proteins and structures are produced in a manipulative parasite and how much is invested in them.

*Allocation Trade-offs*

Allocation trade-offs are a consequence of metabolic costs. Optimal allocation involves optimizing the partitioning of an organism's resources to maximize fitness (Cody 1966); allocation trade-offs involve compromises between competing traits for survival, growth, and

reproductive output (Silvertown and Dodd 1999). This is the logic underpinning Poulin's analyses (1994, 2010).

Demonstrating physiological trade-offs can be notoriously difficult (Stearns 1992, Trumbo 1999). Trade-offs may or may not be observed under certain environmental conditions and in some cases we only observe them when an organism experiences limited resources (Stearns 1992). Two traits that are predicted to conflict may in fact be positively correlated, depending on environmental conditions (Reznik *et al.* 2000). Despite these barriers, there are a number of studies that indicate that the parasite larval stage is a particularly good place to demonstrate trade-offs between traits including growth rates, maturity rates, and fecundity (*e.g.* Crosson *et al.* 2007, Paterson and Barber 2007).

One possible case of a physiological trade-off between manipulation and another fitness-related trait is that presented by Maure *et al.* (2011). The bracionid parasitoid wasp *Dinocampus coccinellae* induces its host, a ladybird beetle, to guard the wasp when it emerges from the host to pupate. The proximate mechanism is unknown, but the authors propose that it is an effect of the wasp's venom (Maure *et al.* 2011). This study found a negative relationship between the duration of guarding behavior by the ladybird beetle and the fecundity of the emerging wasp (Maure *et al.* 2011). One explanation for these results is a trade-off between the size, or perhaps output, of the venom organs and fecundity; larger amounts of venom require more energy and this in turn reduces egg production, particularly if the female does not feed as an adult. However, this case highlights the attention needed when demonstrating trade-offs, as there is an alternate explanation: limited resources available to the growing parasite. I will discuss resource limitation in the next section.

**External Costs of Manipulation**

I will now address the costs of manipulation that do not have an origin within the developing parasite. A parasite acquires energy from an external source, its host, and may pay trade-off costs with the strength of its manipulation. Manipulative parasites may suffer higher mortality in effecting the altered behavior. Competition with other parasites for host resources belongs in this category as well. We have few examples of manipulative costs from the literature, but those that do exist fit best into this external cost category.

*Resource Acquisition Costs*

Energy acquired from a host clearly benefits a parasite, but it comes at a cost in terms of the pathology caused to the host unless resource acquisition increases (Combes 1997). The virulence of a parasite is an optimization among factors including transmission, longevity of the host, and mortality rates (Anderson and May 1978, 1979; May and Anderson 1978).

As an example of resource acquisition costs, let us revisit the parasitoid wasps studied by Maure *et al.* (2011). These authors first suggest that production of the manipulative agent is costly and that fecundity suffers as a result. However, they also posit that a more plausible explanation is that the strength of the manipulative behavior – how long the beetle guards the pupae – depends upon how much energy the ladybird beetle has. That is, the host runs out of energy to guard the pupae. Wasps that cause more damage may have more eggs when they emerge from the pupae because they have taken more from their host, but the beetle will guard them for a shorter period of time.

*Mortality Costs*

Thomas *et al.* (2005) proposed that indirect manipulation pathways had the potential to make manipulation costs very small and questioned whether they could be demonstrated. Poulin

*et al.* (2005) responded that there were in fact at least three documented cases to demonstrate costs. However, these costs differed from those in previous studies (Poulin 1994b, Thomas *et al.* 2005) because they occur in the form of increased mortality, and thus belong in the category of external costs of manipulation. Poulin *et al.* (2005) submitted that individual manipulative parasites suffered greater mortality compared to other parasites in the same animal that cause little or no manipulation. Manipulative parasite died at higher rates because of predation, inability to develop, and host immune system.

The most comprehensive example of mortality costs presented by Poulin *et al.* (2005) is that of two trematode species that both parasitize the cockle *Austrovenus stutchburyi*. Individual parasites of both species can be manipulative when they locate themselves near the edge of the cockle's foot causing malformation, rendering cockles unable to burrow, and increasing the rate at which predators consume infected cockles. A minority of individuals of both species encysts in the middle (25%) or base (10%) of the foot. These individual parasites do not cause manipulation, but reap the benefit. There is a demonstrable cost to this manipulation. Cockles stranded above the substrate have a higher predation rate from unsuitable hosts, such as a predatory whelk. Not only this, but a non-host predator, a labrid fish, feeds by taking bites out of the malformed foot of infected cockles and in doing so ends the lives of those trematodes near the edge of the foot (Mouritsen and Poulin 2003a). Manipulation increases the predation rates of cockles by 5 to 7 times compared to that of buried, healthy cockles (Mouritsen 2002), and the manipulative parasites suffer nearly 20% additional mortality as a result of the labrid fish than conspecifics located at the base of the foot (Mouritsen and Poulin 2003b). In this case Poulin *et al.* (2005) present a thorough example of differential mortality as a cost of manipulation for

which we have estimates of the benefits (rates of increased transmission) and costs (rates of increased mortality).

Two other cases of differential mortality are the trematode *Dicrocoelium dendriticum* and *Microphallus papillorobustus* (Poulin 2005). *Dicrocoelium dendriticum* is a classic example of host manipulation in an ant intermediate host. The ant consumes many cercaria clones, and a single cercaria migrates to the ant's subesophageal ganglion, causes the ant's behavior to change, and then fails to develop into an infective stage. For this individual parasite the direct fitness reward is zero, but it may be compensated by inclusive fitness of its clones that are passed on at higher rates (Wickler 1976, Wilson 1977). In the case of *M. papillorobustus*, the metacercaria stages infect both the brain and body of their gammarid (Amphipoda) intermediate host. Those in the cerebral ganglion suffer an approximately 17% mortality from the host's immune response, while non-manipulative metacercariae in the abdomen suffer around 1% mortality (Thomas *et al.* 2000). These cases of differential mortality offer the potential to quantify one type of cost to manipulation.

It is important to note that these examples assume that mortality creates a selective pressure on the variation in site selection within the host that is in opposition to the selection force for increased transmission from the behavioral manipulation. To my knowledge, we do not know the basis of site selection in these parasites and there is no evidence for a genetic basis of infection site selection.

*Competition Costs*

Exploitation of manipulation effort by non-manipulative parasites has proven an attractive concept (Thomas *et al.* 1998a, Thomas *et al.* 1998b, Vickery and Poulin 2010). These costs proved to be important factors in the game theory analysis of Vickery and

Poulin (2010) and thus warrant mention as potential external costs. If we can demonstrate that individual parasites do pay metabolic costs of manipulation - leading to trade-offs with other traits - then it follows that these parasites may pay additional fitness costs relative to others in the population, but only if the manipulative parasite is in direct competition for resources in the same host. Detecting these negative fitness costs may prove difficult, because one must compare singly and multiply infected hosts, determine which parasites are manipulative and non-manipulative, and thoroughly understand the genetic relationship between the parasites. On this last point, recall that manipulation is predicted to provide benefits to manipulative parasites even when they are being exploited by non-manipulative kin (Vickery and Poulin 2010). As such, competition costs may be prohibitively difficult to demonstrate.

**Conclusions**

We must understand that costs of manipulation are not monolithic in their origin. Researchers must be clear about which costs they wish to address, and realize that these costs will not always be easily measureable or even demonstrable. Many negative fitness effects associated with increased manipulation (e.g. reduced fecundity) could be allocated to more than one of the categories I have proposed. The case presented by Maure *et al.* (2011) demonstrates that it is not always simple to determine the nature and origins of an observed cost. The strongest explanation Maure *et al.* (2011) propose for the observed trade-off between length of host guarding and parasite fecundity lies in an acquisition trade-off in host energy reserves (external cost), but they also suggest that an allocation trade-off in energy for venom gland size can explain their results (internal cost). It is well known that energy acquisition levels can mask

allocation trade-offs so that the trade-offs are difficult to clearly discern (van Noordwijk and de Jong 1986). Certain individuals acquire greater resources and can express high levels of traits on both sides of a trade-off (Reznik *et al.* 2000). On the population level, these individuals can confound a researcher's attempts to elucidate a trade-off. We must be wary of such pitfalls when addressing the cost of manipulation, but this aspect of manipulation is ripe for the lessons and techniques of evolutionary ecology. Cost of manipulation is an important facet in understanding the evolution and maintenance of host-parasite interactions that holds great potential for exploration.

**REFERENCES**

Adamo, S.A. 2002. Modulating the modulators: parasites, neuromodulators and host behavioral change. Brain, Behavior and Evolution. 60, 370-377

Adamo, S.A. 2012. The strings of the puppet master; how parasites change host behavior. *In* Host Manipulation by Parasites (ed. DP Hughes, J Brodeur and F Thomas). Oxford: Oxford University Press.

Adamo, S.A. 2013. Parasites: evolution's neurobiologists. Journal of Experimental Biology 216, 3-10.

Anderson, R.M., and R.M. May. 1978 Regulation and stability of host-parasite population interactions. I. Regulatory processes. Journal of Animal Ecology 47, 219-247

Anderson, R.M, and R.M. May. 1979. Population biology of infectious diseases. Part I. Nature 280, 361-367

Biron, D. G., F. Ponton, L. Marche, N. Galeotti, L. Renault, E. Demey-Thomas, J. Poncet, S.P. Brown, P. Jouin, and F. Thomas. 2006. 'Suicide' of crickets harbouring hairworms: a proteomics investigation. Insect Molecular Biology 15, 731-742

Benesh, D.P., E.T. Valtonen, and O. Seppala. 2008. Multidimensionality and intra-individual variation in host manipulation by an acanthocephalan. Parasitology 135, 617-626

Benesh, D.P. 2008. What are the evolutionary constraints on larval growth in a trophically transmitted parasite? Oecologia 162, 599-608

Boucias, D.G., and J.C. Pendland. 1998. Principles of Insect Pathology. Boston MA: Klewer.

Brown, S.P. 1999. Cooperation and conflict in host-manipulating parasites. Proceedings of the Royal Society B 266:1899–1904

Cody, M.L. 1966 A general theory of clutch size. Evolution 20: 174-184

Combes C. 1997. Fitness of Parasites: Pathology and Selection. International Journal for Parasitology 27, 1-10

Cornet, S., N. Franceschi, A. Baure, T. Rigaud, and Y. Moret. 2009. Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: consequences for the risk of super-infection and links with host behavioural manipulation. International Journal for Parasitology 39, 221-229

Crosson, J., S. Paterson, and A. Fenton. 2007. Host availability and the evolution of parasite life-history strategies. Evolution 61, 275-284

DeWitt T.J., A. Sih, and D.S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution 13, 77-81

Franceschi, N., A. Baure, L. Bollache, and T. Rigaud. 2008. The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. International Journal for Parasitology. 38, 1161-1170

Franceschi, N., S. Cornet, L. Bollache, F-X. Dechaume-Moncharmont, A. Bauer, S. Montreuil, and T. Rigaud. 2010. Variation between population and local adaptation in acanthocephalan-induced parasite manipulation. Evolution 64, 2417-2430

Friberg, I.M., J.E. Bardley, and J.A. Jackson. 2010. Macroparasites, innate immunity and immunoregulation: developing natural models. Trends in Parasitology 26, 540-549

Helluy, S. 1983. Un mode de favorisation de la transmission parasitaire: la manipulation du comportement de l'hote intermediaire. Rev Ecol Terre Vie 38, 211-223.

Helluy, S., and J.C. Holmes. 1990. Serotonin, octopamine and the clinging behavior induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lucustris* (Crustacea). Canadian Journal of Zoology. 68, 1214-1220

Helluy, S., 2013. Parasite-induced alterations of sensorimotor pathways in gammarids: collateral damage of neuroinflammation? Journal of Experimental Biology. 216, 67-77

Hughes, D. 2013. Pathways to understanding the extended phenotype of parasites in their hosts. Journal of Experimental Biology. 216, 142-147

Klein, S., 2003. Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. Physiology and Behavior 79, 441-449.

Lefèvre T., B. Roche, R. Poulin, H. Hurd, F. Renaud, and F. Thomas. 2008. Exploiting host compensatory responses, the 'must' of manipulation? Trends in Parasitology 24:

Lefèvre, T., S.A. Adamo, D.G. Biron, D. Misse, D. Hughes, and F. Thomas. 2009. Invasion of the body snatchers: the diversity and evolution of manipulative strategies in host-parasite interactions. Advances in Parasitology 68, 45-83

Maure, F., J. Brodeur, N. Ponlet, J. Doyon, A. Firlej, E. Elguero, and F. Thomas. 2011. The cost of a bodyguard. Biology Letters 7, 834-846

May, R.M., and R.M. Anderson. Regulation and stability of host-parasite population interactions. II. Destabilzing processes. Journal of Animal Ecology 47, 249-267

Minchella, D.J. 1985. Host life-history variation in response to parasitism. Parasitology 90, 205-216

Moore, J. 2002. Parasites and the Behavior of Animals. New York, NY: Oxford University Press.

Moore, J. 2013. An overview of parasite-induced behavioral alterations – and some lessons from bats. Journal of Experimental Biology 216, 11-17

Moore, J., and N.J. Gotelli. 1990. A phylogenetic perspective on the evolution of altered host behaviours: a critical look at the manipulation hypothesis. *In* Barnard, CJ, Behnke JM (Eds), Parasitism and Host Behaviour. Taylor & Francis, London. pp. 193-233.

Mouritsen, K.N. 2002. The parasite-induced surfacing behaviour in the cockle *Austrovenus stutchburyi*: a test of an alternative hypothesis and identification of potential mechanisms. Parasitology 124, 521–528.

Mouritsen, K.N., and R. Poulin. 2003a. The risk of being at the top: foot- cropping in the New Zealand cockle *Austrovenus stutchburyi*. Journal of Marine Biological Associations UK 83, 497–498.

Mouritsen, K.N., and R. Poulin. 2003b. Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. International Journal for Parasitology. 33, 1043–1050.

Murren, C.J., J. Auld, H. Callahan, C. Ghalambor, C. Handelsman, M. Heskel, J. Kingsolver, H. Maclean, J. Masel, H. Maughan, D. Pfennig, R. Relyea, S. Seiter, E. Snell-Rood, U. Steiner, and C.D. Schlichting. Cost of plasticity or plasticity of cost concepts: revisiting the limits to phenotypic plasticity in the genomics era. In Prep.

van Noordwijk, A., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128, 137–142

Parker, G.A., M.A. Ball, J.C. Chubb, K. Hammerschmidt, and M. Milinski. 2009. When should a trophically transmitted parasite manipulate its host? Evolution 63, 448-458

Patterson, S., and R. Barber. 2007. Experimental evolution of parasite life-history traits in *Strongyloides ratti* (Nematoda). Proceedings of the Royal Society B. 274, 1467-1474

Perrot-Minnot M.J., and F. Cezilly. 2013. Investigating candidate neuromodulatory systems underlying parasitic manipulation: concepts, limitations and prospects. Journal of Experimental Biology 216: 134-141

Poulin, R. 1994a. The evolution of parasite manipulation of host behaviour: a theoretical analysis. Parasitology 109, S109-S118.

Poulin, R., B.L. Fredensborg, E. Hansen, and T.L.F. Leung. 2005. The true cost of host manipulation by parasites. Behavioral Processes 68, 241-244

Poulin, R. 2007. Evolutionary Ecology of Parasites. Princeton University Press, Princeton NJ.

Poulin, R. 2010. Parasite manipulation of host behavior: and update and frequently asked questions. Advances in the Study of Behavior 41, 151-186

Reznik, D., L. Nunny, and A. Tessier. 2000. Big houses, big cars, superfleas and the cost of reproduction. Trends in Ecology and Evolution 15 421-425

Seppälä, O., E.T. Valtonen, and D.P. Benesh. 2008. Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? Proceedings of the Royal Society B 275, 1611–1615

Silvertown, J., and M. Dodd (1999) The demographic cost of reproduction and its consequences in Balsam Fir (*Abies balsamea*). The American Naturalist 29: 321-331

Sparkes, T.C., V.M. Wright, D.T. Renwick, K.A. Weil, J.A. Talkington, and M. Milhalyov. 2004. Intra-specific host sharing in the manipulative parasite *Acanthocephalus dirus*: does conflict occur over host modification? Parasitology 129, 335-340

Stearns, S.C. 1989. Trade-offs in life-history evolution. Functional Ecology 3, 259-268.

Tain, L., M.J. Perrot-Minnot, and F. Cezilly. 2006. Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. Proceedings of the Royal Society B 273, 3039-3045

Tain, L., M.J. Perrot-Minnot, and F. Cezilly. 2007. Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric host species. Biology Letters 3, 68-71

van Tienderen, P.H. 1991. Evolution of Generalists and Specialists in spatially heterogeneous environments. Evolution 45, 1317-1331.

Thomas, F., F. Renaud, and R. Poulin. 1998a. Exploitation of manipulators: 'hitch-hiking' as a parasite transmission strategy. Animal Behaviour 56, 199-206

Thomas, F., M. Villa, I. Montoliu, F. Santalla, F. Cezilly, and T. Rigaud. 1998b. Analyses of a debilitating parasite (*Microphallus papillorobustus*, Trematoda) and its "hitchhiker" parasite (*Maritrema subdolum*, Trematoda) on survival of their intermediate host (*Gammarus insensibilis*, Amphipoda). Journal of the Helminthological Society of Washington 65, 1-5.

Thomas, F., P. Ulitsky, R. Augier, N. Dusticier, D. Samuel, C. Strambi, D.G. Biron, and M. Cayre. 2003. Biochemical and histological changes in the brain of the cricket *Nemobius sylvestris* infected by the manipulative parasite *Paragordius tricuspidatus* (Nematomorpha). International Journal for Parasitology 33, 435-443

Thomas, F., S. Adamo, and J. Moore. 2005. Parasitic manipulation: where are we and where should we go? Behavioral Processes 68, 185-199

Thomas, F., S.A. Brodeur, F. Maure, N. Franceschi, S. Blanchet, and T. Rigaud. 2011. Intraspecific variability in host manipulation by parasites. Infection, Genetics and Evolution 11, 262-269

Trumbo, S.T. 1999. Using integrative biology to explore constraints on evolution. Trends in Ecology and Evolution 14, 5-6

Wickler, W. 1976. Evolution-oriented ethology, kin selection, and altruistic parasites. Zeitschrift fur Tierpsychologie. 42, 206–214

Wilson, D.S. 1977. How nepotistic is the brain worm ? Behavioral Ecology and Sociobiology. 2, 421–425

Zuk, M., R. Thornhill, J.D. Ligon, and K. Johnson. 1990. Parasites and mate choice in red jungle fowl. American Zoologist 30, 235–244

## CHAPTER 3: What does it cost a parasite to manipulate host behaviour? An experimental test*


### Summary

Behavioral manipulation, which occurs when a parasite modifies its host's behavior to increase transmission, is believed to be costly for the parasite, despite lack of empirical demonstrations of such costs. We tested the cost hypothesis with the acanthocephalan parasite *Leptorhynchoides thecatus*, in which juvenile size is an indicator or fitness. Increased manipulation should be correlated with smaller larval size. First, we demonstrate that the parasite causes modification of three behaviors in the amphipod intermediate host *Hyalella azteca*: geotaxis, photophilia, and activity, but does not alter phototaxis. Second, none of these behaviors showed the negative relationship to parasite size predicted by theory. In fact, geotaxis modification is stronger in larger parasites. We suggest that if manipulation is costly, its cost is not evident in this host-parasite system.


