DISSERTATION

PARASITOID-INDUCED BEHAVIORAL ALTERATIONS OF *AEDES AEGYPTI* MOSQUITO LARVAE INFECTED WITH MERMITHID NEMATODES (NEMATODA: MERMITHIDAE)

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MEGAN REBECCA WISE DE VALDEZ ENTITLED PARASITOID-INDUCED BEHAVIORAL ALTERATIONS OF *AEDES AEGYPTI* MOSQUITO LARVAE INFECTED WITH MERMITHID NEMATODES (NEMATODA: MERMITHIDAE) BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

PARASITOID-INDUCED BEHAVIORAL ALTERATIONS OF *AEDES AEGYPTI* MOSQUITO LARVAE INFECTED WITH MERMITHID NEMATODES (NEMATODA: MERMITHIDAE)

Parasite-induced behaviors occur in many insect species and are caused by a wide range of parasites. The majority of studies addressing parasite-induced behavioral alterations have focused on parasites with complex lifecycles and the adaptive nature of such changes. Behavioral changes caused by parasitoids, multi-cellular single-host parasites that kill their host upon emergence, are far less studied and the adaptive nature of these changes is likely to be different. In this dissertation, I investigated behavioral alterations in *Aedes aegypti* mosquito larvae infected with parasitoid nematodes (family Mermithidae). I conducted several sets of experiments.

The first set of experiments allowed me to test the following hypotheses: 1) Mermithid nematodes induce behavioral changes in mosquito larvae and the degree to which they do so depends upon intensity of infection. 2) Different species of mermithid nematodes induce similar changes in mosquito larvae behavior. 3) Behavioral alterations vary with mermithid developmental stage. 4) Mosquito larvae infected with mermithid nematodes behave similarly to uninfected food-deprived mosquito larvae. I found that *Ae. aegypti* infected with *Romanomermis culicivorax* or *Strelkovimermis spiculatus* exhibited resting behaviors significantly more than uninfected controls and that intensity of infection did not affect activity levels. The difference in behavior between infected and uninfected *Ae. aegypti* was more pronounced in *S. spiculatus* infections. Early in development, infected mosquito larvae were significantly more active than uninfected controls in some behaviors associated with feeding. Finally, there was no significant difference between infected and uninfected food-deprived mosquito behaviors. Both infected and uninfected food-deprived larvae differed significantly from well-fed uninfected larvae, however. The increase in feeding activity early in development as well as the lack of difference between food-deprived and infected *Ae. aegypti* behavior may indicate that this increase is a parasitoid adaptation that increases host feeding behaviors in order to acquire sufficient nutrients for successful parasitoid development. The activity of infected *Ae. aegypti* at specific stages in parasitoid development indicate that these behavioral alterations may be parasitoid adaptations that reduce host predation risk and thus increase pre-emergence host survival. However, without definitive evidence of increased fitness, it is difficult to distinguish between an adaptive explanation for these changes and one that indicates these behaviors may be a consequence of impaired nutrition due to infection.

In the second set of experiments I addressed the adaptive nature of mermithid-induced behavioral alterations. I focused specifically on the hypothesis that parasitoids should alter the behaviors of their hosts in ways that decrease the risk of predation, thus increasing the survival of the parasitoid to its free-living stage because my previous results indicated that infection reduced host activity levels. I compared the behaviors of infected *Ae. aegypti* to those of uninfected larvae in the presence and absence of the predatory mosquito larva, *Toxorhynchites rutilus*. Studies have shown that differences in

activity levels between species of mosquito larvae can lead to differential predation rates by *Tx. rutilus* and it is reasonable to expect parallel effects of a single species infected with mermithid nematodes. The experiments I conducted allowed me to answer the following questions: 1) Does the recovery time after an artificial alarm stimulus differ between infected and uninfected *Ae. aegypti* larvae? 2) Do infected and uninfected *Ae. aegypti* larvae facultatively switch to low-risk behaviors in the presence of *Tx. rutilus* and do they do so to a similar degree? 3) Does infection result in differential predation on *Ae. aegypti* by *Tx. rutilus*? I found that infected *Ae. aegypti* did not facultatively switch their behaviors in the presence of *Tx. rutilus* whereas uninfected controls did. This may be because infected larval behaviors in the absence of *Tx. rutilus* is already biased towards those that are low-risk When the behaviors of the infected and uninfected larvae were compared in the presence of *Tx. rutilus*, their behaviors did not differ, indicating that both groups maintain low levels of activity in the presence of a predator. These results were consistent with the results of the experiment on predation rate; there was no difference in predation rate on infected or uninfected *Ae. aegypti* by *Tx. rutilus*. Based on these results, I found no evidence that mermithid-induced behavioral alterations are adaptive in ways that benefit the parasitoid by decreasing the risk of predation.

Eventually, the study of parasite-induced changes of mosquito larvae behavior and the impact these changes could have on various trophic interactions could lead to determining possible mechanisms for how parasite-induced behavioral alterations shape community structure. Because this aspect host-parasite relationship was beyond the investigative scope of my dissertation, I reviewed literature that addresses the ecology

and parasites of container dwelling mosquitoes and the potential effect that these parasites may have on the structure of natural container communities.

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This dissertation is dedicated to

My little one

Whom I will meet in a few short weeks

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INTRODUCTION TO DISSERTATION

Parasite-induced behaviors occur in many insect species and are caused by a wide range of parasites. The majority of studies addressing parasite-induced behavioral alterations have focused on parasites with complex lifecycles and the adaptive nature of such behavioral alterations. Behavioral changes caused by parasitoids, single-host parasites that kill their host upon emergence, are studied less and the adaptive nature of behavioral changes is likely to be different. However, regardless of parasite life-history traits, without definitive evidence of fitness effects, one cannot exclude the possibility that behavioral changes may be side effects of infection. Nonetheless, to the extent that the altered behavior is a predictable part of the suite of effects induced by the parasite, then it will be subject to natural selection, along with other parasite traits.

Mermithid nematodes are parasitoids that readily infect larval mosquitoes as well as other insects. This mosquito-mermithid relationship is an accessible system with which to investigate the nature of parasite-induced behavioral alterations. In addition, because of the unique nature of parasitoid-host relationships, this system offers a perspective on the study of parasite-induced behavioral alterations that differs from that of parasites with complex life cycles that do not kill their hosts.

Phylum Nematoda: Family Mermithidae

Nematodes in the family Mermithidae are world wide in distribution and infect a wide range of invertebrate hosts in marine, freshwater, and terrestrial environments.

Mermithids have been recorded from echinoderms, chelicerates, insects, mollusks, and crustaceans, but are best studied in insects.