### Introduction

Parasitic manipulation occurs when a parasite changes the behaviour of its host so that the parasite benefits from increased transmission rate or survival (Moore, 2002 and Thomas, Adamo, & Moore, 2005). Under the concept of extended phenotype, manipulation of host behaviour is considered a parasite trait, which, like any other trait, must increase fitness to persist (Dawkins, 1982; Combes, 2001). The benefits of manipulation are well documented. Host behaviour modification benefits a parasite's fitness by increasing the probability of successful

*Submitted for publication in the journal Animal Behaviour October 2013

transmission (Bethel & Holmes, 1977; see Moore 2002). Many parasites that rely upon predation for transmission are known to alter behaviours that increase interactions with suitable host predators (see Moore, 2002). Such altered behaviours include, but are not limited to, preferences for illuminated areas, increased activity, and elevation seeking (particularly in aquatic organisms) (Bethel & Holmes, 1973; Moore, 1983; Cezilly, Gregoire, & Bertin, 2000; review in Moore, 2002).

The costs of manipulation are not as well documented or as easily understood as the benefits. Poulin (1994) was the first to link the field of parasite manipulation of hosts with a major area of evolutionary theory, that of cost/benefit trade-offs. He argued that manipulation is costly because energy used on manipulation was not available for growth or reproduction (see also Parker, Ball, Chubb, Hammerschmidt, & Milinski, 2009; Vickery & Poulin, 2010). There have been no empirical tests for the energetic cost-of-manipulation hypothesis, despite its persistence in the literature (Thomas, Renaud, & Poulin, 1998; Vickery & Poulin, 2010). We believe that the costs of behavioural modification may be understood by providing an empirical demonstration of energetic costs and tests of these hypotheses.

The acanthocephalan parasite *Leptorhynchoides thecatus* (Kostylev) is a particularly good candidate for examining fitness cost of manipulation. First, *L. thecatus* has a fairly simple life cycle: it uses a single crustacean species, *Hyalella azteca* (Amphipoda), as an intermediate host and is transmitted to its fish final host when the crustacean is consumed. No altered host behaviours have been previously documented in this host-parasite system but we predict they exist because altered behaviours are seen almost all acanthocephala-amphipod systems (Moore, 2002). Second, acanthocephalan behaviour modification may be relatively costly, because the parasite grows in the amphipod's hemocoel and must remotely affect the amphipod's behaviour

by biochemical secretions. Third, we can quantify one major aspect of fitness in the *L. thecatus* larval stage known as the cystacanth: Steinauer & Nickol (2003) showed that while cystacanth size did not influence an individual's adult fecundity, larger cystacanths survived the transition to adulthood in the intestine of the fish at significantly higher rates than smaller cystacanths – a necessary prerequisite for reproduction. Thus, if behavioural modification is costly for *L. thecatus*, we expect it to be linked to cystacanth size such that cystacanths that cause stronger behavioural changes suffer a greater reduction in size.

This study had two goals: 1) To determine whether parasite-induced behavioural changes occur in the *L. thecatus – H. azteca* system, and 2) to investigate the connection between the strength of behavioural change and the size of the cystacanth causing that change. A negative relationship between strength of manipulation and cystacanth size would support the hypothesis that manipulation is costly, while the absence of a negative relationship would suggest that manipulation is not so costly so as to decrease an important fitness trait, that of establishment (survival).

## Methods

### *General Methods*

### *Collection of Organisms*

We limited our study to parasites from Atkinson Reservoir in Atkinson, Nebraska, a population studied for over 30 years and a local race of the parasite (Ashley & Nickol, 1989).

Green Sunfish (*Lepomis cyanellus* Rafinesque), Bluegill (*Lepomis macrochirus* Rafinesque), and Pumpkinseed Sunfish (*Lepomis gibbosis* Linnaeus) were collected from Atkinson Reservoir, Holt County, Nebraska (42°32'36"N X 98°58'22"W). On the day of capture, fish were dissected and inspected for intestinal worms. All *L. thecatus* were removed from the caeca of the fishes, placed in tap water, and stored at 4°C. Collection was carried out under C.S.U. Animal Care and Use protocol 11-2590A. Amphipods (*Hyalella azteca*) were also collected from aquatic vegetation at the perimeter of the reservoir and transported to the laboratory for culturing.

*Infection and Culture of Amphipods*

Eggs were harvested from gravid female worms dissected in several millilitres of tap water. The resulting egg suspension was standardized so that an average of 1.5 fully-embryonated eggs was present per field of view in 0.05 ml of suspension at 100X (Barger and Nichol, 1998). Fifty (50) *H. azteca* were placed in wide-mouth glass quart jars containing 800ml culture water, 50g gravel, and four grams of filamentous green algae (*Pithophora* sp.). In order to expose amphipods to the parasite, one (1) ml of the egg suspension was pipetted over the algae. Amphipods were allowed to forage for 24-72 hrs and were then transferred to 4.5L Rubbermaid® plastic containers, where they were cultured for 32-40 days at 23°C.

DI water used in experiments and culturing was modified according to the Moderately Hard Water formula (see U.S. EPA, 1994). Amphipods were cultured in 27L and 30L Sterilite® plastic storage containers with 1-2 cm of sand substrate. Cultures were given Tetramin® tropical fish food three times per week and water was changed weekly. All animals were kept in a climate-controlled room a 23°C, on a 15:9 light dark (LD) cycle.

*Behavioural Experiments*

*General Methods*

Six behavioural experiments were designed to determine whether infection changes an amphipod's geotactic preference, its phototactic and photophilic responses, or its activity level. Experiments were conducted in three apparatuses: one for geotaxis, one for phototaxis and photophilia, and a third for activity. This meant that there were three groups of experiments (see Table 1) for each amphipod; within an apparatus, experiment order for each apparatus was similar for all subjects (e.g. 1A, 1B, 1C) but the order of apparatus use (and experiment groups) was randomized to prevent potential order bias. All of the experiments for a given amphipod were conducted back-to-back in a single three-hour period. To increase the number of subjects, tests were conducted with two amphipods in an apparatus at the same time. We chose amphipods easily identified by visibly different size so that both subjects could be reliably identified if they entered darkened areas. Amphipods were removed from culture tanks in hours 3-6 of their photophase and introduced to the experimental apparatus in total darkness; they were allowed 20 minutes to acclimate in complete darkness to the new environment before observations began. A similar acclimation of 20 minutes in darkness was repeated after each transfer to a new apparatus. All light sources were full-spectrum fluorescent bulbs (5500 20W – naturallighting.com). All experiments were conducted in the same climate controlled room, and a test of water temperature found that no detectable difference in temperatures among different areas of water within the apparatuses.

After experiments described below were completed, amphipods were euthanized in 95% ethanol. We measured each amphipod's size from rostrum tip to urostyle by straightening it on a ruler under a dissecting microscope. Each amphipod was dissected under a dissecting microscope to determine the infection status and intensity. Unlike some acanthocephalans, *L. thecatus* cystacanths are not visible in the intact amphipod, so one must dissect the amphipod to reliably determine if it is infected. Cystacanths were placed in aged tap water on a depression slide for two minutes, covered with a cover glass, and their sex determined under a compound microscope. Length and width of the cystacanth were measured using an ocular micrometer on a compound microscope. If the amphipod harboured an acanthella, the infection status was recorded, the worm's length and width measured, and the acanthella preserved. Behaviour of amphipods harbouring acanthellae was analyzed separately from that of amphipods containing cystacanths.

*1. Geotaxis in infected and uninfected amphipods*

*Experimental Apparatus*

A 1L Pyrex ® graduated cylinder (6cm in diameter and 44cm in depth) was filled with one litre of culture water to a depth of 36cm. This apparatus is similar to that used by Cezilly et al. (2000), with which these authors determine differences in "Vertical Distribution;" we are calling this experiment "Geotaxis." The 100ml marks on the side of the cylinder were 3.6cm apart, defining a total of ten contiguous spaces that amphipods could occupy. An upright white plastic 18.9L bucket was suspended one meter above the graduated cylinder. The bottom of the

bucket was lined with 3 layers of black plastic. A light bulb was lowered into the bucket and suspended 0.15 meters above the bottom of the bucket and black plastic barrier. This physical barrier ensured that amphipods did not experience direct light; it provided a diffuse light in the room similar to an overcast day. A second light source was placed 25cm below the base of the graduated cylinder, without any obstruction between the light and the cylinder. Before the experiment started, two *H. azteca* to be tested were placed into the cylinder just below the water surface and allowed 20 minutes to acclimate in darkness.

*Experiment 1A: Geotaxis: Is there a difference in vertical position between infected and uninfected amphipods?*

Observations began when the diffuse light above the apparatus was switched on; the position of each amphipod was recorded at 30-second intervals for 15 minutes, for a total of 30 observations (see Cezilly et al., 2000). For any single observation, an amphipod's score ranged from 1 (bottom 100ml) to 10 (top 100ml). Thus, the total score for an individual amphipod over the 15-minute period was a sum of the scores over 15 minutes and ranged from 30 to 300.

*Experiment 1B: Geotaxis/Phototaxis: Is vertical position influenced by direct light? Is this response different for infected amphipods?*

This experiment is based on that of Bethel & Holmes (1973). Immediately following experiment 1A the physical barriers around the light source (white plastic bucket and opaque plastic) were removed so that direct light could shine on the graduated cylinder from above.

Simultaneously, the light source above the apparatus was switched off and the light bulb below the apparatus turned on (see Bethel & Holmes, 1973); the amphipods' responses over the following 30 seconds were recorded. The light source was reversed again and the response of each amphipod over the following 30 seconds recorded. Light sources were switched such that only one light was on at any one time and amphipods experienced light from above and then below 3 times each – a total of 6 30-second observations.

*2. Responses to light in infected and uninfected amphipods*

In this experiment, we follow Bethel & Holmes (1973) in distinguishing between phototaxis, defined here as a directional movement toward a light source, and photophilia, a preference for lighted environments. Phototaxis was tested by exposing the amphipod to a new source of direct light, while photophilia was measured by observing the amphipod over a period of time during which they were given the choice between a dark and a lighted area.

*Experimental Apparatus*

We covered the top, sides, and bottom of one half an unframed glass aquarium (44cm x 20cm x 22cm) with opaque black plastic. The aquarium was filled to 18cm depth with fresh culture water. An air-stone was mounted 8cm below the surface at the light-dark interface to aerate the water. Flow from this air-stone did not noticeably disturb the amphipods or create strong currents in the tank. A light bulb was placed 25cm above the water surface and another was placed 25cm below the bottom of the aquarium. Both light sources were directly over or

under the centre of the tank so as to simulate full sunlight in the uncovered half of the tank. Following Bethel & Holmes (1973), three zones were delineated on the tank: the upper 2cm (1/9) of the light zone (ULZ), the remainder (8/9) of the light zone (RLZ), and the dark zone (DZ). For some analyses, ULZ and RLZ were combined for one score: the combined light zone (CLZ). Amphipods were transferred by pipette, placed just below the water surface in the centre of the tank, and allowed 20 minutes to acclimate in darkness.

*Experiment 2A: Phototaxis: Is there a difference in phototaxis between infected and uninfected amphipods?*

After acclimation, the light source above the tank was switched on, and 30 seconds later, the location of each amphipod in the DZ, ULZ, or RLZ was determined. We classified the initial reactions in three categories: the amphipod 1) was stationary within the same zone, 2) swam in the same zone (photokinesis), or 3) changed zones.

*Experiment 2B: Photophilia: Is there a difference in photophilia between infected and uninfected amphipods?*

Immediately following experiment 2A, the position of each amphipod was recorded at 30-second intervals for 15 minutes, for a total of 30 observations (after Cezilly et al., 2000). At each 30-second interval, we recorded whether each amphipod was in the ULZ, the RLZ, or not visible (assumed to be in the DZ). If an amphipod was stationary immediately after experiment

2A, it was disturbed with a glass rod by lightly touching it until it moved from its initial location; observation began when it was clear that the amphipod was active and responsive.

*Experiment 2C: Is there a difference in phototaxis between infected and uninfected amphipods that are physically disturbed?*

In some parasite-amphipod associations, altered phototaxis in infected amphipods is less prevalent when the amphipods are physically disturbed (Bethel & Holmes, 1973, 1977). To test this with *L. thecatus*, immediately after experiment 2B the two amphipods were removed from the tank and one was randomly chosen. This animal was placed by pipette into the bottom of the tank in middle of the light zone (CLZ) with the same overhead light source as 2A and 2B, and disturbed by a light touch with a glass rod until it moved (Bethel & Holmes, 1973). The lower light source was then turned on and the upper one extinguished. We recorded the animal's behaviour over the following 15 seconds: it could swim toward or away from the light, or remain stationary. The light source was then switched from bottom to top, the amphipod disturbed again with the rod, and its reaction recorded. Light sources were alternated such that each was used three times, for six total observations, or until the amphipod swam into the dark zone (DZ). After the first amphipod of the pair was tested in this way it was removed, the other placed in the apparatus, and the experiment performed using the second amphipod.

*3. Activity levels of infected and uninfected amphipods*

*Experimental Apparatus*

A Pyrex® 1L beaker with a diameter of 10cm was filled with one litre of culture water. A line was drawn across the underside of the beaker to separate it into two equal sections. One indirect light source was provided above the apparatus as described in experiment 1 (above).

*Experiment 3: Is there a difference in activity levels between infected and uninfected amphipods?*

After acclimation, the light was turned on and the number of times each amphipod crossed the centreline during a 15-minute period was recorded. This count served as the activity score for the amphipod.

*Statistical Analysis*

Statistical analyses were conducted using Stata/IC Version 11.0 (StataCorp LP, College Station, Texas). Graphical representations of the data were created using the R statistical program.

*Behavioural Alterations*

*All Behavioural Experiments: 1A, 1B, 2A, 2B, 2C, 3*

First, to eliminate the effects of a possible bias in experiment order, for results from each experiment, a two-way ANOVA with Tukey adjustment was conducted including factors of infection status and experiment order. A similar test was done to see if the larger amphipod in each pair of subjects scored differently than the smaller amphipod. In every case no significant effect was found for apparatus order, relative size, or interaction terms. These variables were ignored for further analyses and each experiment used more simple and precise tests to investigate differences in behaviour between infected and uninfected amphipods.

*Experiment 1A: Geotaxis*

Scores from this experiment ranged from 30-300, so data were log transformed to adjust for this scoring system. Data for this test had a very strong skew toward lower scores. Log transformation helped reduce skew in the data, but persisted and we used a non-parametric two-sample rank sum Wilcoxon (Mann-Whitney U) test to determine differences between infected and uninfected amphipods.

*Experiment 1B: Phototaxis*

This experiment produced simple proportion of amphipods that were negatively phototactic (predicted behaviour) and positively phototactic. Differences between infected and uninfected groups were determined using a Chi-squared test.

*Experiment 2A: Phototaxis*

Chi-squared tests were used to determine differences in proportions of infected and uninfected amphipods that moved between zones or stayed within the zone in which they were initially observed.

*Experiment 2B: Photophilia*

Data from this experiment proved to be highly skewed toward large numbers of observations in the dark zone (DZ) of the apparatus for both infected and uninfected amphipods. For this reason, a non-parametric two-sample rank sum Wilcoxon (Mann-Whitney U) test was used to determine differences in behaviour between infected and uninfected amphipods.

*Experiment 2C: Phototaxis and disturbance*

A chi-squared test was conducted using the proportion of infected amphipods that responded with negative phototaxis to the proportion of uninfected amphipods that did likewise.

*Experiment 3: Activity*

This experiment produced count data met the assumptions of normality for parametric tests. A one-way ANOVA was used to detect differences in activity between infected and uninfected amphipods.

*Correlation Analyses between Behavioural results and Cystacanth Volume*

To ask if larval size was related to the strength of behavioural change, we used correlation analyses on experimental results of geotaxis (1A), photophilia (2B), and activity (3). The other three tests, all of phototaxis (1B, 2A, 2C), did not indicate significant altered behaviour due to parasitism and therefore could not be used to determine whether behavioural change was costly, i.e. whether there was a relationship between behavioural modification and cystacanth size. Cystacanth volume was log transformed for analyses (see also Steinauer & Nickol 2003). The models incorporated other attributes that influence cystacanth volume: amphipod sex, amphipod length, and cystacanth sex (Steinauer & Nickol, 2003). All models were tested using a one-sided test against the a priori prediction of a negative relationship between cystacanth volume and strength of behavioural change. For experiment 1A (Geotaxis) we used the non-parametric Spearman's R, while experiment 2B and 3 used parametric assessments of the relationship.

## Results

### *Behavioural Alteration*

We found significant differences in behavioural scores between infected and uninfected amphipods. Geotaxis (1A), photophilia (2B), and activity levels (3) are all modified when *H. azteca* is infected with *L. thecatus*. However, there were no effects of infection on phototaxis (experiments 1B, 2A, 2C).

### *Experiment 1A: Geotaxis: Is there a difference in vertical position between infected and uninfected amphipods?*

Geotaxis scores differed significantly between uninfected and infected amphipods. For this test, the subject animal had a minimum score of 30 (1 for each of the 30 observations). The majority of uninfected amphipods spent their time on or near the bottom (Figure 1). These amphipods had an average geotaxis score of 38 ± 5.4 ($N = 58$); that is, their average was 8 above the minimum of 30 observations. Infected amphipods averaged a score of 57 ± 8.9 ($N = 30$), or 27 above the minimum of 30 observations. Thus, infected amphipod scores were approximately 3½ times those of uninfected amphipods. The difference in means of infected and uninfected amphipods was statistically significant ($U = 556$, $N_1=30$, $N_2= 58$, $P = 0.001$).