Two basic life cycles of mermithids exist, both of which involve free living and parasitic stages. In both cases, the parasitic stage is the only stage at which the mermithid feeds. The first, more direct life cycle involves pre-parasitic, non-feeding juveniles that hatch from eggs, and then locate and burrow into an appropriate host, where they develop. After going through several molts within the host, the post-parasitic nematode emerges, molts several more times, mates, then deposits eggs in the environment. The duration of each of these stages ranges from five days to several months depending on the species (Poinar 1983). All mermithids of mosquitoes fall into this group.

The second type of life cycle involves a paratenic host. The eggs do not hatch until they have been ingested by a paratenic host. The infective juvenile stage then hatches within the paratenic host and remains coiled up in the host tissue, initiating development after the paratenic host is ingested by the developmental host. The cycle then proceeds as above (Poinar 1983).

Within the phylum Nematoda, mermithids can be distinguished both structurally and physiologically. The main structural distinction is the degeneration of the complete digestive system as the nematode matures. During the infective stage of the mermithid, the intestine and pharynx are functional, but as the mermithid develops within the host, the pharynx and intestine separate. The pharynx becomes a stichosome that contributes to protein synthesis and the intestine becomes a solid mass of cells, called a trophosome, which serves as a food storage organ. The anus degenerates and becomes nonfunctional

(Poinar 1983). The food, which the trophosome has stored during the parasitic development stage, is then used by the free-living post-parasitic adult (Gordon 1981).

Mermithids most often have detrimental effects on their host by causing either complete tissue degeneration, or suppressed development of normal host tissues and developmental stages, presumably due to nutrient depletion (Gordon 1981). When the parasite emerges from the host, the host usually dies or is rendered sterile. Other effects on the host are behavioral alterations, sex changes, induced inter-sexuality, and decreased size (Gordon 1981).

Mermithid Nematodes of Mosquitoes

A Brief History of Research

Studies on mermithids of mosquitoes prior to the late 1960s were mainly descriptive and offered minimal information about the host-parasite interaction. During the late 1960s through the early 1980s, scientists explored the lethal effects of mermithid nematodes and applied this information to developing a biocontrol agent against mosquitoes. Much of what is known about the life cycles and host specificity of mermithid nematodes was elucidated through lab and field work of these scientists. They investigated issues such as parasitoid developmental rate, optimal conditions for development, and which and when species of mosquitoes were most susceptible (Trpiš et al., 1968, Tsai and Grundman 1969, Petersen and Willis 1970, 1972, Petersen, 1972, 1975a, b, Ignoffo et al. 1973, Gordon et al. 1974, 1981, Hughes and Platzer 1977, Harlos et al. 1980, Paily and Jayachandran 1987, Gaugler et al 1984).

Early field studies tested mermithids as a form of biocontrol and determined the efficacy of mermithids at controlling mosquito populations, as well as the persistence of mermithid populations in nature (Petersen and Willis 1970, 1972, 1974a, b, 1975). Later, field studies focused on other factors, such as environment, temperature, pollution, species susceptibility in nature, and host-parasite population dynamics (Brown et al. 1977, Gaugler et al. 1984, Galloway and Brust 1985, Paily and Jayachandran 1987). The number of biocontrol studies on mermithids and mosquitoes has decreased over the past 20 years, although new species are still being considered (Paily 1990, Camino and Garcia 1991, Paily and Balaraman 1994, 2000, Becnel and Johnson 1998).

Little work outside of biocontrol has been done with these nematodes. As a result, we know little about parasite-host ecology, behavioral ecology, and population or community ecology. However, a small number of relatively recent studies have addressed the natural distribution of many species of mosquito mermithids (Blackmore et al. 1993), and the population dynamics of several mermithid species and their hosts, as well as the host-parasite interactions in nature (Blackmore 1989, 1991, 1992, 1994, Olds et al. 1989, Blackmore and Nielsen 1990).

The majority of studies that have concerned the interaction of mosquitoes and mermithid nematodes have been conducted using the mermithid *Romanomermis culicivorax* Ross and Smith 1976 (syn. *Reesimermis nielseni*, Tsai and Grundmann 1969, in part) (Platzer 1981, Petersen 1984). *Romanomermis culicivorax* is the only species of mermithid that has been reared commercially for use in mosquito biocontrol. *Romanomermis culicivorax* naturally infects 17 species of mosquitoes and can infect an additional 78 species when exposed in the laboratory or field (Petersen and Chapman 1979). *Aedes aegypti*, a mosquito species commonly used in the laboratory for the study of *R. culicivorax*, has been successfully infected both in the laboratory and in the field (Petersen and Chapman 1979); however, natural infection has not been documented.

Life Cycles

The complete life cycles of mermithids in multivoltine mosquitoes can take anywhere from 3 to 6 weeks and in univoltine mosquitoes can take up to a year depending on the species (Petersen 1984). Mermthids of mosquitoes exhibit two types of life cycles: development within the mosquito larvae and development within the adult mosquito. In both life cycles, eggs are released into an aquatic environment. After hatching, the free-living juvenile seeks out a host and if none is found within 36-48 hours, the juvenile dies. If a suitable host is found, the pre-parasite burrows into the host using a stylet and enters the haemocoel, an activity which takes approximately five minutes (Petersen 1985).

The two types of life cycles diverge after the mermithid burrows into the host. Mermithids that develop in mosquito larvae migrate to the thoracic region and initiate development almost immediately (Petersen 1985). The development of mermithids within mosquito larvae has been most extensively studied using *Romanomermis culicivorax*. After developing slowly for three to four days post infection, mermithids undergo rapid development and molt several times, all the while depleting host metabolites (Gordon et al. 1974, Petersen 1984).

In contrast, mermithids that develop in the adult mosquito migrate to the head capsule of the larva where they await host pupation and eclosion. At this time the mermithid migrates to the abdomen of the newly emerged adult mosquito (Blackmore

1993a). If the mermithid survives its own initial growth period, the parasite emerges from the host and burrows into the soil where it matures and mates (Petersen 1984).

Distribution

More than 25 species of mermithids have been described from over 80 species of mosquitoes (Blackmore 1993a). Some host genera include *Aedes, Anopheles, Culex*, and *Culiseta* (Petersen 1975b, 1985). Mermithids naturally occurring in mosquitoes are unable to infect other aquatic insects (Ignoffo et al. 1973, Becnel and Johnson 1998). Host specificity of mosquito mermithids varies. Mermithids that develop in larval mosquitoes are less host specific than those that develop in adult mosquitoes (Petersen 1984, Blackmore 1993a). The geographic distribution of mermithids in mosquitoes is cosmopolitan, with the majority of reports being from North America and Eurasia, a fact that may reflect the relatively large amount of research done there (Blackmore 1993a).