*Experiment 1B: Phototaxis/Geotaxis: Is vertical position influenced by direct light? Is this response different for infected amphipods?*

The majority of amphipods responded to direct light sources by swimming away from them but a small number of animals did not respond to light source changes or were very slow in responding (three of 30 infected; four of 59 uninfected). There was no statistical difference between the proportions of uninfected and infected amphipods that failed to swim away from the light source ($X^2_2 = 0.36$, $P = 0.55$).

*Experiment 2A. Phototaxis: Is there a difference in phototaxis to a direct light source between infected and uninfected amphipods?*

There was no difference in phototaxis between infected and uninfected amphipods during the first 15 seconds after the overhead light source was illuminated. Amphipods started in the LZ of the aquarium in 46% of trials and these amphipods ($N = 41$) were significantly more likely to change zones ($X^2_2 = 4.04$, $P = 0.04$) and significantly less likely to remain in the same zone ($X^2_2 = 4.45$, $P = 0.03$) than those amphipods starting the dark zone ($N = 48$). There was no difference between infected and uninfected amphipods ($X^2_2 = 0.08$, $P = 0.77$).

*Experiment 2B. Photophilia: Is there a difference in photophilia between infected and uninfected amphipods?*

We used the combined light zone (CLZ) data for number of times an amphipod was observed in the light during the 15-minute period, because divisions within the light zone were intended to detect altered phototaxis; the results of experiments 1B, 2A, and 2C failed to demonstrate any altered phototaxis. There was a significant difference in the number of observations in the light zone between uninfected (4.2 ± 1.0) and infected (8.6 ± 7.6) amphipods ($U = 459$, $N_1=30$, $N_2= 58$, $P < 0.001$). Although all amphipods were photophobic, infected ones were much less so, and were nearly twice as likely to be observed in the CLZ (Table 2; Figure 2).

*Experiment 2C: Is there a difference in phototaxis between infected and uninfected amphipods that are physical disturbed?*

Disturbance with a glass rod did not alter phototaxis in either infected or uninfected amphipods. There was no significant difference between the proportions of infected (3 of 53) and uninfected amphipods (1 of 23) that failed to swim away from the light source ($X^2_2 = 0.08$, $P = 0.74$).

*Experiment 3: Is there a difference in activity levels between infected and uninfected amphipods?*

There was a significant difference in the activity scores of infected (112 ± 20) and uninfected (80 ± 17) amphipods, $t$ = -2.2405, $P$ = 0.028 (Table 2; Figure 3). On average, infected amphipods were 40% more active than uninfected amphipods in this test.

*Behavioural scores for acanthella-infected amphipods*

Behaviours of six amphipods infected by acanthella were compared to those of the uninfected amphipods (n=50). There was no difference in geotaxis scores (experiment 1A: acanthella-infected amphipods (38±5), uninfected amphipods (42±7), $P$ = 0.7), photophilia scores (experiment 2B: 4±1 and 8±7 respectively; $P$ = 0.27), and activity scores (experiment 3; 80±17 and 98±21; $P$ = 0.26) of acanthella and uninfected amphipods. All six acanthella-infected amphipods showed negative phototaxis in experiments 1B, 2A, and 2C. These measures were not significantly different from those of uninfected amphipods. The number of amphipods infected with acanthellae did not allow for reliable analysis of parasite size.

*Cystacanth Size and Behavioural Alteration*

*Correlation Analyses*

The relationship between cystacanth size and altered behaviour is key in demonstrating cost of altering host behaviour in this study. A negative relationship supports the hypothesis that

a cost is incurred for altering behaviours. We analyzed the results of the behavioural experiments that demonstrated parasite induced behavioural alteration: geotaxis (1A), and photophilia (2B), activity (3). Regression analyses used log-transformed cystacanth volume. Factors correlated with cystacanth size (see Methods) were included in the models to account for other influences on our primary measure of fitness.

*Geotaxis (1A): Do geotaxis scores of infected amphipods correlate with cystacanth volume?*

There is a positive relationship between geotaxis scores and cystacanth size; that is, larger cystacanths are found in more negatively geotactic amphipods (Figure 4). Geotaxis and cystacanth size were significantly correlated ($r_s = 0.68$, $N = 18$, $P = 0.002$).

*Photophilia (2B): Do photophilia scores of infected amphipods correlate with cystacanth volume?*

There is also a positive correlation between cystacanth size and the number of times an infected amphipod was observed in the light zone (Figure 5). This relationship is significant for our one-tailed analysis ($r = 0.46$, $P = 0.043$) and is not negative as predicted a priori.

*Activity (3): Do activity scores of infected amphipods correlate with cystacanth volume?*

There is no clear relationship between activity and cystacanth size (Figure 6). The full statistical model indicates that activity is not a significant ($r$ = -0.16, $P$ = 0.56) and does not support the predicted negative correlation.

**Discussion**

*Leptorhynchoides thecatus* can be added to the list of acanthocephalans that modify amphipod behaviours (Bethel & Holmes, 1973; Bakker, Mazzi, & Zala, 1997; Maynard, Wellnitz, Zanini, Wright, & Dezfuli, 1998; Cezilly et al., 2000; Bauer, Trouve, Gregoire, Bollache, & Cezilly, 2000; Dezfuli, Giari, & Poulin, 2001; Bauer, Haine, Perrot-Minnot, & Rigaud, 2005) and this parasite does so with no detectable impact on its future survival, as indicated by cystacanth volume. First, this study addressed whether infected amphipods exhibit altered behaviour. Experimentally infected *Hyalella azteca* spent more time higher in the water column, more time in lighted areas, and were more active than uninfected amphipods. However, both infected and uninfected amphipods were negatively phototactic to a direct light source. Our results also suggest that amphipods harbouring the acanthella stage, which cannot infect a fish, behave similarly to uninfected amphipods. The number of acanthellae-infected amphipods in this study is small, so we cannot definitively rule out the possibility that they alter amphipod behaviour, but our results are consistent with the prediction that in manipulative parasite's onset of behavioural changes and parasite infectivity to the definitive host should be simultaneous (Bethel & Holmes, 1973; Dianne et al., 2011). These altered behaviours, along with the normal

behaviour in acanthella-infected amphipods, support the hypothesis that this parasite is manipulation.

The second major question addressed in this study is the cost of these behavioural changes for *Leptorhynchoides thecatus*. In this study, cost can be viewed in two ways. Both can be linked to size: Larger cystacanths show increased survival and larger cystacanths require greater energy to reach their size than smaller cystacanths. Following the assumptions of previous theoretical analyses, which postulate an energetic cost for acanthocephalan manipulations, we predicted a negative relationship between cystacanth size and behavioural changes. Our results provide no evidence to support this prediction. If anything, altered behaviour is related to increased, not decreased, fitness – as reflected in survival. If there is a cost for the parasite, we did not detect it with this straightforward indicator of parasite survival. Of course there are countless ways to measure fitness (e.g. mating success, egg production, etc.), which were not addressed here, but before any of these can be measured the acanthocephalan must establish in the definitive host.

Poulin (1994) proposed that, in the absence of other factors, a negative relationship should exist between increased manipulation and other fitness components such as growth or reproductive output. In other words, he suggested that manipulation is subject to a physiological trade-off based on an allocation of energy to competing life history traits (Stearns 1992). Our results run counter to this logic: cystacanths that grow larger have higher survival rates and are associated with greater behavioural alterations than smaller conspecifics. Larger cystacanths are associated with greater negative geotaxis; the opposite would be true if altering geotaxis was costly in terms of size and/or survival. In addition, neither photophilia nor activity is higher for smaller cystacanths. In sum, there is no evidence that behaviours altered by *L. thecatus* cost the

parasite in terms of survival, as indicated by size. Not only do we conclude that the cost of altering behaviour does not match that predicted by theory, but that the opposite is true: larger cystacanths tend to alter behaviour more than smaller ones do. Larger cystacanths are associated with increasing negative geotaxis and they tend to be associated with increasing photophilia. Larger cystacanths are demonstrably not associated with decreased photophilia or decreased activity.

An organism's fitness is a consequence of its survival and reproduction, and we have shown that the extent to which a cystacanth alters behaviour does not affect its adult survival, a parameter that is predicted by cystacanth size. In this parasite, reproduction – the second component of fitness – occurs in the adult stage. This takes place in the fish, and it is unclear how increased ability to alter behaviour by the cystacanth – a different stage in a different host – could have negative consequences for adult fecundity or egg quality, especially as cystacanth size itself is not related to adult fecundity (Steinauer & Nickol 2003). Although *L. thecatus* probably uses chemical intermediaries in order to alter its host's behaviour (Helluy 2013), these have not been identified, and it is not clear that they are expensive to produce. Even if they are, our results suggest that the benefit from altering behaviour exceeds that of cost.

Cystacanth size not only predicts adult survival, it also can be seen as an indicator of energy sequestration on the part of the cystacanth. Even if we ignore the linkage between cystacanth size and adult survival, and the fact that behavioural modification does not reduce either, we are left with the observation that manipulation does not require an energy expenditure large enough to affect size itself. In the case of geotaxis, it is quite the opposite.

At this point, we can consider other possible fitness costs associated with manipulation, such as decreased competitive ability for the parasite causing behavioural change (Vickery &

Poulin 2010). For *L. thecatus*, this particular measure is unlikely because cases of amphipods with multiple cystacanths are exceedingly rare in nature (Ashley & Nickol 1989). It is also possible that manipulative parasites experience increased mortality (Poulin, Fredensborg, Hansen, & Leung, 2005). There are intriguing indications that some parasites may even use the host immune system to alter behaviour (Helluy 2013).

The mechanisms of behavioural modification can greatly influence the cost (Thomas et al., 2005). For instance, altered behaviours may result from active parasite effort (e.g. a secretion) or mechanical influence (e.g. nervous system interference). Unfortunately, we have few study systems that demonstrate proximate mechanisms of host behaviour modification (Helluy & Holmes 1990; Helluy 2013).

In short, there are countless ways to measure fitness, and because of this, we do not claim to have shown that manipulation is cost-free for *L. thecatus*. Instead, we have noted that despite nearly two decades of theory based on the idea that manipulation must be costly, such costs have yet to be empirically identified. We have attempted to identify a cost of manipulation using an acanthocephalan in which greater cystacanth size confers greater survival in the final host. Based on the cost hypothesis, we expected to see more manipulative cystacanths pay a price in terms of size. Instead, we found the opposite: if anything, larger cystacanths cause greater behavioural changes. Whatever the cost of altering behaviour, it is invisible to our system and the result for the parasite seems to be a net benefit.

The benefit of altered behaviour to parasite fitness is increased transmission. We have not demonstrated transmission directly, because infected amphipods can only be identified upon dissection. However, we do have strong evidence that behaviours such as those we investigated increase transmission in other amphipod systems (Bethel & Holmes, 1977; Cezilly et al., 2000).

In addition, modified behaviours, such as activity and refuge use in the presence of predator odours, can be reliable indicators of manipulation (Baldauf et al., 2007; Perrot-Minnot, Kaldonski, & Cezilly, 2007; Benesh, Kitchen, Pulkkinen, Hakala, & Valtonen, 2008). Preliminary results from odour tests suggest that *L. thecatus* infections reduce amphipod anti-predatory behaviours (Study authors, in prep).

What should we make of normal phototactic behaviour in *H. azteca* infected by *L. thecatus*, when other acanthocephalans seem to alter this behaviour? In order to understand this, we need to consider the difference between phototaxis and photophilia. Bethel & Holmes (1973) usefully distinguished between phototaxis and photophilia, with phototaxis seen as directed movement in relation to a light source and photophilia seen as a preference or aversion for lighted areas. This distinction is important because the same parasite-host association (e.g. *Polymorphus marilis* in Bethel & Holmes, 1977) yielded increased photophilia while having no effect on phototaxis. Given this definition, most subsequent studies on acanthocephalans and amphipods have shown increases in photophilia, but not tested phototaxis sensu Bethel & Holmes (1977) (Table 3). Thus, the results of most studies of acanthocephalans and amphipods are consistent with ours in that they shot increased photophilia as defined by Bethel & Holmes (1977) but have not examined phototaxis in that context.

This has significant ecological relevance to manipulation. Bethel & Holmes (1977) suggested that the distinction between phototaxis and photophilia is important because surface feeders are likely to encounter and positively phototactic animals, whereas for sub-surface feeders, increased photophilia may be sufficient to cause increased predation. Surface-feeding mallards consumed amphipods harbouring phototaxis-altering parasites but ate no amphipods harbouring parasites that did not alter phototaxis (Bethel & Holmes, 1977). For acanthocephalans

exploiting fish, such as *L. thecatus*, strong reversal of normal negative phototaxis may reduce transmission, because it would increase encounters with non-host surface-feeding predators. Fish parasites appear to rely on other altered behaviours, such as increased activity and photophilia, to increase predation rates. A review of amphipod-acanthocephalan study systems is presented in Table 3. It demonstrates that our findings are consistent the patterns of light preferences and reactions in these other systems, and suggests that lack of altered phototaxis in intermediate hosts represents a wider trend among parasites using fish definitive hosts.

In conclusion, this study demonstrates that *L. thecatus* infections cause altered behaviour in their intermediate host and provides an important test of the cost hypothesis associated with studies of manipulation and parasite-induced behavioural change. This study of *L. thecatus* suggests that these costs of behavioural modification are not large enough to be detected in the fitness indicator of cystacanth size. While we could not explore every possible indicator of parasite fitness, our results suggest that costs related to establishment and survival are not substantial and that the benefits of behavioural modification outweigh the costs. We recognize that this is the first test of the cost hypothesis and look forward to future studies that will explore costs and how they are manifested in the balance with the benefits that manipulation brings. Exploration of cost and benefits of a trait is a key component of understanding how that trait has evolved and is maintained.

Table 3.1. Experimental apparatus and the behaviours observed in each. Columns indicate the apparatus used in experimental tests, and the associated behaviours for the subject *H. azteca* observed in each. "Geotaxis" is reaction to gravity, or vertical positioning, "phototaxis" is a directed reaction to a single light source, "photophilia" is the preference for light areas over a period of time, and "activity" is a measure of horizontal movement.

| | **Geotaxis** | **Phototaxis/Photophlia** | **Activity** |
|---|---|---|---|
| Apparatus | 1L Graduated cylinder with 10 vertical zones | 20L Unframed glass aquarium 50% covered with opaque plastic | 1L Beaker with line to indicate two equal halves |
| Behaviours Tested | **1A.** Geotaxis | **2A.** Phototaxis | **3.** Activity level |
| | **1B.** Geotaxis & phototaxis | **2B.** Photophilia | |
| | | **2C.** Phototaxis & disturbance | |

Table 3.2. Mean scores and analysis results of effects of infection for each experiment. Summary of average scores (± S. D.) of uninfected amphipods (*H. azteca*) and amphipods infected with *L. thecatus* cystacanths. *P*-values indicate the results of the statistical test for each experiment: geotaxis (1A) and photophilia (2B) used ranked-sum Wilcoxon tests, while a one-way ANOVA was used for activity (experiment 3).

| Experiment | 1A. Geotaxis | 2B. Photophilia | 3. Activity |
|---|---|---|---|
| Uninfected Mean | 38 ± 5.4 | 4.2 ± 1.0 | 80 ± 17 |
| Infected Mean | 57 ± 8.9 | 8.6 ± 7.6 | 112 ± 21 |
| P-value | 0.001 | <0.001 | 0.023 |

Table 3.3. Summary of studies on light responses altered by acanthocephalans using amphipods and infecting mammals, birds, and fish. Photophilia is defined as a preference/avoidance for lighted areas measured by time spent in such areas and phototaxis as the positive or negative movement in reaction to a direct light stimulus. This distinction is based on Bethel & Holmes (1973, 1977).

| **Acanthocephalan species** | **Definitive Host** | **Behaviour Altered*** | **Nature of change** | **Author(s)** |
|---|---|---|---|---|
| *P. minutus* | Dabbling ducks | Photophilia | Increased | Hindsbo, 1972 |
| *P. paradoxus* | Muskrats | Phototaxis | Positive | Bethel & Holmes, 1973 |
| *C. constrictum* | Dabbling and Diving ducks | Phototaxis | Positive | Bethel & Holmes, 1977 |
| *P. marilis* | Diving Ducks | Photophilia | Increased | Bethel & Holmes, 1977 |
| | | Phototaxis | None | Bethel & Holmes, 1977 |
| *P. laevis* | Fish | Photophilia | Increased | Maynard et al., 1998 |
| *P. laevis* | Fish | Photophilia | Increased | Cezilly et al., 2000 |
| *P. minutus* | Bird | Photophilia | Increased | Cezilly et al., 2000 |
| *P. laevis* | Fish | Photophilia | Increased | Franceschi et al., 2007 |
| *P. tereticolis* | Fish | Photophilia | Increased | Tain et al., 2006 |
| *A. galaxii* | Fish | Photophilia | Increased | Rauque et al., 2011 |

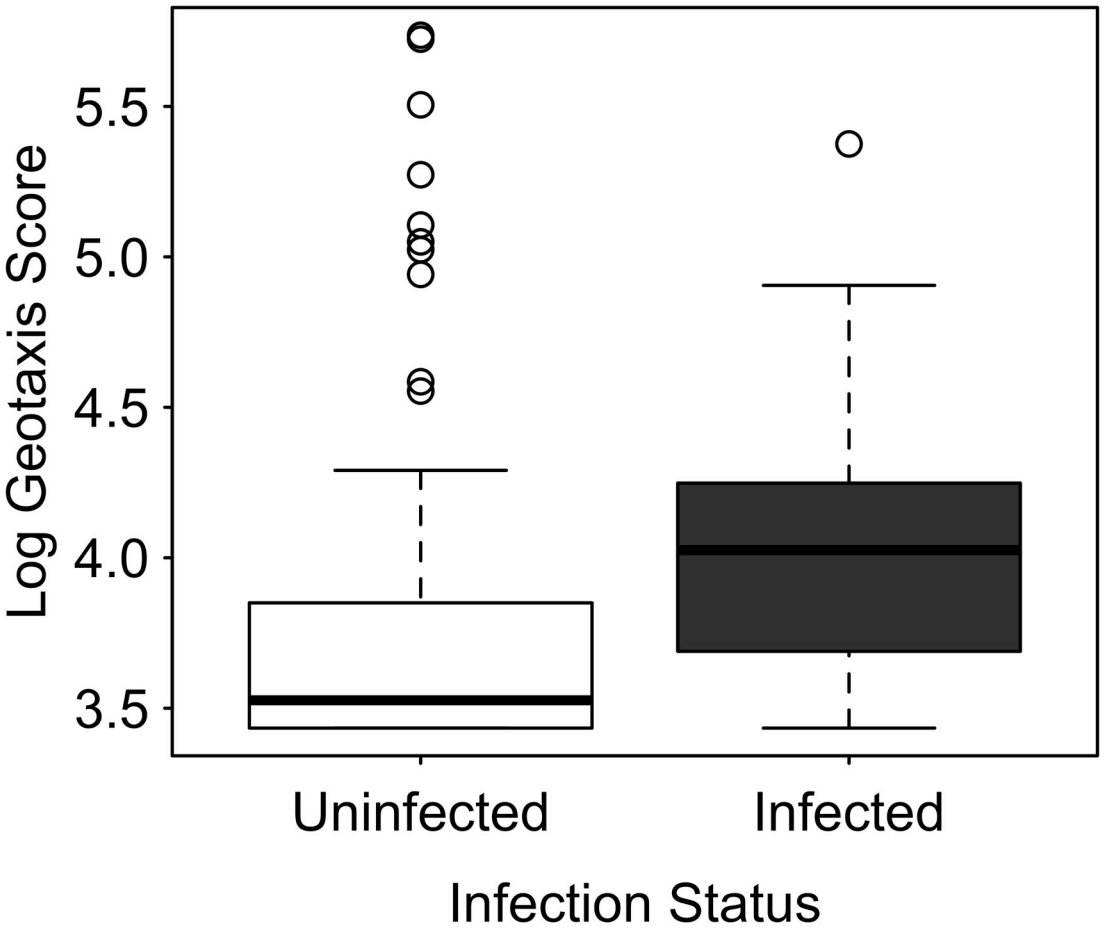


Figure 3.1. Median geotaxis score by infection status. Geotaxis scores represent the total vertical scores of an amphipod (*H. azteca*) over a 15-minute period. Scores were log transformed because of non-normality. These groups are significantly different ($P = 0.001$).