Factors Affecting Parasitism

In nature, many factors affect parasitism of mosquitoes by mermithids. Temperature influences rate of development, life span, sex ratio, and size of mermithids (Trpiš et al. 1968, Petersen 1975a, 1985, Paily and Balaraman 1994). The range of temperatures at which mermithids can successfully infect mosquitoes varies with the species of mermithid (Hughes and Platzer 1977, Paily and Balaraman 1994). Pollutants and salinity can also affect parasitism and again, mermithid species vary in their response to these conditions (Paily and Jayachandran 1987, Camino and Garcia 1991).

Within the group of suitable mosquito hosts, susceptibility can vary (Harlos et al. 1980, Becnel and Johnson 1998, Paily and Balaraman 2000). The basis for difference in susceptibility is not always known, but may be attributed to avoidance behavior (Petersen

1975b, Woodard and Fukuda 1977), activity level of the mosquito larvae, thickness of the cuticle (Petersen 1975b) or the instar of the mosquito (Petersen and Willis 1970, Petersen 1975b). For example, when second instar larvae were exposed to *Romanomermis iyengari, Culex sitiens* was most susceptible, but when fourth instar larvae were exposed, *Armigeres subalbatus* was most susceptible (Paily and Balaraman 2000). In addition to age-related differences in susceptibility, there may be strain differences (Blackmore 1993b). Populations that have been exposed to mermithids over long periods of time show an evolutionary shift to resist parasitism through parasitoid-avoidance behavior (Woodard and Fukuda 1977).

After parasite entry, the host diet can affect mermithid development. Because mermithids acquire nutrients directly from host haemolymph and fat body (Gordon 1981), nutritionally deprived mosquito hosts restrict the nutrients available to the developing mermithid (Gordon et al. 1981). Mermithids emerging from nutrient deprived hosts are smaller and male-biased compared to mermithids emerging from well-fed mosquito hosts. Mermithid development also becomes delayed and asynchronous in nutrient-deprived hosts (Gordon et al. 1981). Other influences on mermithid development include parasite intensity (more parasites/host = more males and/or smaller mermithids), host species (less susceptible = more males and/or smaller mermithids) (Petersen 1972, Hughes and Platzer 1977, Gordon et al. 1981, Blackmore 1991, Paily and Balaraman 1994) and host sex (Harlos et al. 1980).

Development of the mermithid can be stopped completely by the encapsulation defense of the host if the mermithid does not migrate to the appropriate site immediately upon infection (Trpiš et al. 1968, Harlos et al. 1980, Gaugler et al. 1984, Blackmore

1993b, 1994). Ability to encapsulate mermithids varies among mosquito species (Blackmore 1989, 1993b, 1994, Gaugler et al. 1984, Blackmore and Neilsen 1990) and is inversely related to parasite intensity (Blackmore and Nielsen 1990).

Physiological Effects of Parasitism on Host

The most significant effect of mermithids is host mortality upon parasite emergence. Those that emerge from adults usually cause death, and when they do not, they reduce host fitness by rendering their hosts sterile (Gordon et al. 1974, Petersen 1984, Blackmore 1993a).

Mermithids can also delay host development or inhibit development of certain host structures. Mermithids that develop within adult mosquitoes may suppress ovarian development and may castrate males (Steiner 1924, Trpiš et al. 1968, Harlos et al. 1980, Galloway and Brust 1985, Blackmore 1989). During mermithid development in mosquito larvae, mermithids place significant nutritional demands on their host. Specifically, mermithids reduce fat body, deplete haemolymph proteins, and use all storage metabolites, including glycogen, of the host. Also, because mermithids cannot use trehalose as an energy source, mermithids modify host carbohydrate metabolism to produce glucose (Gordon 1981). These nutritional demands result in delayed host development and reduction in head capsule and body size, as well as the inability to develop significant fat body (Gordon et al. 1974, Gordon 1981, Petersen 1984, Galloway and Brust 1985). The severity of the effects of mermithids on their hosts depends on infection intensity (Baily and Gordon 1973).

Mosquito Larvae Behavioral Ecology

Larval Feeding Behavior

Although feeding behavior of mosquito larvae has been studied in a variety of species both in the lab and the field, ethological details remain obscure (Walker and Merritt 1991). Larval feeding behaviors can be classified into five general groups: collecting-filtering, collecting-gathering, scraping, shredding, and predatory (Clements 1999). Collecting-filtering is associated with suspended particles in the water column or surface, collecting-gathering is the re-suspension and removal of particles loosely attached to substrates, scraping is used to remove biofilm, shredding involves gnawing and biting fragments of tissue from plants, filamentous algae or dead invertebrates, and predation is the consumption of other aquatic invertebrates (may include cannibalism). Mosquito larvae usually use several of these feeding strategies with one being predominant (Clements 1999). Walker and Merritt (1991) classified mosquito larvae behavior and developed a specific list of 14 larval behaviors using fourth instar *Ochlerotatus triseriatus* larvae. Feeding behaviors included suspension feeding, feeding at the air-water interface, brushing container and leaf surfaces, and chewing leaf veins. The occurrence of specific feeding behaviors varies with the species (Yasuda and Mitsui 1992, Grill and Juliano 1996, Yee et al. 2004), the amount of food available (Juliano et al. 1993, Eisenberg et al. 2000, Yee et al. 2004), and the presence of predators (Sih 1986, Juliano and Reminger 1992, Juliano and Gravel 2002, Kesavarju and Juliano 2004).

Larval Predator Avoidance Behavior

Natural predators of mosquito larvae include hemipterans, dytiscid beetles, larval odonates, other mosquito larvae (*Toxorhychites* spp. and cannibalism), stoneflies

(Plecoptera), *Hydra*, flatworms, tadpoles, and fish (Collins and Washino 1985, Legner 1985, Sjöström 1985, Blaustein and Byard 1993, Church and Sheratt 1996, Finke et al. 1997). The importance of certain species of predators to mosquito populations is habitat-dependent (Collins and Washino 1985). Predators of mosquitoes also differ in how they feed upon mosquito larvae; some actively search while others sit and wait (Linley 1995).

Mosquitoes exhibit two categories of anti-predator behaviors: 1) avoidance mechanisms, which are usually passive and occur regardless of the presence of a predator, and 2) escape responses, which are active in response to a predator stimulus (Clements 1999). Avoidance behaviors range from taking refuge and using protective coloration to changing activity and aggregation patterns. Escape behavior most often involves rapid diving (Clements 1999).