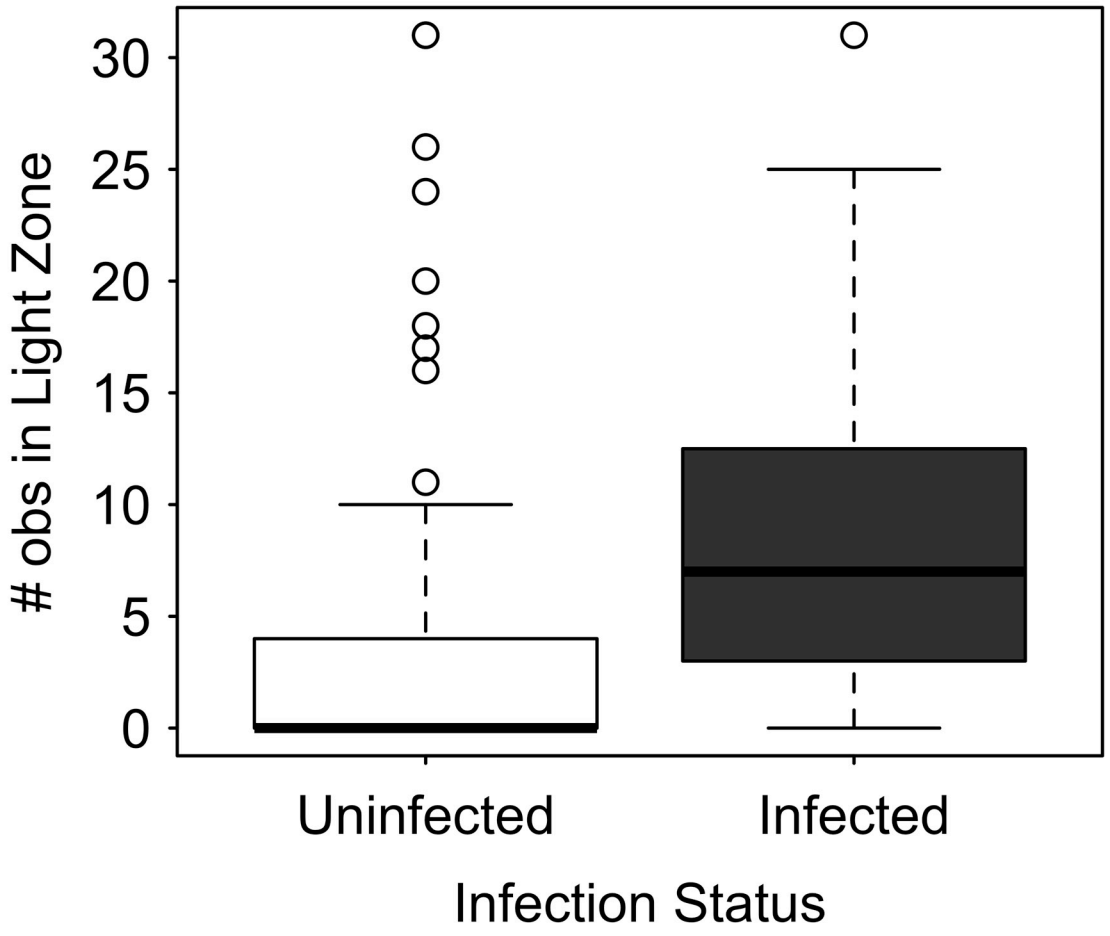


Figure 3.2. Median photophilia score by infection status. Photophilia scores represent the total number of times an amphipod (*H. azteca*) was observed in the light zone (LZ) of the experimental apparatus over a 15-minute period. These groups are significantly different ($P < 0.001$).

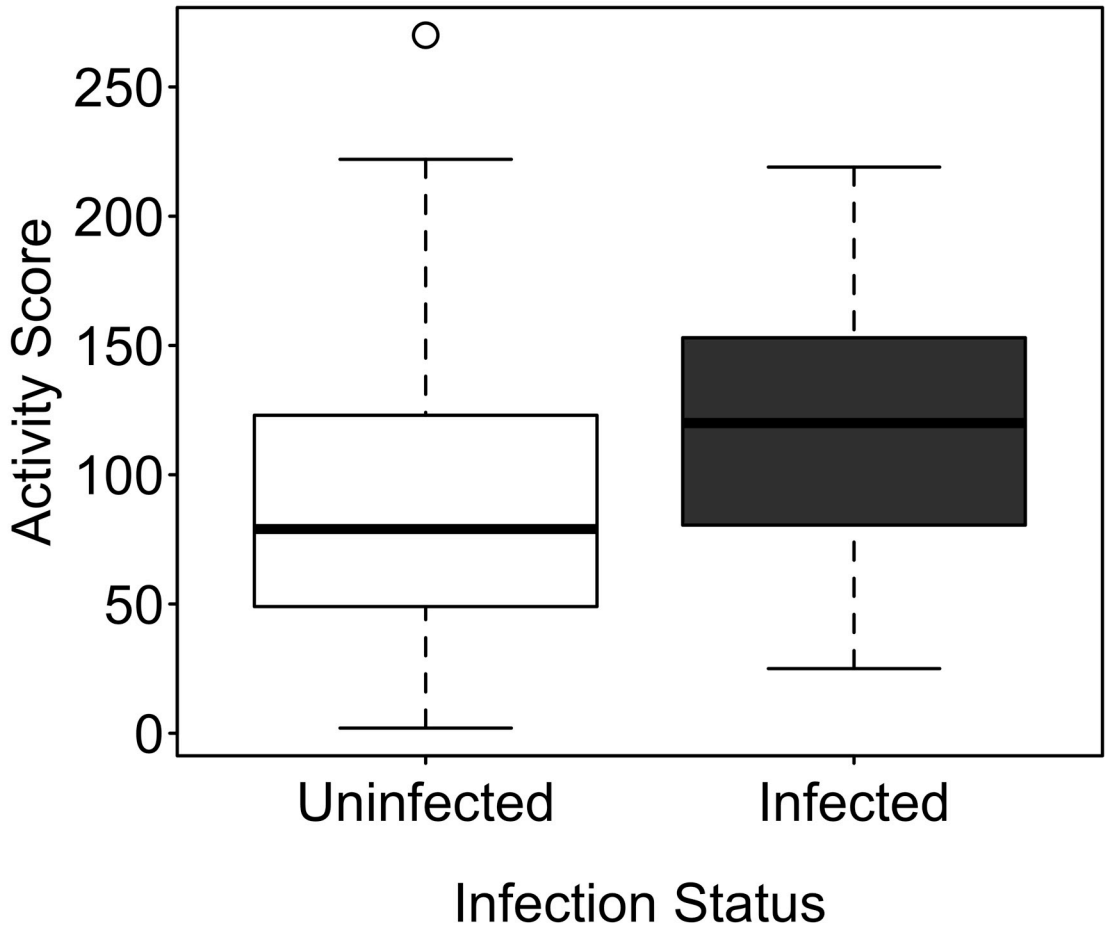


Figure 3.3. Median activity score by infection. Activity score is the total number of times an amphipod (*H. azteca*) crossed a line dividing a 1L beaker in two equal halves over a 15-minute period. These groups are significantly different ($P = 0.028$).

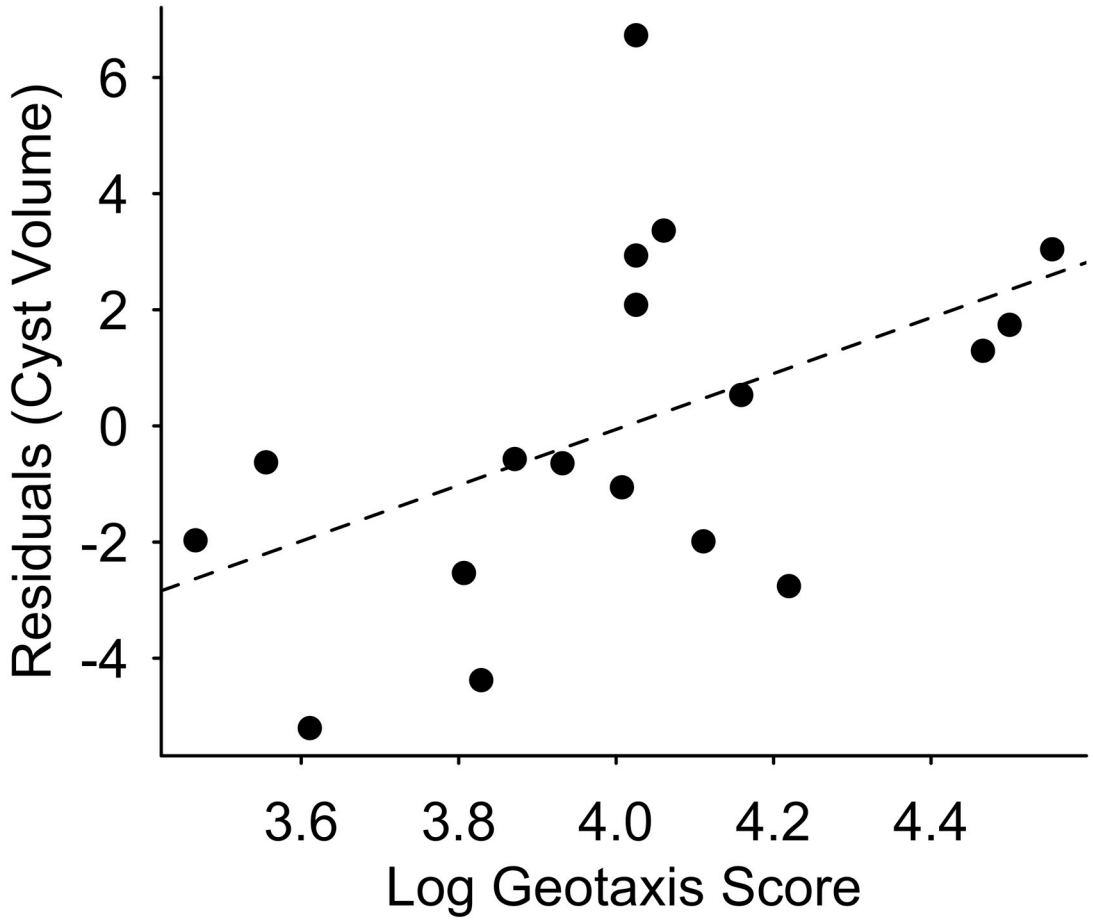


Figure 3.4. Geotaxis scores by cystacanth volume. Each point represents behaviour of an amphipod infected by a single cystacanth and the residual of cystacanth volume accounting for other factors in the model (n=18). The geotaxis score is based on a total of 30 scores of height in the water column over a 15-minute observation period. There is a significant positive relationship between geotaxis scores and cystacnth size ($r_s = 0.68$, $N = 11$, $P = 0.002$).

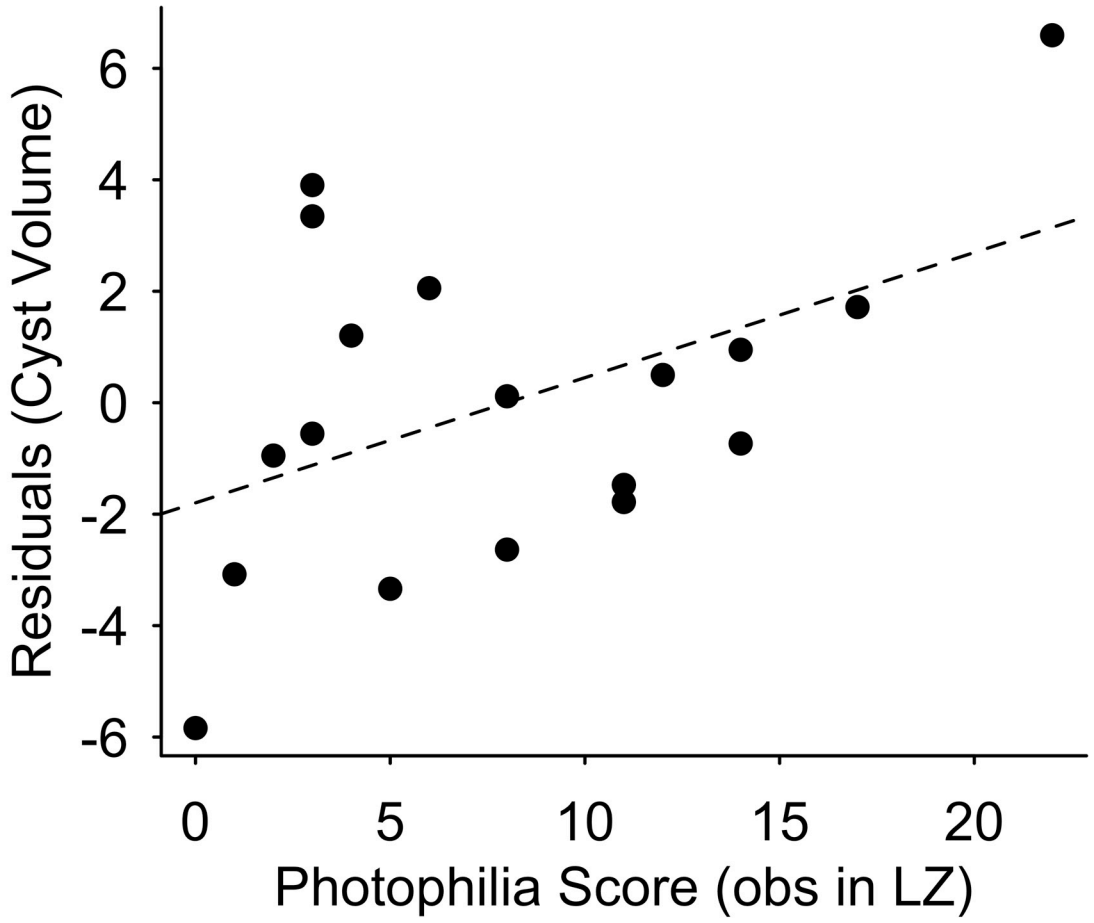


Figure 3.5. Photophilia and cystacanth volume. Each point represents behaviour of an amphipod infected by a single cystacanth and the residual of cystacanth volume accounting for other factors in the model (n=18). Each amphipod's position was recorded every 30 seconds for 15 minutes. The photophilia score is the number of times an amphipod was observed in the uncovered half of the aquarium and ranges from 0-30. Photophilia was not negatively correlated ($r$ = 0.46, $P$ = 0.043) with cystacanth size.

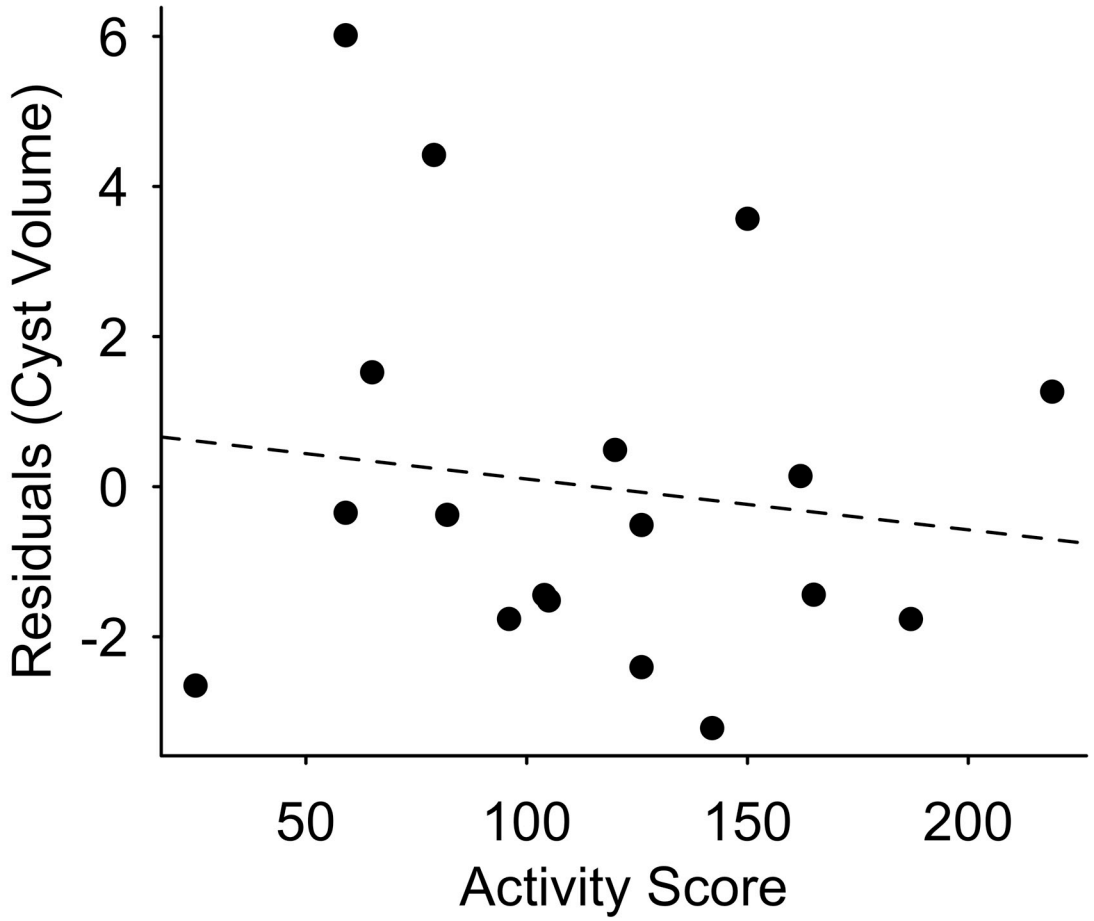


Figure 3.6. Activity score and cystacanth volume. Each point represents behaviour of an amphipod infected by a single cystacanth and the residual of cystacanth volume accounting for other factors in the model (n=18). The activity score is the total number of times that an amphipod crossed from one half of a 1L circular beaker to the other during a 15-minute observation period. This relationship was not significant ($r$ = -0.16, $P$ = 0.56).

## REFERENCES


Ashley, D.C. & Nickol, B.B. **(**1989). Dynamics of the *Leptorhynchoides thecatus* (Acanthocephala) suprapopulation in a Great Plains reservoir. *Journal of Parasitology, 75,* 46-54.

Bakker, T.C.M., Mazzi D. & Zala, S. **(**1997). Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology, 78,* 1098-1104.

Baldauf, S.A., Thünken, T., Frommen, J.G., Bakker, T.C.M., Heupel, O. & Kullmann, H. (2007). Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology, 37,* 61-65.

Bauer, A., Trouve, S., Gregoire, A., Bollache, L. & Cezilly, F. (2000). Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. *International Journal for Parasitology*, *30,* 1453-1457.

Bauer, A., Haine, E.R., Perrot-Minnot M.J. & Rigaud, T. (2005). The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *Journal of Zoology*, *279,* 39-43.

Barger, M.A. & Nickol, B.B. (1998). Effects of coinfection with *Pomphorhynchus bulbocolli* on development of *Leptorhynchoides thecatus* (Acanthocephala) in amphipods (*Hyalella azteca*). *Journal of Parasitology*, *85,* 60-63.

Bethel, W.M. & Holmes, J.C. (1973). Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. *Journal of Parasitology, 59,* 945–956.

Bethel, W.M. & Holmes, J.C. (1977). Increased vulnerability of amphipods to predation owing to altered behavior induced by larval acanthocephalans. *Canadian Journal of Zoology, 55,* 110-115.

Benesh, D.P., Kitchen, J., Pulkkinen, K., Hakala, I. & Valtonen, E.T. **(**2008). The effect of *Echinorhynchus borealis* (Acanthocephala) infection on the anti-predator behavior of a benthic amphipod. *Journal of Parasitology, 94,* 542-545.

Cezilly, F., Gregoire, A. & Bertin, A. **(**2000). Conflict between co-occurring manipulative parasites? An experimental study of the joint influence of two acanthocephalan parasites on the behaviour of *Gammarus pulex*. *Parasitology, 120,* 625-630.

Combes, C. (2001). *Parasitism: The Ecology and Evolution of Intimate Interactions*. London: University of Chicago Press, Ltd.

Dawkins, R. **(**1982). *The Extended Phenotype*. New York: W. H. Freeman.

Dezfuli, B.S., Giari, L. & Poulin, R. **(**2001). Cost of intraspecific and interspecific host sharing in acanthocephalan cystacanths. *Parasitology, 122,* 483-489.

Dianne, L., Perrot-Minnot, M.J., Bauer, A., Gailard, M., Leger, E. & Rigaud, T. (2011). Protection first then facilitation: a manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution, 65,* 2692-2698.

Franceschi, N., Bauer, A., Bollache, L. & Rigaud, T. **(**2007). The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal for Parasitology*, **38**, 1161-1170.

Helluy, S. **(**2013). Parasite-induced alterations of sensorimotor pathways in gammarids: collateral damage of neuroinflammation? *Journal of Experimental Biology, 216,* Special Issue, 67-77.

Helluy, S. & Holmes, J.C. **(**1990). Serotonin, octopamine, and the clinging behavior induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustrus* (Crustacea). *Canadian Journal of Zoology, 68,* 1214-1220.

Hindsbo, O. **(**1972). Effects of *Polymorphous* (Acanthocephala) on color and behavior of *Gammarus lacustris*. *Nature, 238,* 333.

Maynard, B.J., Wellnitz, T.A., Zanini, N., Wright, W.G. & Dezfuli, B.S. (1998). Parasite-altered behavior in a crustacean intermediate host: Field and laboratory studies. *Journal of Parasitology, 84,* 1102–1106.

Moore, J. **(**1983). Responses of an avian predator and its isopod prey to an Acanthocephalan parasite. *Ecology, 64,* 1000-1015.

Moore, J. **(**2002). *Parasites and the behavior of animals*. New York: Oxford University Press.

Parker, G.A., Ball, M.A., Chubb, J.C., Hammerschmidt, K. & Milinski, M. (2009). When should a trophically transmitted parasite manipulate its host? *Evolution, 63,* 448-458.

Perrot-Minnot, M.J., Kaldonski, N. & Cezilly, F. **(**2007). Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *International Journal for Parasitology, 37,* 645-651.

Poulin, R. **(**1994). The evolution of parasite manipulation of host behaviour: a theoretical analysis. *Parasitology, 109,* 109–118.

Poulin, R., Fredensborg, B.L., Hansen, E. & Leung, T.L.F. **(**2005). The true cost of host manipulation by parasites. *Behavioral Processes, 68,* 241-244.

Rauque, C.A., Patterson, R.A., Poulin, R. & Tompkins, D.M. (2011). Do different parasite species interact in their effects on host fitness? A case study on parasites of the amphipod *Paracalliope fluviatilis*. *Parasitology, 138,* 1176-1182.

Stearns, S.C. (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.

Steinauer, M.L. & Nickol, B.B. (2003). Effect of cystacanth body size on adult success. *Journal of Parasitology, 89,* 251-254.

Tain, L., Perrot-Minnot, M.J. & Cezilly, F. (2006). Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proceedings of the Royal Society B, 273,* 3039-3045.

Thomas, F., Renaud, F. & Poulin, R. (1998). Exploitation of manipulators: 'hitch-hiking' as a parasite transmission strategy. *Animal Behaviour, 56,* 199-206.

Thomas, F., Adamo, S. & Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behavioral Processes, 68,* 185-199.

U.S. Environmental Protection Agency. 1994. *Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms*. 3rd ed. Cincinnati, OH. EPA-600/4-94-002.

Vickery, W.L. & Poulin, R. (2010). The evolution of host manipulation by parasites: a game theory analysis. *Evolutionary Ecology, 24,* 773-788.

## CHAPTER 4: Parasite induced manipulation of odor responses in an amphipod-acanthocephalan system*


### Summary

Odor-related behaviors in aquatic invertebrates are important and effective anti-predator behaviors. Parasites often alter invertebrate host behaviors to increase transmission to hosts. This study investigated the responses of the amphipod *Hyalella azteca* when presented with two predator chemical cues: (1) alarm pheromones produced by conspecifics and (2) kairomones produced by a predatory Green Sunfish (*Lepomis cyanellus*). We compared the responses of amphipods uninfected and infected by the acanthocephalan parasite *Leptorhynchoides thecatus*. Uninfected amphipods reduced activity and increased refuge use after detecting the odors with a stronger reaction to alarm pheromones than predator kairomones. Infected amphipods spent significantly more time active and less time on the refuge than uninfected amphipods, and behaved as if they had not detected the chemical stimulus. Therefore, *L. thecatus* infections disrupt the amphipod's normal anti-predator behaviors and likely make their hosts more susceptible to predation.


### Introduction

Many aquatic invertebrates rely heavily upon chemosensory perception for survival, particularly when in turbid and low-light environments. Two types of chemical cues alert prey to the presence of predators: alarm pheromones and kairomones (Wisenden, 2000). Prey species

*Submitted for publication in the International Journal for Parasitology, October 2013

release alarm pheromones during or after predator attacks to alert conspecifics to impending danger (Chivers and Smith, 1998). Prey can also sense chemicals that predators unintentionally produce (kairomones) and alter their behavior as they become aware of the threat.

Freshwater amphipods (Crustacea) feature prominently in research on odors and associated anti-predator behavior. Amphipods in the genus *Gammarus* (Gammaridae) are less active and less likely to drift in the water column in streams containing predatory fish kairomones than in streams without kairomones (Williams and Moore, 1985, Anderson et al., 1986, Holomuzki and Hoyle, 1990, Wudkevich et al., 1997). Fish kairomones also cause amphipods to spend more time in refuges, to favor less exposed habitats, to swim down to substrates, to aggregate, and to select smaller, less noticeable mates (Holomuzki and Hoyle, 1990, Mathis and Hoback, 1997, Wudkevich et al., 1997, Kullmann et al., 2008). Similar behaviors are observed in gammarids exposed to alarm pheromones (Wudkevich et al., 1997). These behaviors benefit amphipods by reducing predation rates. Amphipods previously exposed to either alarm pheromones or predator kairomones were less likely to be found and consumed by Green Sunfish (*Lepomis cyanellus* Rafinesque) than unexposed amphipods (Holomuzki and Hoyle, 1990, Wisenden et al., 2001). These studies demonstrate that decreased activity, increased refuge use, and increased geotaxis by amphipods in response to alarm pheromones and kairomones are effective anti-predator behaviors.

Many animals behave abnormally when infected by parasites and anti-predator behaviors are among those affected (see Moore, 2002). Parasites can benefit from altered host behavior if the behavioral changes increase the parasite's transmission rates. These types of parasitic manipulations have been documented in a wide variety of host-parasite interactions (Poulin, 1994, Moore, 2002, Thomas et al., 2005). Aquatic arthropods serve as intermediate hosts for

several parasite species (see review in Moore 2002). Many of these parasites can only grow to adulthood after a predator consumes their intermediate host. Thus, interference in host anti-predator behaviors may be a parasite strategy to increase predation rates by suitable definitive hosts (Dianne *et al.* 2012).

Amphipods feature prominently in manipulation research, particularly amphipods infected by worms from phylum Acanthocephala. These thorny-headed worms are known to alter activity (Thünken et al., 2010), orientation to light (Bethel and Holmes, 1973), drift rate (Dezfuli et al., 2003), and refuge use (Perrot-Minnot et al., 2007) in amphipods. This slate of behaviors is strikingly similar to those that influence predation rates and infected amphipods can be at greater risk of predation than uninfected amphipods (Bethel and Holmes, 1977).

Acanthocephalans also affect amphipod responses to odors. In choice experiments, uninfected amphipods avoided areas with high concentrations of predatory fish kairomones while amphipods infected by acanthocephalans did not. Similarly, given a choice between two incurrent water sources, one containing fish kairomones and one free of kairomones, uninfected amphipods avoided the stream containing fish kairomones, while infected amphipods did not discriminate between streams (Baldauf et al., 2007).

These alterations in predator-related odor behavior can be remarkably specific. For an acanthocephalan, predation by an inappropriate host leads to the parasite's death and these parasites can be quite specific in the odor-related behaviors they manipulate. For instance, an acanthocephalan of fish altered its host's responses to fish kairomones and infected amphipods had higher fish predation rates, but bird acanthocephalans in the same amphipod population did not alter amphipod responses to fish kairomones or result in higher fish predation (Kaldonski et al., 2007).

This study asks whether the acanthocephalan *Leptorhynchoides thecatus* (Kostylev) alters its amphipod host's odor-related anti-predator behavior. *Leptorhynchoides thecatus* is a common fish parasite found in eastern and central North America. It uses a single amphipod species, *Hyalella azteca* (Saussure), as an intermediate host and a broad range of centrarchid fish as definitive hosts (Linton, 1949). The amphipod is infected when it consumes the parasite egg, which hatches and then develops in the amphipod's hemocoel from a non-infective acanthella stage into an infective cystacanth. A fish acquires the parasite when it consumes an amphipod hosting a cystacanth. The acanthocephalan establishes in the fish's intestinal ceca, matures, finds a mate, and produces eggs that pass with the feces to continue the life cycle.

Our previous study of this *L. thecatus* population found that the infected amphipods exhibit altered photophilia, geotaxis, and activity, behaviors that may reasonably be thought to influence encountering predators (Stone and Moore, in prep). This study aimed to determine whether *L. thecatus* alters *H. azteca* behaviors that are directly implicated in predation and transmission rates, that is, anti-predator behaviors. Because dissection is required to reliably determine amphipod infection, we cannot easily conduct predation experiments to determine whether a fish captures infected amphipods at a higher rate than uninfected amphipods. We therefore investigated two chemical cues that stimulate anti-predator behavior: alarm pheromones from *H. azteca* and kairomones from *L. cyanellus*. To our knowledge, this is the first study to address parasite-altered responses to alarm pheromones in amphipods.

## Materials and Methods

### *Collection of organisms*

All organisms used in this study were collected from Atkinson Reservoir, Holt County, Nebraska (42°32’36”N X 98°58’22”W). This population of *L. thecatus* appears unique in its host use patterns with Green Sunfish (*L. cyanellus*) providing the primary definitive host in this location (Ashley and Nickol, 1989). Capture, collection, and culture of animals were carried out under CSU Animal Care and Use protocol 11-2590A and Nebraska Game and Parks Commission scientific collection permits. Green Sunfish (*L. cyanellus*), Pumpkinseed Sunfish (*Lepomis gibbosus* Linnaeus), and Bluegill (*Lepomis macrochirus* Rafinesque) were captured by seine net on the littoral portion of the lake. On the day of capture, fish were dissected and adult *L. thecatus* worms were removed from the host’s alimentary tract, placed in aged tap water, and stored at 4°C. Three juvenile *L. cyanellus* sunfish were captured and transported to the laboratory at Colorado State University, Fort Collins, CO, where they served as kairomone sources. Amphipods were also collected from aquatic vegetation found in the same littoral zone as fish sampling and transported to the laboratory.

### *Culturing of organisms*

Fish captured and transported to the laboratory were cultured in individual 10-gallon aquaria with a hang-on-the-back filtration system. The fish were fed to satiety on Tetramin® tropical fish food once per day and water was changed once every two weeks. Because the

carbon component of the filtration system removes the organic compounds including fish kairomones sensed by amphipods, the carbon filter was removed from the culture tanks 24hrs before behavioral tests.

Amphipods were cultured in 27L and 30L Sterilite® plastic storage containers with one 1.25cm of sand substrate and approximately 20L of culture water. Culture water was formulated from DI water according to the Moderately Hard Water formula (see U.S. EPA, 1994). Cultures were given Tetramin® tropical fish food three times per week and water changed weekly. All animals were kept in a climate-controlled room a 23°C, on a 15:9 light dark (LD) cycle.

*Infection of Amphipods*

Eggs were harvested from gravid female worms dissected in several milliliters of tap water. The resulting egg suspension was standardized so that an average of 1.5 fully-embryonated eggs was present per field of view in 0.05 ml of suspension at 100X (Barger and Nichol, 1998). Fifty (50) *H. azteca* were placed in wide-mouth glass quart jars containing 800ml culture water, 50g gravel, and four grams of filamentous green algae (*Pithophora* sp.). Amphipods were exposed to one (1) ml of the egg suspension, which was pipetted over the algae. The amphipods foraged for 24-72hrs and were then transferred to 4.5L Rubbermaid® plastic containers, where they were maintained for 32-40 days at 23°C.

*Test apparatus*

A 5-gallon aquarium (40cm x 20cm x 25cm) was separated into two equal halves by a single clear glass partition. This division allowed the one aquarium to serve as two equally sized test areas, so that preparing a single apparatus could be used to test two amphipods. For each behavioral test, the area was filled to 15cm depth with fresh culture water so that the focal animal was observed in 20cm X 20 cm X 15cm of water, with a substrate of sand just thick enough to cover the bottom of the tank. A single air stone was suspended against one wall approximately 5cm above the substrate. The flow from the air stone mixed and aerated the water, but was not so strong as to hinder amphipod movement. A single 60W full-spectrum fluorescent light bulb suspended 25cm above the surface of the water provided illumination. Following the design of Wisenden et al. (1999), a single refuge was created by a 3cm x 6cm piece of opaque black glass supported by legs 2cm above the substrate. The refuge was placed in the center of the experimental space, equidistant from all sides and directly under the light source.

*Behavioral tests*

Amphipods were allocated to one of three treatments: exposure to alarm pheromone, exposure to predator kairomone, and exposure to no cue (culture water only). Treatment solutions were prepared prior to testing each pair of amphipods. Alarm pheromones were obtained by removing 3 amphipods from a culture tank and placing them in a small eyeglass with 10ml of culture water. Following Wudkevich et al. (1997), these amphipods were homogenized with a glass rod. Two 10ml solutions were prepared simultaneously because two amphipods

were tested sequentially in the test apparatus. The chemical stimulus for a second amphipod awaited application while the first amphipod was observed. Chemical cues do degrade over time, but the introduction of the second alarm pheromone occurred well before degradation and within the efficacy time, so there is no reason to believe that results would differ between amphipods tested first or second (Wisenden et al., 2009). A two-way ANOVA for difference in activity between first and second applications of the predator kairomone and between infection groups did not detect a degradation of kairomone potency ($n = 86$, $P = 0.43$).

Predator kairomones were collected by removing 10ml of water from a *L. cyanellus* culture aquarium in which the water had been circulating for 24hrs without the carbon filter (see above). The individual fish chosen as a kairomone source was not recorded because amphipod reaction to kairomones from different individual fish tends not to vary (Wudkevich et al., 2009). The control treatment consisted of 10ml of newly formulated culture water put aside while preparing the test apparatus.

Each amphipod was introduced to the experimental apparatus in darkness and allowed to acclimate for ten minutes before the light was switched on, signaling the commencement of a 5-minute observation period. We recorded the amount of time the amphipod was active (swimming or crawling not on or under the refuge) and the time it spent in refuge use (on or under the refuge), using a stopwatch dedicated to each behavior. The total number of seconds spent in each state served as scores for the two behavior categories, activity and refuge use.