Mosquito larvae can shift their behaviors from high risk (those associated with feeding) to low risk (resting, non-feeding behaviors) in the presence of a predator or in the presence of chemical cues associated with predation (Sih 1986, Juliano and Gravel 2002). Like feeding behaviors, predator avoidance behaviors are affected by food resource levels: as hunger increases so do high risk behaviors (Juliano et al. 1993).

Mosquito species differ in their ability to avoid predation. This can be due to species-specific differences in larval behavior or to the evolutionary relationship between predator and prey. Several studies have addressed the effect of interspecific differences in larval anti-predator behavior using *Toxorhynchites* sp., a sit and wait ambush predator. Yasuda and Mitsui (1992) showed that an active species, *Aedes albopictus*, was preyed upon more readily by *Toxorhynchites towadensis* than *Orthopodomiya anopheloides*, a less active species with strong escape responses. Similarly, the survival time of *Ae.*

aegypti, a highly active species, in the presence of *Toxorhynchites rutilus* was less than that of *Oc. triseriatus*, a less active species (Grill and Juliano 1996).

The length of evolutionary time during which predator and prey are associated can affect response to predators. Sih (1986) found that *Culex pipiens*, which has a long evolutionary history with its notonectid predator, was killed at a much lower rate than was *Ae. aegypti*, which does not normally encounter notonectids. The behavioral shifts of *Oc. triseriatus* and *A. albopictus* when exposed to *Tx. rutilus* differed; *Oc. triseriatus* adopted low-risk behaviors in the presence of *Tx. rutilus* while *Ae. albopictus* did not, presumably because *Ae. albopictus* has been recently introduced into the United States and therefore has a short evolutionary history with *Tx. rutilus* (Kesavarju and Juliano 2004).

Larval Competitive Behavior

Mosquito populations can be regulated through both intra- and interspecific competitive interactions (Chambers 1985). Competition can be mediated by species-specific response to food resources, intra- and interspecific fluctuation densities, and presence of a predator. For example, *Ae. albopictus* out-competes *Ae. aegypti* when food resources are low and densities are high in tire environments (Juliano 1998). *Aedes aegypti* is found in these environments only when alone at low density and with high resource availability (Juliano 1998). *Ochlerotatus triseriatus* is more sensitive to intra-specific densities than it is to the densities of its competitor, *Orthopodomyia signifera*, whereas *Or. signifera* was more sensitive to the interspecific densities than to its own (Chambers 1985). In addition, Grill and Juliano (1996) found that in the presence of a predator, *Ae. aegypti* failed to produce adults whereas *Oc. triseriatus* always produced adults. This indicated that *Oc. triseriatus* experienced a competitive advantage over *Ae. aegypti* in the presence of a predator, most likely due to increased predation rates on *Ae. aegypti* compared to *Oc. triseriatus.* In the absence of a predator however, neither species of mosquito had a competitive advantage. Similarly, *Toxorhynchites sp.*, a larval mosquito predator, reversed the competitive interactions between *Or. anopheloides* and *Ae. albopictus* (Yasuda 1996). *Aedes albopictus* is the superior competitor in the absence of a predator, however, when a predator is present, *Or. anopheloides* becomes the dominant mosquito species. These switches in competitive interactions are usually due differential vulnerabilities to predation.

Effect of Parasites on Mosquito Larvae Behavior

While studies of the effects of parasites on adult mosquito behavior continue to be conducted (Koella and Packer 1996, Koella et al. 1998, 2002, Anderson et al. 1999, Hurd 2003, Ferguson and Read 2004), only three studies have quantitatively addressed behavior of mosquito larvae infected with parasites (Welch 1960, Webber et al. 1987a,b). Welch (1960) presented limited quantitative evidence that *Ae. aegypti* larvae infected with *R. neilseni* (Nematoda: Mermithida) were half as active as uninfected larvae. Welch (1960) used the number of times the anal siphon of the mosquito larvae broke the surface of the water as an indicator of larval activity. Welch proposed that mermithid nutritional requirements may have an impact on mosquito larval feeding. Webber et al. (1987a) found that *Ae. aegypti* infected with more than three *Plagiorchis noblei* metacercariae spent more time at the surface of the water and less time in the bottom half of the water column than did uninfected larvae. Later, Webber et al. (1987b) found that because of this behavioral difference between infected and uninfected larvae, infected larvae were preyed upon by the parasite's definitive host (meadow vole) more often than uninfected larvae.

Parasites can mediate host competition through influencing survivorship and reproduction of both host species (Park 1948, Rouault 1979, Jaenike 1995, Yan 1996, Hudson and Greenman 1998, Yan et al. 1998, Bollache et al. 2001, Tompkins et al. 2000). Confounding factors that influence the degree to which parasites impact competition are host growth rate, host carrying capacity, susceptibility, parasite pathogenicity, and the degree of parasite aggregation (Yan 1996). Parasites can also act as predators, removing the individual from the population, thus reducing competitive interactions among the remaining hosts (Hochberg, 1991). In mosquito larvae, the effect of apicomplexan parasites on interspecific competition has been studied (Blackmore et al. 1995, Juliano 1998, Aliabadi and Juliano 2002) and ciliates have been shown to alter intraspecific competition (Washburn et al. 1991). However, neither of these host-parasite interactions affected competition via altered behaviors; rather, they released the uninfected individuals from competition by removing infected individuals from the population. Effects on competitive interactions as a result of parasite-induced behavioral alterations have been reported from several other host species (Minchella and Scott 1991 and references therein, Yan et al. 1998, Bauer et al. 2000, Feener 2000, MacNeil et al. 2003, Wellnitz et al. 2003) and there is no reason to expect their absence in mosquitoes.

Mermithid-Induced Behavioral Alterations

In Non-mosquito Hosts

Mermithid-induced behavioral alterations in insects other than mosquitoes have been reported. Maeyama et al. (1994) found that *Colobopsis* ants, which live in ant-plants, committed suicide by drowning when infected with mermithid nematodes (*Mermis* sp.). The authors hypothesize that this suicidal behavior of ants is a parasitoid adaptation that delivers it to the appropriate microhabitat (aquatic) for emergence to its free living stage.

Several studies have addressed mermithid-induced behavioral alterations in mayflies. Vance (1996a) found that not only did mermithid parasitism feminize adult male mayflies, but that these males adopted upstream dispersal and ovipositioning behavior similar to that of females. This behavioral alteration is also thought to be a parasitoid adaptation to ensure delivery to an appropriate aquatic environment for emergence.

Changes in predator avoidance behaviors of mayflies infected with mermithids have also been investigated. Vance (1996b) reported that infected mayfly larvae drifted less than uninfected larvae and were less likely to encounter fish predators. However, other studies have shown that infected mayfly larvae increased risky behaviors (Benton and Pritchard 1990, Williams et al. 2001) or were unable to escape approaching predators (Vance and Peckarsky 1997) and thus were more likely to encounter predators.

In Mosquito Hosts

Mermithid-induced behavioral alterations in mosquitoes have been noted in several studies; however, only one quantitative study has been conducted (Welch 1960). The majority of studies on mermithid-induced behavioral changes in adult mosquitoes showed that mermithid parasitism rarely affected adult behavior (Petersen et al., 1967, Tripiš et al., 1968, Harlos et al., 1980, Ewing et al., 1989, Reardon and Lunt, 1989). However, Blackmore (1994) found that infected females take blood meals less often than uninfected conspecifics. Blackmore (1994) suggested that the change in behavior may be the result of nutritional stress place upon the host during mermithid development. Doucet et al. (1979) reported reduced mating activity in infected males, but proposed no mechanism.

Studies that report behavioral changes in mermithid infected larvae are less common. Gaugler et al. (1984) reported that although there appeared to be no adverse affects on larvae of *Aedes sp.* infected with mermithids that mature in the adult mosquitoes, the pupae appeared somewhat sluggish. Shamseldean and Platzer (1989) showed that larvae become paralyzed for a few minutes after the mermithid pierces the cuticle, but then begin to clean the entry hole. In the only study that compared infected larvae to uninfected larvae, Welch (1960) found that infected mosquito larvae were half as active as uninfected mosquito larvae.

Parasite-Induced Behavioral Alterations

Adaptive Changes in Host Behavior – Benefit to the Host

Parasite-induced behavioral alterations that benefit the host include avoidance behaviors by the host prior to infection or behaviors that lead to retarding or stopping parasite development once infection occurs. Parasite avoidance behaviors are often seen in insects that are hosts to parasitoids (Gross 1993). For example, the beetle *Popillia japonica* uses aggressive and evasive behaviors such as brushing with the legs and rubbing with the abrasive raster to ward off attack by the entomopathogenic nematode, *Heterorhabditis bacteriophora* (Gaugler et al. 1994). Similarly, mosquitoes bend and groom to ward off infection by mermithid nematodes (Vance, unpublished, Welch 1960, Gaugler et al 1984, Woodard and Fukuda 1977). Checkerspot caterpillars deter braconid wasps by knocking parasitoids away from their web (Stamp 1982). Organisms that are hosts to blood sucking vectors can use physical defenses such as swatting or they can move or switch to different habitats (Moore 2002).

Once the parasite has infected the host, other types of behaviors are involved in attempts to rid the host of parasites. Both behavioral fever and behavioral chills, in which the host chooses temperatures outside the range of successful parasite development, can be used. *Musca domestica* infected with the fungus *Entomophtera muscae* and grasshoppers infected with the fungus *Beauveria bassiana* choose high temperatures (behavioral fever) to retard parasite development (Watson et al. 1993, Inglis et al. 1996). Cockroaches infected with the acanthocephalan *Moniliformis moniliformis* choose colder temperatures than uninfected cockroaches in order to suppress parasite development (Moore and Freehling 2002). Likewise, bumble bees infected with conopid parasitoids spend the night outside the nest where temperatures drop to levels below that of normal parasitoid development (Müller and Schmid-Hempel 1993).

Adaptive Changes in Host Behavior – Benefit to the Parasite

Altered host behaviors in which parasites benefit usually involve those that increase transmission or survival. Behaviors that increase transmission are varied and depend on the life-history of the host and the parasite (Moore 2002). Some of the behaviors that can be altered are the following: habitat shift, increased activity, color change, predator avoidance, phototactic behavior, and response to stimuli. In complex parasite life cycles, alterations in these behaviors may lead to increased predation by the next host in the life cycle.

An example of habitat shift is isopods infected with *Plagiorhynchus cylindraceus* that switch their habitat preference from sheltered humid areas to those that are less humid and exposed (Moore 1983). Probably as a result of this habitat shift, isopod-starling encounters increased among infected isopods, which were preyed upon more readily by starlings (the definitive host) than were uninfected conspecifics (Moore 1983). Mosquito larvae infected with more than three *Plagiorchis noblei* were found more often on the surface of the water than below; this habitat switch put them in a more favorable spot to be preyed upon by voles (Webber et al. 1987a,b).

Parasite-induced changes in activity and phototactic behavior can lead to increased predation. Amphipods infected with the trematode *Microphallus papillorobustus* were hyperactive and positively phototactic when disturbed allowing gulls to feed more readily on the infected individuals than the uninfected individuals (Helluy 1982). Bethel and Holmes (1973) showed that the inability of *Corynosoma constrictum* infected with *Polymorphus paradoxus* to move to a safe area when disturbed as well as their preference for lighted areas increased their risk of predation. Changes in these behaviors, as well as others, can benefit the parasite by enhancing transmission to the vertebrate host (Moore 2002, Hurd 2003). However, quantitative evidence supporting increased transmission is lacking.

The presence of an intermediate host and a predatory definitive host does not necessarily mean that behavioral alteration of the intermediate host will occur. A study by Knudsen et al. (2001) compared two different parasite species, a trematode and a nematode. Both species use the same amphipod/fish system; however, only the trematode altered the intermediate host behavior and was thus preyed upon by fish more readily than either uninfected or nematode infected amphiods. This suggests that parasite life history may influence the ability to alter host behavior. Host species with similar life histories may also influence the manner in which parasites alter host behavior. In a comparative study by Moore and Gotelli (1996), the effect of the acanthocephalan *Moniliformis moniliformis* on cockroach host behavior was found to be different among cockroach species.

Adaptive Changes in Host Behavior – Benefit to the Parasitoid

Differences in life histories of parasites have implications for the adaptive nature of host behavioral modifications. Parasitoids, unlike parasites with complex life cycles, have direct life cycles and usually kill their host upon emergence to their free-living stage. Therefore, adaptive behavioral modifications caused by parasitoids are likely to differ from those caused by parasites with complex life cycles. For example, behavioral modifications in which predation on the host is increased is not as likely to evolve in parasitoids; however, they might in cases of kin-selected suicide (Poulin 1992). If parasitoid-induced behavioral alterations are adaptive, parasitoids should alter host behavior in ways that enhance parasite development, ensure delivery to appropriate microhabitats for emergence (Maeyema et al. 1994, Vance 1996) or increase parasite survival (Fritz 1982, Poulin et al. 1994, Brodeur and Boivin 2004).