Immediately following the first 5-minute observation period, 10ml of one of the three stimuli (alarm pheromones, predator kairomones, no cue) was pipetted into the water above the bubbling air stone, approximately 3-5cm below the surface and in the path of rising bubbles. We allowed 30 seconds for the added liquid to disperse, and then observed the amphipod for 5

minutes. The number of seconds active and in refuge use was recorded and scores calculated as above.

*Measurement of infection status and organisms metrics*

After the behavioral test, amphipods were euthanized in 95% ethanol. Each amphipod was measured from rostrum tip to urostyle by straightening it on a ruler under a dissecting microscope; it was subsequently dissected to determine presence and intensity of infection. Cystacanths were placed in aged tap water on a depression slide for two minutes, covered with a cover glass, and their sex, length (not including proboscis), and width determined under a compound microscope with an ocular micrometer. If the amphipod harbored an acanthella (uninfective immature stage), the infection status was recorded, and the worm's length and width measured and recorded.

*Statistical Analysis*

Statistical analyses were conducted using R: R Development Core Team (2013). R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

To determine differences in reactions to cues, a difference score was calculated by subtracting the post-cue score in a behavioral category (Activity or Refuge Use) from the score in that category before the cue was added. Difference scores with negative values indicated a decrease in that activity after the cue was added and positive values indicated an increase.

First, uninfected amphipods exposed to no chemical cue were tested with t-tests to determine whether there was a difference in the mean scores before and after the application. We used two-way ANOVAs with Tukey adjustment to investigate differences in behavior before and after exposure to the cue and by infection group. We conducted this test using difference scores for both activity and refuge use as response variables. Not all infections matured to the cystacanth stage and there were a small number of infected amphipods that harbored acanthellae. The ANOVA model tested how difference scores in each behavior category were influenced by infection status (uninfected, acanthella-infected, and cystacanth- infected), chemical cue (kairomones and alarm pheromones), and an interaction between term infection status and chemical cue. Pairwise t-test comparisons were carried out to clarify differences between infection groups and whether an infection group changed behavior before and after the addition of the cue.

**Results**

Uninfected amphipods reduced activity and increased refuge use when presented with the chemical cues. Alarm pheromones elicited stronger responses than predator kairomones. Infected amphipods harboring cystacanths differed significantly from uninfected amphipods, and the strength of altered response was stronger for alarm pheromone responses than kairomones (Figures 1-4).

As expected, the "no cue" treatment had no effect on active or refuge use scores ($n = 29$, active: $P = 0.61$; refuge use: $P = 0.43$; Table 1). In other words, the addition of 10ml of culture liquid to the environment does not change amphipod behavior. In contrast, when exposed to alarm pheromones, uninfected amphipods reduced their activity, on average, by -60 (±58)

seconds. Cystacanth-infected amphipods exhibited no significant difference in time active before or after the alarm pheromone was added (-1.6 ± 27 seconds; $P$ =0.74). The difference in activity scores was statistically significant for these two infection groups ($F$ = 115.5, $P$ =0.037; Table 2). In the refuge use category, the average score of uninfected amphipods doubled in the presence of alarm pheromones (44 ± 46 seconds) and cystacanth-infected amphipods did not statistically increase refuge use (2.5±25 seconds; $P$ = 0.48); the difference in refuge use was significantly different between uninfected and infected amphipods ($F$ = 30.33, $P \leq 0.001$, Table 2). For both chemical stimuli, cystacanth-infected amphipods were more similar to uninfected amphipods exposed to no chemical cue than uninfected amphipods given alarm pheromones (Alarm pheromones: $F$ = 1.34, $P$ = 0.45; kairomones: $F$ = 0.85, $P$ = 0.54; Figures 1&2).

Predator kairomones significantly altered the activity and refuge use of uninfected amphipods, but not of cystacanth-infected amphipods. Both uninfected amphipods and infected amphipods decreased activity, on average, when exposed to kairomones, but uninfected amphipods did so to a significantly greater extent (-30 ± 59 seconds) than infected amphipods (-8.1 ± 52; $P$ = 0.003; Table 1). There was also a significant difference in refuge use; uninfected amphipods increased refuge use 14.6 (±55) seconds on average, while infected amphipods actually decreased refuge use by -2.7 (±49) seconds ($P$ = 0.036). These scores were significantly different between groups ($F$ = 8.65, $P < 0.001$).

The interaction term between infection status and chemical in the two-way ANOVA was significant for activity, but not for refuge use. For activity, the effects test found the interaction term between chemical and infection to be significant ($F$ = 3.3, $P$ = 0.039; Table 3). This indicates that cystacanths alter amphipod activity more strongly for alarm pheromones than

kairomones. The same analysis did not find the interaction term for refuge use significant ($F$ = 1.6, $P$ = 0.20; Table 3).

Behavior of acanthella-infected amphipods was compared to both uninfected and cystacanth-infected amphipods. For alarm pheromones, acanthella-infected amphipods ($n$ = 5) were indistinguishable from uninfected amphipods in activity ($P$ = 0.5) and refuge use ($P$ = 0.67). Acanthella-infected and cystacanth infected amphipods differed significantly in their responses to alarm pheromones in activity and refuge use ($P < 0.001$ for both categories). For kairomones, acanthella-infected amphipods ($n$ = 10) did not differ significantly from uninfected amphipods in difference scores for activity ($P$ = 0.5) or refuge use ($P$ = 0.67), nor could difference scores be distinguished from cystacanth-infected amphipods for activity ($P$ = 0.5) and refuge use ($P$ = 0.67).

**Discussion**

Amphipods infected with *Leptorhynchoides thecatus* cystacanths reacted to predation-related odors differently than uninfected amphipods did. Uninfected *H. azteca* reduced their activity and increased their refuge use when presented with both alarm pheromones and predator kairomones. This behavior was stronger for alarm pheromones than kairomones. Cystacanth-infected amphipods did not significantly decrease activity or increase refuge use when these cues were present – it is as if cystacanths-infected amphipods did not sense the stimuli. We believe that this is the first time that parasite-induced alteration of intermediate host response to alarm pheromones has been demonstrated.

These behaviors are directly relevant to amphipod-predator interactions. For instance, this study is modeled on demonstrations that these odor-related behaviors reduced predation in

uninfected amphipods (*Gammarus lacustris*; Wudkevich et al., 1997, Wisenden, 2000). Our uninfected *H. azteca* decreased average activity to the same extent as *Gammarus lacustris* exposed to predator kairomones and alarm pheromones (Wudkevich et al., 1997). In predation tests, the behavioral responses to alarm pheromones directly benefitted *G. lacustris* by increasing the time it took for a *L. cyanellus* to find and strike amphipods (Wisenden, 2000). The same predation tests have not been conducted using predator kairomones, but the similarity of response to both cues - decreased activity - suggests that the behavioral response to kairomones is an equally effective anti-predator behavior. Other studies on the efficacy of anti-predator reactions to odors support these findings (Bakker *et al.* 1997, Wisenden, 2000, Baldauf et al., 2007, Médoc and Beisel, 2007). The qualitative and quantitative similarities between the responses of *H. azteca* and other amphipod species to chemical cues, and the predation study results in other species suggest that reduced activity and increased refuge use decrease predation for *H. azteca* much as they do for other amphipod species.

It is therefore reasonable to suggest that risk of fish predation is increased by the reduced activity and refuge use associated with *L. thecatus* infections. These changes fit the definition of manipulation proposed by Thomas et al. (2005): parasite-induced changes in behavior or physiology that increase parasite transmission.

The timing (ontogeny) of behavioral change in this host-parasite association is also consistent with parasite-induced manipulation. Bethel and Holmes (1977) pointed out that manipulative parasites were likely to alter behavior at the time that they became infective, when transmission would be beneficial, and not before that time. In our study, the onset of infectivity matches the pattern of behavioral change in amphipods harboring acanthellae and cystacanths. Acanthellae-infected amphipods exposed to alarm pheromones exhibit anti-predator behavior

similar to that of uninfected amphipods and respond to the stimulus, while cystacanth-infected amphipods do not change their behavior. For kairomones, the results for acanthellae-infected amphipods are not distinguishable from those found with cystacanth-infected amphipods. One reason may be that the response to kairomones in general was not as strong as the response to alarm pheromones and this weaker response makes it more difficult to distinguish differences between groups. Although the sample sizes of acanthella-infected amphipods are not large, sample size is addressed in the analyses; it is therefore reasonable to interpret the data from acanthellae-infected amphipods as similar to that of uninfected amphipods. The fact that *L. thecatus* acanthellae do not alter *H. azteca* as cystacanths do is expected for a manipulative parasite.

This study sheds light on an ecologically important aspect of the parasite-host relationship, the manipulation by *Leptorhynchoides thecatus* of *Hyalella azteca*'s reaction to conspecific alarm pheromones and predator kairomones. Cystacanth-infected amphipods do not react to these chemical cues as their uninfected conspecifics do, and were more similar to amphipods exposed to no chemical stimulus. Normal behavior of uninfected amphipods effectively reduces predation (Wisenden, 2000), so it is reasonable to assume that these parasites increase predation rates through these altered behaviors.

Human sensory biases make it easy for researchers of parasitic manipulation to overlook chemosensory perception (see Moore, 2013). However, the chemical environment plays an essential role for many animals, particularly aquatic invertebrates. It is likely that as we move beyond our attraction to easily observed behavioral changes (e.g. photoreactions and activity), we will find a wealth of possible olfactory manipulations. We add alarm pheromones to odor-related manipulations in amphipods (Baldauf et al., 2007, Kaldonski et al., 2008, Médoc and

Beisel, 2009, Thünken et al., 2010), in other crustaceans including isopods and copepods (Hechtel et al., 1993, Jakobsen and Wedekind, 1998), and in other host phyla such as mammals, particularly mouse responses to predator odors infected by protozoans *Toxoplasma* and *Eimeria* (Kavaliers and Colwell, 1995, Kavaliers et al., 1998, Berdoy et al., 2000). It is likely that additional odor-related behavioral manipulations will be documented; there is much to be explored in this field. We do not yet know the specificity and limits of olfactory alteration in our *L. thecatus* system. However, if previous studies are an indication (Kaldonski et al., 2007), manipulative parasites are likely to surprise us with their ability to target specific, ecologically important odor-related behaviors to achieve increased transmission.

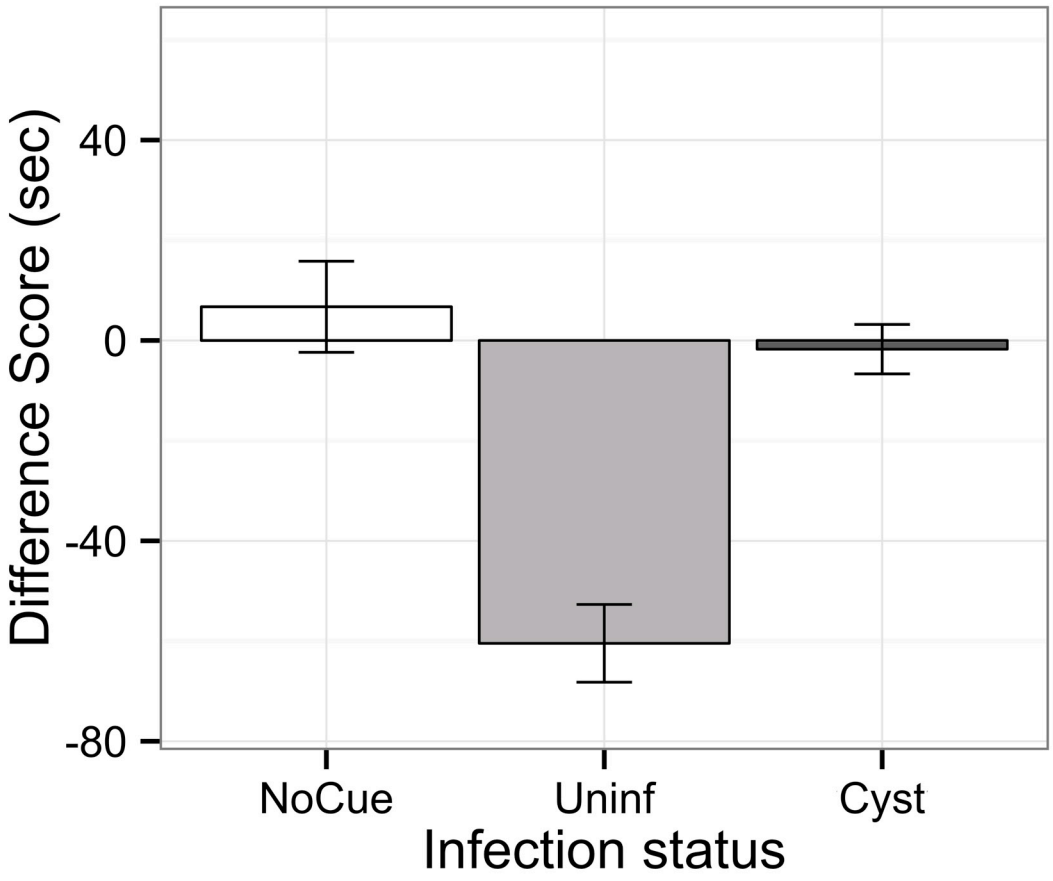


Figure 4.1. Mean difference scores in time active response to alarm pheromones. Difference scores represent the total time active after the chemical stimulus minus total time active before chemical stimulus exposure, such that negative values indicate a decrease in that behavior between trials. Alarm pheromones consisted of 3 pulverized amphipods (*H. azteca*) in 10ml culture water. The "Cyst" column represents amphipods infected with *L. thecatus* cystacanths.

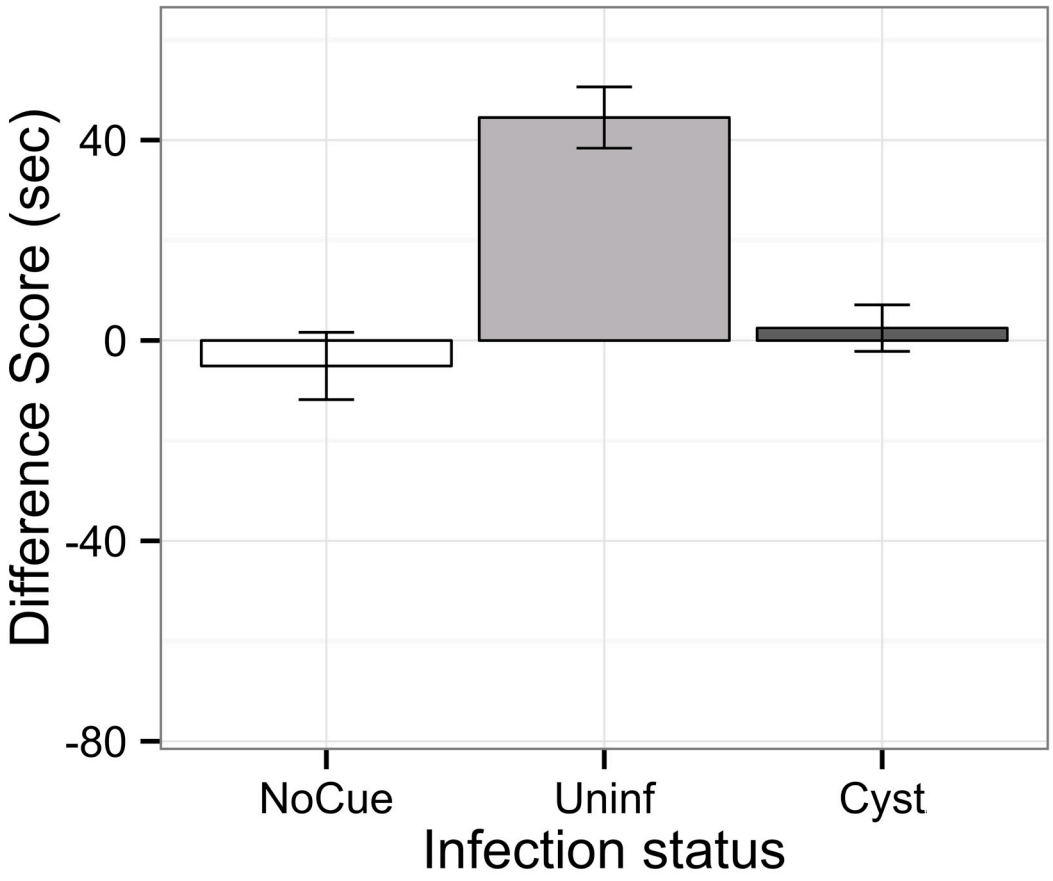


Figure 4.2. Mean difference scores in time of refuge use response to alarm pheromones. Difference scores represent the total time of refuge use after the chemical stimulus minus total time of refuge use before chemical stimulus exposure, such that positive values indicate an increase in that behavior between trials. Alarm pheromones consisted of 3 pulverized amphipods (*H. azteca*) in 10ml culture water. The "Cyst" column represents amphipods infected with *L. thecatus* cystacanths.

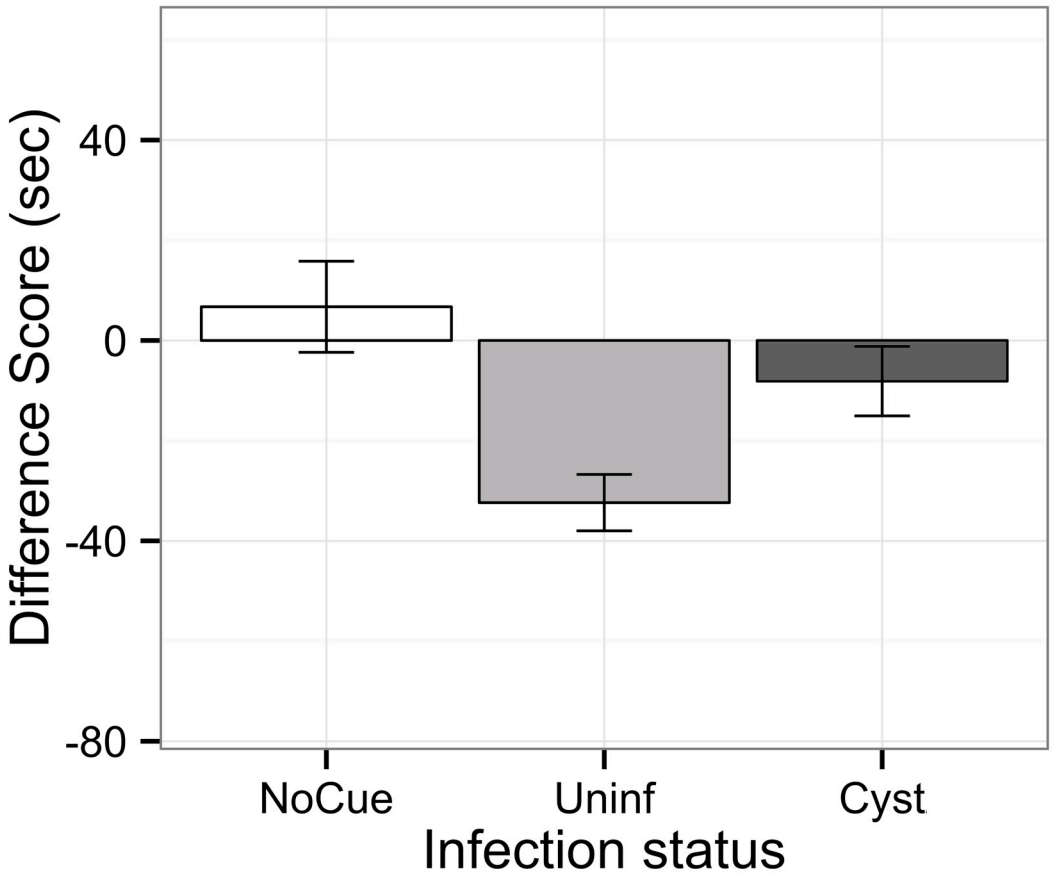


Figure 4.3. Active response to predator kairomones, as measured by mean difference score times. Difference scores represent the total time active after the chemical stimulus minus total time active before chemical stimulus exposure, such that negative values indicate a decrease in that behavior between trials. Predator kairomones were added in 10ml of Green Sunfish (*L. cyanellus*) conditioned water. The "Cyst" column represents amphipods infected with *L. thecatus* cystacanths.