Hyperparasitism negatively affects developing parasitoids (Brodeur and Boivin 2004 and references therein), thus changes in behavior that decrease hyperparasitism would benefit the parasitoid. For example, aphids parasitized by diapausing parasitioid wasps left the colony to mummify in concealed locations which thus reduced hyperparasitism (Brodeur and McNeil 1989). Müller (1994) found that conopid parasitoids alter bumble bee behaviors in ways that increase their own development. Infected bumble bees dug into the soil before they died which resulted in a protected hibernation site for the parasitoid. Parasitoids emerging from bumble bees that had buried themselves were larger and heavier than those emerging from bumble bees that had not.

Parasitoids require specific habitats in which to emerge to their free-living stage. If the habitat of the host differs from the habitat needed for parasitoid emergence, parasitoids should alter the behavior of their host to ensure delivery to appropriate habitat. This change in behavior has been seen in two mermithid nematode-host systems described earlier (Maeyema et al. 1994, Vance 1996a).

Host survival is necessary for developing parasitoids. If the host dies prior to parasitoid emergence, so does the parasitoid. Therefore, parasitoids should alter the predator avoidance behaviors of their hosts in ways that reduce predation (Fritz 1982). However, it might be identical to uninfected hosts which also benefit if they avoid predation. Alteration of predator avoidance behaviors is converse to how parasites with complex life-cycles may alter predator avoidance behaviors of their hosts. Although several studies have reported that parasitoids alter predation rates on their hosts (Fritz 1982 and references therein, Benton and Pritchard 1990, Vance 1996b, Vance and Peckarsky 1997, Williams et al. 2001, Brodeur and Boivin 2004 and references therein), very few have provided definitive evidence that these behavioral alterations are parasitoid adaptations (Brodeur and Boivin 2004).

Poulin et al. (1994) indicate that the timing of parasitoid-induced behavioral alterations affects how parasitoids might benefit. They suggest that once infection occurs, the parasitoid could benefit by 1) immediately altering host behavior, 2) not altering host behavior, or 3) by altering host behaviors later in development. Bethel and Holmes (1973) noted that gammarids infected with an acanthocephalan exhibited altered predator avoidance behavior only during the stage of the acanthocephalan that was transmittable to the next host. Similarly, Koella et al. (2002) showed that *Plasmodium gallinaceum* affected the behavior of *Ae. aegypti* differently depending on its stage of development. The oocyst stage, which is not infective to the definitive host, decreased feeding activity, which is considered risky behavior. During the sporozoite stage, which is transmitted to the vertebrate host, host feeding activity was increased, and presumably enhanced transmission success.

Likewise, timing of parasitoid-induced behavioral alterations can affect how parasitoids benefit. Koinobiont parasitoids should alter their host's behaviors in times of host vulnerability (Brodeur and Boivin 2004). Foraging is an example of behavior that increases predation risk. Parasitoids that have their nutritional requirements met early in development would benefit from reducing these soon after infection. Parasitoids that require additional nutrients not already provided may benefit from reducing these behaviors later after sufficient nutrients have been acquired by their host.

Changes in Host Behavior as Side Effects of Infection

Although parasite-induced behavioral alterations as adaptations are the most evolutionarily intriguing, without definitive evidence (see Poulin 1995), pathology and stress due to infection cannot be discarded as an explanation (Holmes and Zohar 1990, Thompson 1990, Horton and Moore 1993). Several pathological effects of parasite/parasitoid infection of invertebrates that could cause a change in host behavior are organ malfunction due to direct tissue damage, modification of the neuroendocrine control system, or impaired nutrition (Holmes and Zohar 1990). In host-parasitoid systems pathological effects that may cause changes in behavior are likely due to the physiological stress parasitoids place on their hosts (Vinson and Iwantsch 1980, Slansky 1986).

Pathological effects on host behavior can be viewed in two ways. Pathology can be considered a mechanism of adaptation if it occurs routinely and has adaptive value or it can be considered an accidental alteration if there is no adaptive value. In the case of host-parasitoid interactions, this dichotomy is not easy to separate. Because parasitoids kill their host, changes in host behavior as a result of pathology are intuitively thought to be accidental side effects of a lethal infection, especially if the behavioral alterations occur close to the time of host death. However, the same pathological effects that eventually cause host death may result in altered behaviors that promote the survival of the parasitoid. Again, even with the knowledge that modified host behavior is a result of pathology, the adaptive nature of the behaviors cannot be determined without experimental evidence of increased fitness of the parasite.

Determining Adaptive Nature of Parasite-Induced Behavioral Alterations

Poulin (1995) proposed four criteria that might reveal the nature of parasite-induced behavioral alterations: 1) if behavioral alterations are complex, they are more likely to have arisen as an adaptation than by chance 2) behavioral alterations should have a purposive design, 3) behavioral alterations are likely to be adaptations if they have arisen independently in several lineages of host or parasite, and 4) parasite-induced behavioral alterations should lead to an increase in fitness of either the host or the parasite. These criteria provide a framework with which to address the adaptive nature of parasite-induced behavior alterations, although behaviors that fall outside these criteria may also be adaptations. The complexity of behavioral changes is highly subjective and behaviors that are considered simple may be the result of complex physiological mechanisms (Poulin 1995). Although behavioral modifications that arise in several different host lineages can be more easily confirmed as adaptive, those that arise within a single lineage may also be adaptations (Poulin 1995).

Brodeur and Boivin (2004) propose similar approaches to determine the adaptive nature of parasitoid-induced behavioral alterations: 1) identification of proximate mechanism and a thorough account of the pathology associated with damage inflicted by immature parasitoids, 2) assessment of fitness benefits and their variations for both host and parasitoid, and 3) study the influence of parasitoid and host phylogeny. Knowledge of the proximate mechanism of a behavior may provide evidence as to whether the behavioral modification is truly adaptive or is coincidentally beneficial (Poulin 1995). Both Poulin (1995) and Brodeur and Boivin (2004) highlight the lack of studies in which these criteria have been experimentally tested. Definitive experimental evidence addressing at least some of these criteria could support the hypothesis that parasite-induced behavioral alterations are indeed adaptations.

REFERENCES CITED

Aliabadi, B. W., and S. A. Juliano. 2002. Escape from gregarine parasites affects the competitive interactions of an invasive mosquito. Biological Invasions 4:283-297.

Anderson, R. A., J. C. Koella, and H. Hurd. 1999. The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. Proceedings of the Royal Society of London Series B-Biological Sciences 266:1729-1733.

Bailey, C. H., and R. Gordon. 1973. Histopathology of *Aedes aegypti* (Diptera-Culicidae) larvae parasitized by *Reesimermis nielseni* (Nematoda-Mermithidae). Journal of Invertebrate Pathology 22:435-441.

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

Becnel, J. J., and M. A. Johnson. 1998. Pathogenicity tests on nine mosquito species and several non-target organisms with *Strelkovimermis spiculatus* (Nemata mermithidae). Journal of Nematology 30:411-414.

Benton, M. J., and G. Pritchard. 1990. Mayfly locomotory responses to endoparasitic infection and predator presence - the effects on predator encounter Rate. Freshwater Biology 23:363-371.

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

Blackmore, M. S. 1989. The efficacy of melanotic encapsulation as a defense against parasite-induced mortality in snowpool mosquitos (Diptera, Culicidae) infected by mermithid nematodes. Canadian Journal of Zoology 67:1725-1729.

Blackmore, M. S. 1991. Host influence on interpopulation differences in size of adult *Romanomermis* (Nematoda, Mermithidae). Zoologischer Anzeiger 226:319-324.

Blackmore, M. S. 1992. Host effects on *Romanomermis* (Nematoda, Mermithidae) parasites of snowpool *Aedes* mosquitos (Diptera, Culicidae). Canadian Journal of Zoology 70:2015-2020.

Blackmore, M. S. 1993a. Ecology of mosquito-parasitic mermithid nematodes. Vector Control Bulletin 2:5-7.

Blackmore, M. S. 1993b. Strain-specific differences in susceptibility of *Aedes albopictus* to infection by mermithid and filarid nematodes. Vector Control Bulletin N. C. States 2: 67-73.

Blackmore, M. S. 1994. Mermithid parasitism of adult mosquitoes in Sweden. American Midland Naturalist 132:192-198.

Blackmore, M. S., and L. T. Nielsen. 1990. Observations on the biology of *Romanomermis* Sp (Nematoda, Mermithidae) parasites of *Aedes* in Western Wyoming. Journal of the American Mosquito Control Association 6:229-234.

Blackmore, M. S., R. L. Berry, W. A. Foster, E. D. Walker, T. R. Wilmot, and G. B. Craig. 1993. Records of mosquito-parasitic mermithid nematodes in the North-Central United-States. Journal of the American Mosquito Control Association 9:338-343.

Blackmore, M. S., G. A. Scoles, and G. B. Craig. 1995. Parasitism of *Aedes aegypti* and *Ae. albopictus* (Diptera, Culicidae) by *Ascogregarina* Spp (Apicomplexa, Lecudinidae) in Florida. Journal of Medical Entomology 32:847-852.

Blaustein, L., and R. Byard. 1993. Predation by a cyprinodontid fish, *Aphanius mento*, on *Culex pipiens* - Effects of alternative prey and vegetation. Journal of the American Mosquito Control Association 9:356-358.

Bollache, L., G. Gambade, and F. Cezilly. 2001. The effects of two acanthocephalan parasites, *Pomphorhynchus laevis* and *Polymorphus minutus*, on pairing success in male *Gammarus pulex* (Crustacea: Amphipoda). Behavioral Ecology and Sociobiology 49:296-303.

Brodeur, J., and J. N. McNeil. 1989. Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behavior. Science 244:226-228.

Brodeur, J., and G. Boivin. 2004. Functional ecology of immature parasitoids. Annual Review of Entomology 49:27-49.

Brown, B. J., E. G. Platzer, and D. S. Hughes. 1977. Field trials with the mermithid nematode *Romanomermis culicivorax* in California. Mosquito News 37:603-608.

Camino, N. B., and J. J. Garcia. 1991. Influencia de la salinidad el pH en el parasitismo de *Strelkovimermis spiculatus*. Neotropica 37:107-112.

Chambers, R. C. 1985. Competition and predation among larvae of three species of treehole-breeding mosquitoes, Pages 25-53 *in* L. P. Lounibos, J. H. Frank, and Rey, eds. Ecology of Mosquitoes. Vero Beach, FL, Florida Medical Entomology Laboratory.

Church, S. C., and T. N. Sherratt. 1996. The selective advantages of cannibalism in a Neotropical mosquito. Behavioral Ecology and Sociobiology 39:117-123.

Clements, A. N. 1999, The Biology of Mosquitoes Volume 2 Sensory Reception and Behaviour. New York, CABI Publishing.

Collins, F. H., and R. K. Washino. 1985. Insect Predators, Pages 25-41 *in* H. C. Chapman, ed. Biological Control of Mosquitoes. Fresno, CA, American Mosquito Control Association.

Doucet, M. M., C. Laumond, and O. Bain. 1979. *Empidomermis riouxi* n. sp. (Nematoda, Mermithidae). Annales De Parasitologie Humaine Et Comparee 54:341-351.

Eisenberg, J. N. S., J. O. Washburn, and S. J. Schreiber. 2000. Generalist feeding behaviors of *Aedes sierrensis* larvae and their effects on protozoan populations. Ecology 81:921-935.

Ewing, S. A., J. J. Peterson, and J. S. Afolabi. 1989. Prevalence of parasitism of adult *Aedes vexans* by a mermithid (Nematoda, Mermithidae) in Oklahoma. Journal of the American Mosquito Control Association 5:106-108.

Feener, D. J. 2000. Is the assembly of ant communities mediated by parasitoids? Oikos 90:79-88.

Ferguson, H. M., and A. F. Read. 2004. Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts. Malaria Journal 3.

Fincke, O. M., S. P. Yanoviak, and R. D. Hanschu. 1997. Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. Oecologia 112:244-253.

Fritz, R. S. 1982. Selection for host modification by insect parasitoids. Evolution 36:283-288.

Galloway, T. D., and R. A. Brust. 1985. The effects of parasitism by *Romanomermis culicivorax* (Nematoda, Mermithidae) on growth and development of *Aedes vexans* (Diptera, Culicidae) in laboratory and field-tests. Canadian Journal of Zoology 63:2437-2442.

Gaugler, R., S. Wraight, and D. Molloy. 1984. Bionomics of a mermithid parasitizing snow-pool *Aedes* spp mosquitos. Canadian Journal of Zoology 62:670-674.

Gaugler, R., Y. Wang, and J. F. Campbell. 1994. Aggressive and evasive behaviors in *Popillia japonica* (Coleoptera, Scarabaeidae) larvae - Defenses against entomopathogenic nematode attack. Journal of Invertebrate Pathology 64:193-199.

Gordon, R. 1981. Mermithid nematodes - Physiological relationships with their insect hosts. Journal of Nematology 13:266-274.