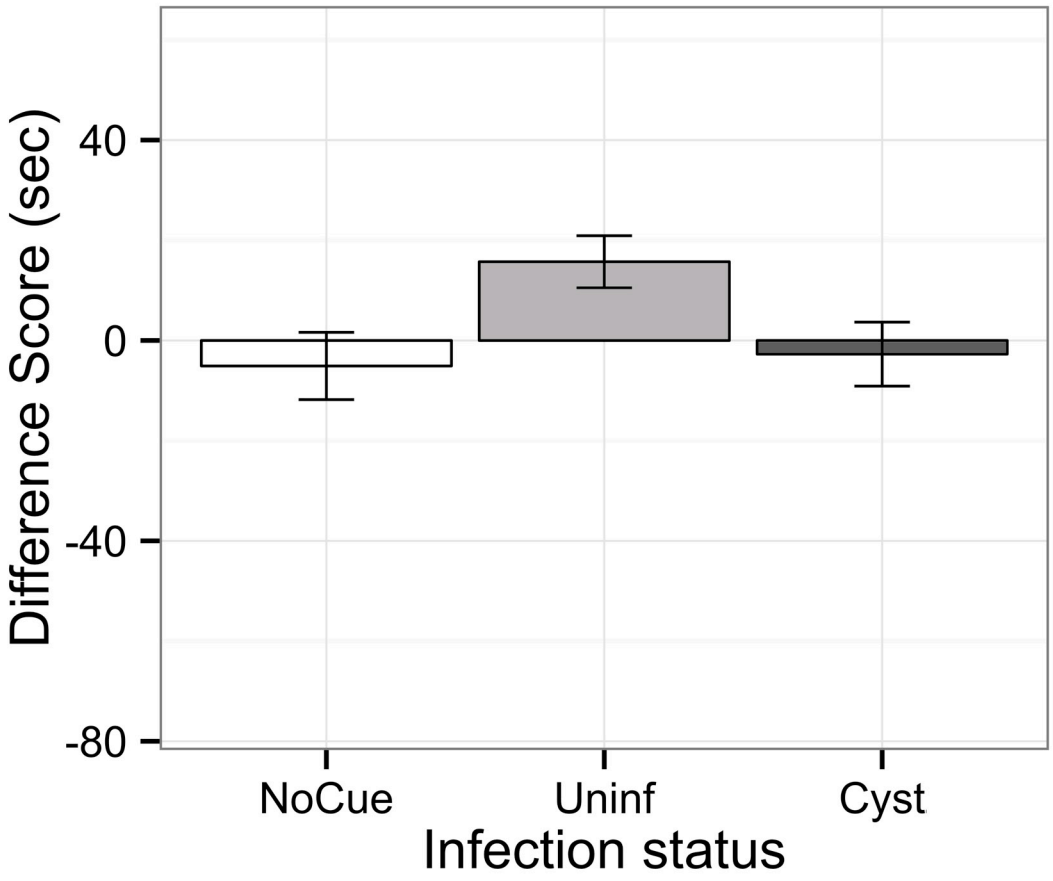


Figure 4.4. Mean difference score in times of refuge use response to predator kairomones. Difference scores represent the total time of refuge use after the chemical stimulus minus total time of refuge use before chemical stimulus exposure, such that positive values indicate an increase in that behavior between trials. Predator kairomones were added in 10ml of Green Sunfish (*L. cyanellus*) conditioned water. The "Cyst" column represents amphipods infected with *L. thecatus* cystacanths.

**REFERENCES**


Andersson, K.G., Bronmark, C., Herrmann, J., Malmqvist, B., Otto, C., Sjorstrom, P., 1986. Presence of sculpins (*Cottus-gobio*) reduces drift and activity of *Gammarus-pulex* (amphipoda). Hydrobiologia 133, 209-215.

Ashley, D.C., Nickol, B.B., 1989. Dynamics of the *Leptorhynchoides thecatus* (Acanthocephala) suprapopulation in a Great Plains reservoir. J. Parasitol. 75, 46-54.

Bakker, T.C.M., Mazzi, D., Zala. S., 1997. Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. Ecology 78, 1098-1104.

Baldauf, S. A., Thünken, T., Frommen, J. G., Bakker, T. C. M., Heupel, O. & Kullmann, H., 2007. Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. Int. J. Parasitol, 37, 61–65.

Barger, M.A., Nickol, B.B., 1998. Effects of coinfection with *Pomphorhynchus bulbocolli* on development of *Leptorhynchoides thecatus* (Acanthocephala) in amphipods (*Hyalella azteca*). J. Parasitol. 85, 60-63.

Berdoy, M., Webster, J.P., MacDonald, D.W., 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. Proc. R. Soc. Lond. B Biol. Sci. 267, 1591-1594.

Bethel, W.M., and J.C. Holmes. 1973. Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. J. Parasitol. 59, 945–956.

Bethel, W. M., Holmes, J. C., 1977. Increased vulnerability of amphipods to predation owing to altered behavior induced by larval acanthocephalans. Can. J. Zool. 55, 110–115.

Chivers, D.P., Smith, R.J.F., 1998. Chemical alarm signaling in aquatic predator-prey systems: A review and prospectus. Ecoscience 5, 338-352.

Dezfuli, B.S., Maynard, B.J., Wellnitz, T.A., 2003. Activity levels and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis.* Folia Parasit. 50, 129-134.

Hechtel, L.J., Johnson, C.L., Juliano, S.A., 1993. Modification of antipredator behavior of *Caecidotea intermedius* by its parasite *Acanthocephalus dirus*. Ecology 74, 710-713.

Holomuzki, J.R., Hoyle, J.D., 1990. Effect of predatory fish presence on habitat use and diel movement of the stream amphipod, Gammarus-minus. Freshwater Biol. 24, 509-517.

Jakobsen, P.J., Wedekind, C., 1998. Copepod reaction to odor stimuli influenced by cestode infection. Behav. Ecol. 9, 414-418.

Kaldonski, N., Perrot-Minnot, M.J., F. Cezilly., 2007. Differential influence of two acanthocephalan parasites on the antipredator behaviour of their common intermediate host. Anim. Behav. 74, 1311-1317.

Kavaliers, M., Colwell, D.D., 1995. Decreased predator avoidance in parasitized mice: neuromodulatory correlates. Parasitology 11, 257-263.

Kavaliers, M., Colwell, D.D., Choleris, E., 1998. Analgesic responses of male mice exposed to the odors of parasitized females: effects of male sexual experience and infection status. Behav. Neurosci. 112, 1001-1011.

Kullmann, H., Thünken, T., Baldauf, S.A., Bakker, T.C.M., Frommen, J.G., 2008. Fish odour triggers conspecific attraction behaviour in an aquatic invertebrate. Biol. Letters 5, 458-460.

Mathis, A., Hoback, W.W., 1997.The influence of chemical stimuli from predators on precopulatory pairing by the amphipod, *Gammarus pseudolimnaeus*. Ethology 103, 33-40.

Médoc, V., Beisel, J.-N., 2008. An acanthocephalan parasite boosts the escape performance of its intermediate host facing non-host predators. Parasitology 135, 1–8.

Moore, J., 2002. Parasites and the behaviour of animals. Oxford University Press, New York.

Moore, J., 2013. An overview of parasite-induced behavioral alterations - and some lessons from bats. J. Exp. Biol. 216, S.I.: 11-17.

Perrot-Minnot, M.J., Kaldonski, N., Cezilly. F., 2007. Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. Int. J. Parasitol. 37, 645-651.

Poulin, R., 1994. Meta-analysis of parasite-induced behavioral changes. Anim. Behav. 48, 137-146.

Thomas, F., Adamo, S., Moore. J., 2005. Parasitic manipulation: where are we and where should we go? Behav. Process. 68, 185-199.

Thünken, T., Baldauf, S.A., Bersau, N., Bakker, T.C.M., Kullmann, H., Frommen, J.G., 2010. Impact of olfactory non-host predator cues on aggregation behaviour and activity in *Polymorphus minutus* infected *Gammarus pulex*. Hydrobiologia 654, 137–145.

U.S. Environmental Protection Agency (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd ed. EPA-600/4-94-002, Cincinnati, OH.

Williams, D.D., Moore, K.A., 1985. The role of semiochemicals in benthic community relationships of the lotic amphipod *Gammarus pseudolimnaeus* – a laboratory analysis. Oikos 44, 280-286.

Wisenden, B.D., 2000. Olfactory Assessment of Predation Risk in the Aquatic Environment. Philos. T. R. Soc. B. 355, 1205-1208.

Wisenden, B.D., Cline, A., Sparkes, T.C., 1999. Survival Benefit to Antipredator Behavior in the Amphipod *Gammarus minus* (Crustacea: Amphipoda) in Response to Injury-released Chemical Cues from Conspecifics and Heterospecifics. Ethology 105, 407-414.

Wisenden, B.D., Pohlman, S.G., Watkin, E.E., 2001. Avoidance of conspecific injury-released chemical cues by free-ranging *Gammarus lacustris* (Crustacea: Amphipoda). J. Chem. Ecol. 27, 1249-1258.

Wisenden, B.D., Rugg, M.L., Korpi, N.L., Fuselier, L.C., 2009. Lab and field estimates of active time of chemical alarm cues of a cyprinid fish and an amphipod crustacean. Behaviour 146, 1423-1442.

Wudkevich, K., Wisenden, B.D., Chivers, D.P., Smith, R.J.F., 1997. Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. J. Chem. Ecol. 23, 1163-1173.

## CHAPTER 5: Host Use Patterns of *Leptorhynchoides thecatus* in Atkinson Lake, Nebraska: Comparison of Samples 30 Years Apart*


### Summary

This study compares current population metrics from a unique population of the acanthocephalan parasite *Leptorhynchoides thecatus* in Atkinson Lake, Nebraska to data from a study 30 years ago. This *L. thecatus* population was originally documented to use Green Sunfish (*Lepomis cyanellus*) and Pumpkinseed Sunfish (*Lepomis gibbosus*) as its predominant hosts; Largemouth Bass (*Micropterus salmoides*) was a less frequent host and Bluegill Sunfish (*Lepomis macrochirus*) rarely hosted viable parasites. My data on gravid female parasite prevalence confirm *L. cyanellus* and *L. gibbosus* as primary hosts. While both of these fish are suitable hosts, there are more *L. cyanellus* in the lake that provide a greater base for this parasite population. Contrary to the previous study, *L. macrochirus* had the third highest prevalence of gravid worms and *M. salmoides* hosted a single gravid female worm. The current study confirms *L. cyanellus* as the primary host and *L. gibbosus* as an equally suitable, but less frequent, host for this unique acanthocephalan population.


### Text

There are few longitudinal comparisons of parasite populations. This study compares current population metrics from a unique acanthocephalan population to the results of sampling nearly 30 years previous. The population of *Leptorhynchoides thecatus* (Kostylev) in Atkinson Lake, Nebraska differs in its host use patterns from populations in other parts of the parasite's


*Submitted as a Research Note to Journal of Parasitology
October 2013

range (Ashley and Nickol, 1989; Steinauer et al., 2007). This acanthocephalan parasitizes a wide variety of fish hosts, with centrarchid fishes as the most common hosts (Lincicome and Van Cleave, 1949). For most *L. thecatus* populations, Smallmouth Bass (*Micropterus dolomieu*) is the most frequent host, and Largemouth Bass (*Micropterus salmoides*) and Rock bass (*Ambloplites rupestris*) rank second and third (Steinauer et al., 2006). The *L. thecatus* population in Atkinson differs from others in that it is found most frequently in sunfish and has low prevalence in bass. Green Sunfish (*Lepomis cyanellus*) is the most reliable host for reproductive female worms; the congeneric Bluegill Sunfish (*Lepomis macrochirus*) does not appear to be successful hosts for reproductive worms (Ashley and Nickol, 1989).

The current study documents the persistence and current host use patterns of the Atkinson population and compares them to the findings of Ashley and Nickol (1989). Ashley and Nickol (1989) used monthly data from 1979 and 1980 and the current study uses collection data from 4 years of sampling (2008-2011). Because gravid females are the key to population persistence, I was especially interested if and how the ecological parameters associated with gravid females changed over time. I therefore focused on collecting parasites from the species and the months identified in the previous study as most likely to yield gravid females by Ashley and Nickol (1989).

Collections occurred at Atkinson Reservoir, Holt County, Nebraska (elevation 2,125 ft, at 42°33'N, 98°58'W). Collection and dissection of fishes was done in accordance with CSU Animal Care and Use protocol 11-2590A and with Nebraska Game and Parks Commission (NGPC) scientific collection permits. Parasites were collected twice each year at peak times for gravid female *L. thecatus*: April-May and October (Ashley and Nickol, 1989). One additional collection occurred in July 2011 because of very low fish numbers in the May 2011 sample. A

10.7m seine net was used to collect young-of-year (5-20cm) *L. cyanellus*, *L. gibbosus*, *L. macrochirus*, and *M. salmoides*. Every *L. gibbosus* (3 fish) and *L. cyanellus* captured was kept for dissection because these species were likely to harbor gravid worms. Most *L. macrochirus* and *M. salmoides* captured were returned to the lake, but a small number were retained for dissection. Fewer of these two species were kept because they were less likely to harbor gravid female worms.

Worms were collected from fishes within 24 hours of capture. Fishes were identified to species. The designation *Lepomis* sp. was used for cases in which it was not possible to determine the species because adult coloration was not present in young sunfish, or if the fish was a *L. cyanellus* - *L. macrochirus* hybrid (Tomelleri and Eberle, 1990). Each fish was euthanized and dissected. Worms were removed from the intestinal caeca and anterior intestine, and reproductive condition recorded. Further confirmation of gravid status occurred in the laboratory.

Data from each collection were tabulated and population statistics calculated for each month as defined by Margolis et al. (1982) and Bush et al. (1997), and consistent with Ashley and Nickol (1989). Because I sampled in April, May, July, and October, comparisons were made only for these months. Graphs for population metrics from these months were formatted in the style of Ashley and Nickol (1989).

In the course of this study 132 fish were inspected for *L. thecatus*. Overall, 69% of fish were infected with 1-16 *L. thecatus* with an intensity of 2.4 (± 2.7) worms. *Leptorhynchoides thecatus* prevalence was highest in *L. gibbosus* (n=3, 1.00), followed by *L. cyanellus* (n=47, 0.94, 95% Confidence Interval: 87-100%), *Lepomis* sp. (n= 26, 0.67, 95% CI: 47-86%), *M. salmoides* (n=15, 0.53, 95% CI: 27-79%), and *L. macrochirus* (n=41, 0.46, 95% CI: 29-

59%)(summarized in Table I). The fish species that hosted the highest percentage of gravid females were, in decreasing order: *L. cyanellus* (*n*♀ worms = 100, 37% gravid), *Lepomis* sp. (*n*♀ worms = 24, 33% gravid), *L. gibbosus* (*n*♀ worms = 7, 29% gravid), *L. macrochirus* (*n*♀ worms = 20, 15% gravid) and *M. salmoides* (*n*♀ worms = 7, 0 gravid) (Table II).

These results indicate that *L. thecatus* continues to inhabit Atkinson Reservoir and the pattern of hosts of highest prevalence is consistent between studies. In both studies, the majority of worms and gravid females are found in *L. cyanellus* and *L. gibbosus* (Table II). There is some possible shift in prevalence of secondary hosts between *M. salmoides* and *L. macrochirus*.

The *L. thecatus* in Atkinson Lake between 2008 and 2011 were most prevalent and most likely to reach patency in *L. cyanellus* and *L. gibbosus* (Table II, Figures 2&3). Overall, prevalence and gravid female prevalence for *L. gibbosus* was similar to that of *L. cyanellus*, but these measures fail to encompass the importance of each species in the persistence of the parasite population. Only three *L. gibbosus* were captured in four years and these were larger, more mature fish than most *L. cyanellus* sampled. Among the hosts with lower prevalences, prevalence was slightly higher for *M. salmoides* than *L. macrochirus,* but *M. salmoides* had a lower mean intensity (Table I). More importantly, *L. macrochirus* hosted a small number of gravid females, while no gravid female worms were found in *M. salmoides* (Table II).

I contend that *L. cyanellus* remains the most important fish species for this *L. thecatus* population. *Lepomis gibbosus* was slightly more likely to harbor gravid females but this host species was far less abundant, suggesting that the *L. gibbosus* population was less important for maintaining the parasite population. Without a comprehensive sampling effort to yield a larger number of *L. gibbosus*, it is not possible to calculate a reliable metric of relative flow rates for these species (per Holmes et al., 1977; Ashley and Nickol, 1989; Rauque et al., 2003).

Across fish species, the prevalence and intensity of *L. thecatus* in this study closely match those described by Ashley and Nickol (1989) for 1979-1980. The monthly calculations of prevalence are strikingly similar between studies (Figure 1). *Lepomis cyanellus* continues to be the most commonly infected species and is the host species most likely to harbor gravid female worms (Figure 2). *Lepomis gibbosus* was a second reliable host in the 1979-1980 data. All three *L. gibbosus* I captured were infected with multiple worms and had a similar mean intensity *L. cyanellus* (Table II). Based on previous data and my small sample, it is reasonable to assume that *L. gibbosus* continues to be a high quality host that supports gravid females worms (Ashley and Nickol, 1989), but its population size may not be sufficient to sustain the parasite in the absence of other hosts.

When examining the occurrence of gravid females, there are a few notable differences between these two studies. In 1979-1980, *M. salmoides* exhibited high overall prevalence and intensity of both non-reproductive and gravid female worms. Ashley and Nickol (1989 – Table III, pg 47) calculated that *M. salmoides* was the largest contributor to egg production for this *L. thecatus* population during the month of May, and in April and October matched or exceeded *L. gibbosus* in this metric. In contrast, I did not find a single gravid female in any of the *M. salmoides* from corresponding months. *Lepomis macrochirus* showed differences in parasite numbers between the two studies as well. Whereas Ashley and Nickol (1989) found *L. macrochirus* the least suitable of the four fish species for *L thecatus* and only 1 gravid female from 139 dissected fish, I found *L. macrochirus* harbored more parasites than *M. salmoides* and 3 gravid females in 41 fish (Figure 5, Table II). In both studies the mean intensity in *L. macrochirus* was close to 2.0. Acanthocephalans are dioecious, so a low mean intensity may limit the chance of sharing a host with suitable mates and complicate egg production. While

these two data sets differ in the extent to which *M. salmoides* or *L. macrochirus* is more likely to host gravid female worms in Atkinson, both of these species are poorer quality hosts than *L. cyanellus* or *L. gibbosus*.

There are some limitations to the present study. The data were collected with the goal of obtaining gravid female worms, so there was species bias in my sampling effort. And as a result, the sample size of 132 fish is not as large as the 517 used by Ashley and Nickol (1989) and I am not able to provide an equivalent resolution of population metrics. Nonetheless, these data provide observations of the same parasite population separated by 30+ years, and the close similarities between the datasets provide important information about the long-term population dynamics of this parasite species at this location.

For instance, this study demonstrates that this *L. thecatus* population is fairly consistent in overall prevalence, gravid female prevalence, and host use patterns despite a number of changes to the lake's ecology in the period between studies. One major change in lake ecology was the stocking of fish by game agencies. *A priori*, it was suggested that stocking was a potentially detrimental perturbation to the host community for this parasite population (pers. comm. Dr. B.B. Nickol). Records from NGPC indicate that the agency stocked two host species in 1991: Bluegill (*L. macrochirus*) and Largemouth Bass (*M. salmoides*). After 1994, Channel Catfish (*Ictalurus punctatus*) was the sole fish stocked. In addition, it is highly unlikely that the definitive host community has been consistent. Ashley and Nickol (1989) did not report the ratio of *L. macrochirus* to *L. cyanellus* but I found a very high ratio my sampling. A high *L. macrochirus:L. cyanellus* ratio may be associated with an increase in hybridization rates between these fish species (Tomelleri and Eberle, 1990). It is not known whether or not these hybrids are suitable hosts for *L. thecatus*, but the hybrid question is a topic that warrants further research.

Finally, despite similarities in prevalence and gravid female prevalence, both mean intensity and relative density of *L. thecatus* in my study were lower than those documented by Ashley and Nickol (1989), particularly in April and May (Figures 1&2). The lower number of parasites per host suggests that this parasite population may not be as robust as it once was. Despite such environmental changes, this parasite population appears resilient to perturbations and consistent in its relative prevalence across host species, and one explanation for this could be that the reservoir is an open system that receives host immigrants from other areas.

This study confirms that Atkinson Reservoir continues to be a suitable ecosystem for *L. thecatus* and the parasite continues to use *L. cyanellus* as its primary host species. The population of *L. thecatus* in Atkinson Reservoir persists and the pattern of prevalence remains similar over time. The majority of worms and gravid females are found in *L. cyanellus* and *L. gibbosus*. There is some possible shift in importance of secondary hosts between *M. salmoides* and *L. macrochirus*.

Table 5.1. *Leptorhynchoides thecatus* occurrence by fish species for the study period (2008-2011).

| Species | n (fish) | Prevalence | Prevalence C.I.* | Intensity | Mean Intensity ± St. Dev.† | Relative Density |
|---|---|---|---|---|---|---|
| *L. gibbosus* | 3 | 1.00 | 100% | 3-5 | 4.3±1.2 | 4.33 |
| *L. cyanellus* | 47 | 0.94 | 87-100% | 1-16 | 4.4±3.2 | 3.82 |
| *Lepomis* sp. | 24 | 0.67 | 47-86% | 1-10 | 3.3±2.6 | 2.56 |
| *L. macrochirus* | 41 | 0.46 | 29-59% | 1-4 | 2.2±1.4 | 1.19 |
| *M. salmoides* | 30 | 0.53 | 27-79% | 1-2 | 1.4±2.6 | 0.73 |

* 95% Confidence Interval
† Standard Deviation

Table 5.2. Monthly totals of *Leptorhynchoides thecatus* from each fish species

| | *L. Cyanellus* (n* = 47) | | *Lepomis sp.* (n*=26) | | *L. macrochirus* (n* = 41) | | *M. salmoides* (n* = 15) | | *L. gibbosus* (n* = 3) | | Combined | n*=132 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month† | No. ♀ (♂) | % ♀ gravid | No. ♀ (♂) | % ♀ gravid | No. ♀ (♂) | % ♀ gravid | No. ♀ (♂) | % ♀ gravid | No. ♀ (♂) | % ♀ gravid | No. ♀ (♂) | % ♀ gravid |
| April '08 | 10 (10) | 60 | 1 (2) | 100 | 3 (4) | 0 | 2 (0) | 0 | 2 (3) | 50 | 18 (19) | 44 |
| April '79-'80 | 55 (51) | 29 | - | - | 0 (0) | - | 32 (30) | 9 | 50 (41) | 12 | 137 (122) | 18 |
| May '08-'11 | 25 (18) | 45 | 8 (9) | 25 | 3(5) | 33 | 2 (1) | 0 | 5 (3) | 20 | 54 (51) | 28 |
| May '79-'80 | 78 (66) | 13 | - | - | 2 (2) | 0 | 15 (9) | 33 | 36 (31) | 11 | 131 (108) | 15 |
| July '11 | 5 (3) | 40 | 9 (5) | 33 | 3 (1) | 0 | - | - | - | - | 17 (9) | 29 |
| July '79-'80 | 3 (7) | 33 | - | - | 4 (3) | 0 | 10 (19) | 0 | 22 (38) | 36 | 39 (67) | 23 |
| Oct '08-'11 | 49 (47) | 33 | 6 (10) | 33 | 11 (10) | 18 | 3 (2) | 0 | - | - | 68 (69) | 32 |
| Oct '79-'80 | 38 (54) | 55 | - | - | 7 (7) | 14 | 46 (51) | 20 | 39 (15) | 49 | 132 (177) | 39 |

*n = total number fish examined. Number examined each month shown in Figures 1-5.

† Shaded rows report the findings of Ashley and Nickol (1989) collected from 1979-1980 for those same months.

‡ *Lepomis* sp. includes those fish that I could not reliably determine to species. Ashley and Nickol (1989) did not report this category

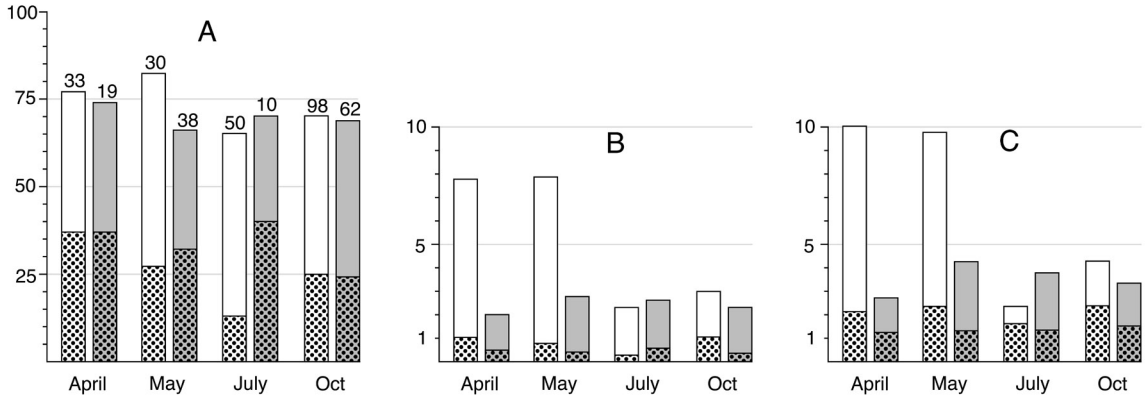


Figure 5.1. Monthly occurrence of *Leptorhynchoides thecatus* from all centrarchid fishes in Atkinson Reservoir. A, prevalence (%); B, relative density; C, mean intensity (Bush et al. 1997). Two bars represent each month: White bars depict all parameters from Ashley and Nickol (1989); grey bars depict the same month's data collected 2008-2011 and reported here. Stippled bars depict gravid females for both studies. Solid bars depict all other worms captured. Numbers above bars indicate the number of fishes sampled.

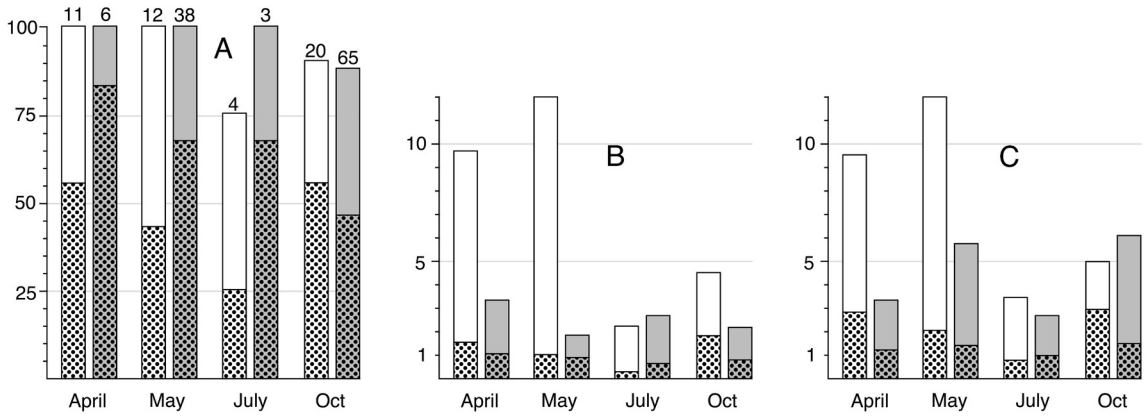


Figure 5.2. Monthly occurrence of *Leptorhynchoides thecatus* from *Lepomis cyanellus* in Atkinson Reservoir. A, prevalence (%); B, relative density; C, mean intensity (Bush et al. 1997). Two bars represent each month: White bars depict all parameters from Ashley and Nickol (1989); grey bars depict the same month's data collected 2008-2011 and reported here. Stippled bars depict gravid females for both studies. Solid bars depict all other worms captured. Numbers above bars indicate the number of fishes sampled.

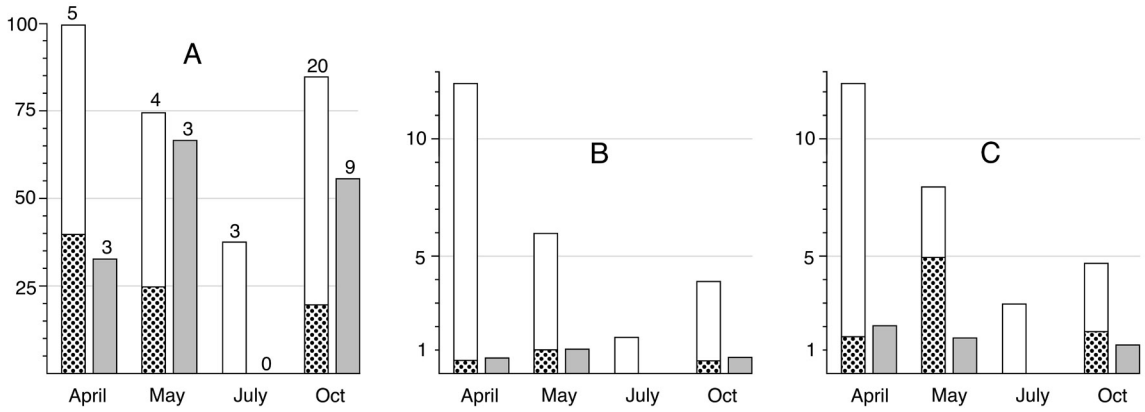


Figure 5.3. Monthly occurrence of *Leptorhynchoides thecatus* from *Micropterus salmoides* in Atkinson Reservoir. A, prevalence (%); B, relative density; C, mean intensity (Bush et al. 1997). Two bars represent each month: White bars depict all parameters from Ashley and Nickol (1989); grey bars depict the same month's data collected 2008-2011 and reported here. Stippled bars depict gravid females for both studies. Solid bars depict all other worms captured. Numbers above bars indicate the number of fishes sampled.

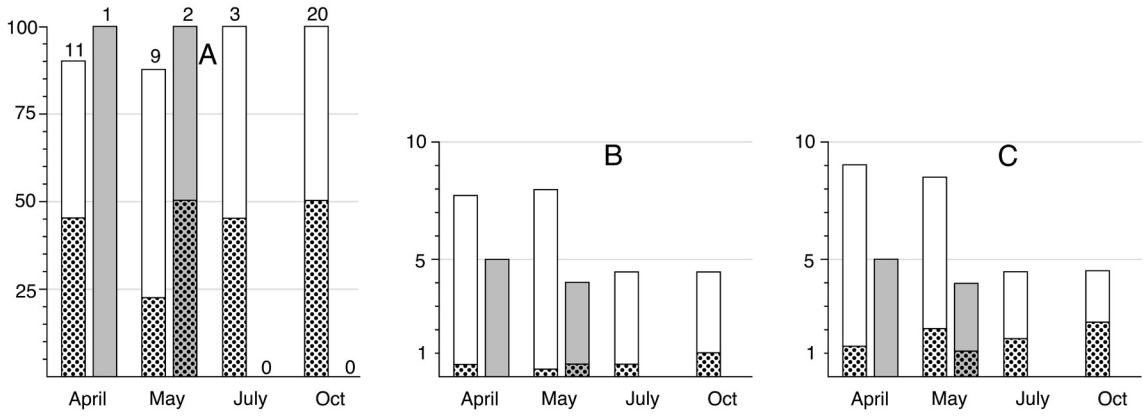


Figure 5.4. Monthly occurrence of *Leptorhynchoides thecatus* from *Lepomis gibbosus* in Atkinson Reservoir. A, prevalence (%); B, relative density; C, mean intensity (Bush et al. 1997). Two bars represent each month: White bars depict all parameters from Ashley and Nickol (1989); grey bars depict the same month's data collected 2008-2011 and reported here. Stippled bars depict gravid females for both studies. Solid bars depict all other worms captured. Numbers above bars indicate the number of fishes sampled.

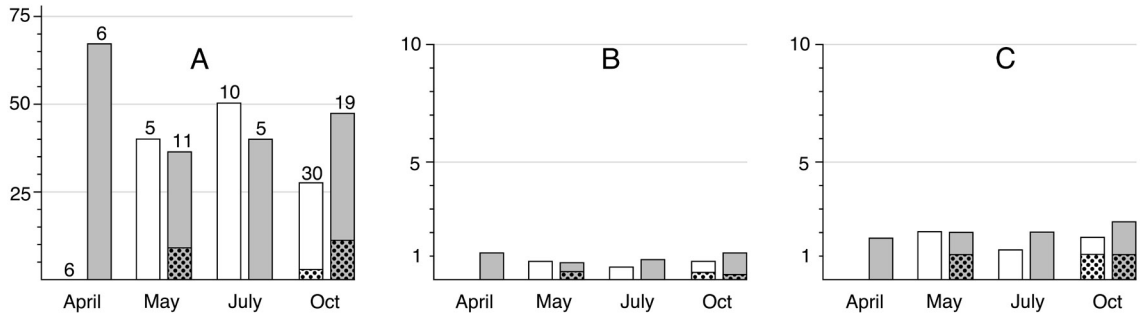


Figure 5.5. Monthly occurrence of *Leptorhynchoides thecatus* from *Lepomis macrochirus* in Atkinson Reservoir. A, prevalence (%); B, relative density; C, mean intensity (Bush et al. 1997). Two bars represent each month: White bars depict all parameters from Ashley and Nickol (1989); grey bars depict the same month's data collected 2008-2011 and reported here. Stippled bars depict gravid females for both studies. Solid bars depict all other worms captured. Numbers above bars indicate the number of fishes sampled.

## REFERENCES


Ashley, D.C., and B.B. Nickol. 1989. Dynamics of the *Leptorhynchoides thecatus* (Acanthocephala) suprapopulation in the Great Plains reservoir. Journal of Parasitology **75:** 46-54.

Bush, A.O., K.D. Lafferty, J.M. Lotz, and A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al.*, revisited. Journal of Parasitology **83:** 575-583.

Holmes, J.C., R.P. Hobbs, and T.S. Leong. 1977. Populations in perspective: Community organization and regulation of parasite populations. *In* Regulation of parasite populations, G.W. Esch (ed.) Academic Press, Inc., New York, p. 209-245.

Lincicome, D.R., and H.J. Van Cleave. 1949. Distribution of *Leptorhynchoides thecatus*, a common acanthocephalan parasitic in fishes. American Midland Naturalist **41:** 421-431.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris, and G.A. Schad. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). Journal of Parasitology **68:** 131-133.

Rauque, C.A., G.P. Viozzi, and L. G. Semenas. 2003. Component population study of *Acanthocephalus tumescans* (Acanthocephala) in fishes from Lake Moreno, Argentina. Folia Parasitologica **50:** 72-78

Steinauer M.L., J.E. Parham, and B.B. Nickol. 2006. Geographic analysis of host use, development, and habitat use of an acanthocephalan species, *Leptorhynchoides thecatus*. Journal of Parasitology **92:** 464-472.

Steinauer M.L., B.B. Nickol, and G. Orti. 2007. Cryptic speciation and patterns of phenotypic variation of a highly variable acanthocephalan parasite. Molecular Ecology **16:** 4097-4109.

Tomelleri J.R., and M.E. Eberle. 1990. Fishes of the Central United States. University Press of Kansas, Lawrence, Kansas.