Gordon, R., C. H. Bailey, and J. M. Barber. 1974. Parasitic development of mermithid nematode *Reesimermis nielseni* in larval mosquito *Aedes aegypti*. Canadian Journal of Zoology 52:1293-&.

Gordon, R., J. M. Squires, S. J. Babie, and I. R. Burford. 1981. Effects of host diet on *Romanomermis culicivorax*, a mermithid parasite of mosquitos. Journal of Nematology 13:285-290.

Grill, C. P., and S. A. Juliano. 1996. Predicting species interactions based on behaviour: Predation and competition in container-dwelling mosquitoes. Journal of Animal Ecology 65:63-76.

Gross, P. 1993. Insect behavioral and morphological defenses against parasitoids. Annual Review of Entomology 38:251-273.

Harlos, J., R. A. Brust, and T. D. Galloway. 1980. Observations on a nematode parasite of *Aedes vexans* (Diptera, Culicidae) in Manitoba. Canadian Journal of Zoology 58:215-220.

Helluy, S. 1982. Relations hotes-parasite du trematode *Microphallus papillorobustus* (Rankin, 1940). I. Penetration des cercaires et rapports des metacercaires avec le tissu nerveux des *Gammarus*, hotes intermediaires. Annales de Parasitologie 57:263-270.

Hochberg, M. E. 1991. Population-dynamic consequences of the interplay between parasitism and intraspecific competition for host-parasite systems. Oikos 61:297-306.

Holmes, J. C., and S. Zohar. 1990. Pathology and host behaviour, Pages 35-63 *in* C. J. a. B. Barnard, J. M., ed. Parasitism and Host Behaviour. London, Taylor & Francis Ltd.

Horton, D. R., and J. K. Moore. 1993. Behavioral effects of parasites and pathogens in insect hosts, Pages 107-124 Parasites and Pathogens of Insects.

Hudson, P., and J. Greenman. 1998. Competition mediated by parasites: Biological and theoretical progress. Trends in Ecology and Evolution 13:387-390.

Hughes, D. S., and E. G. Platzer. 1977. Temperature effects on parasitic phase of *Romanomermis culicivorax* in *Culex pipiens*. Journal of Nematology 9:173-175.

Hurd, H. 2003. Manipulation of medically important insect vectors by their parasites. Annual Review of Entomology 48:141-161.

Ignoffo, C. M., K. D. Biever, W. W. Johnson, H. O. Sanders, H. C. Chapman, J. J. Petersen, and D. B. Woodard. 1973. Susceptibility of aquatic vertebrates and invertebrates to infective stage of mosquito nematode *Reesimermis nielseni.* Mosquito News 33:599-602.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1996. Effects of temperature and thermoregulation on mycosis of *Beauveria bassiana* in grasshoppers. Biological Control 7:131-139.

Jaenike, J. 1995. Interactions between mycophagous *Drosophila* and their nematode parasites - From physiological to community ecology. Oikos 72:235-268.

Juliano, S. A., and R. L. Reminger. 1992. The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. Oikos 63:465-476.

Juliano, S. A., L. J. Hechtel, and J. R. Waters. 1993. Behavior and risk of predation in larval treehole mosquitoes: effects of hunger and population history of predation. Oikos 68:229-241.

Juliano, S. A. 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? Ecology 79:255-268.

Juliano, S. A., and M. E. Gravel. 2002. Predation and the evolution of prey behavior: an experiment with tree hole mosquitoes. Behavioral Ecology 13:301-311.

Kesavaraju, B., and S. A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. Annals of the Entomological Society of America 97:194-201.

Knudsen, R., H. M. Gabler, A. M. Kuris, and P. A. Amundsen. 2001. Selective predation on parasitized prey - A comparison between two helminth species with different life-history strategies. Journal of Parasitology 87:941-945.

Koella, J. C., and M. J. Packer. 1996. Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. Parasitology 113:105-109.

Koella, J. C., L. Rieu, and R. E. L. Paul. 2002. Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. Behavioral Ecology 13:816-820.

Koella, J. C., and F. L. Sorensen. 2002. Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*. Medical and Veterinary Entomology 16:316-320.

Legner, F. E. 1985. Invertebrate predators other than insects, Pages 46-50 *in* H. C. Chapman, ed. Biological Control of Mosquitoes. Fresno, American Mosquito Control Association Inc.

Linley, J. R. 1995. Behavior on approach to surface prey by larvae of *Toxorhynchites amboinensis* and *T. brevipalpis* (Diptera, Culicidae). Journal of Medical Entomology 32:53-65.

MacNeil, C., N. J. Fielding, J. T. A. Dick, M. Briffa, J. Prenter, M. J. Hatcher, and A. M. Dunn. 2003. An acanthocephalan parasite mediates intraguild predation between invasive and native freshwater amphipods (Crustacea). Freshwater Biology 48:2085-2093.

Maeyama, T., M. Terayama, and T. Matsumoto. 1994. The abnormal behavior of *Colobopsis* sp. (Hymenoptera: Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. Sociobiology 24:115-119.

Minchella, D. J., and M. E. Scott. 1991. Parasitism - A cryptic determinant of animal community structure. Trends in Ecology & Evolution 6:250-254.

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: Ecology and Evolution. New York, Oxford University Press.

Moore, J., and N. J. Gotelli. 1996. Evolutionary patterns of altered behavior and susceptibility in parasitized hosts. Evolution 50:807-819.

Moore, J., and M. Freehling. 2002. Cockrach hosts in thermal gradients suppress parasite development. Oecologia 133:261-266.

Müller, C. B., and P. Schmidhempel. 1993. Exploitation of cold temperature as defense against parasitoids in bumblebees. Nature 363:65-67.

Müller, C. B. 1994. Parasitoid induced digging behavior in bumblebee workers. Animal Behaviour 48:961-966.

Olds, E. J., R. W. Merritt, and E. D. Walker. 1989. Sampling, seasonal abundance, and mermithid parasitism of larval *Coquillettidia perturbans* in South-Central Michigan. Journal of the American Mosquito Control Association 5:586-592.

Paily, K. P. 1990. An improved method of mass culturing of *Romanomermis iyengari*, a mermithid nematode parasite of mosquito larvae. Indian Journal of Medical Research Section a-Infectious Diseases 91:298-302.

Paily, K. P., and S. Jayachandran. 1987. Factors inhibiting parasitism of mosquito larvae by the mermithid nematode (*Romanomermis iyengari*) in a polluted habitat. Indian Journal of Medical Research 86:469-474.

Paily, K. P., and K. Balaraman. 1994. Effect of temperature on different stages of *Romanomermis iyengari*, a mermithid nematode parasite of mosquitos. Memorias Do Instituto Oswaldo Cruz 89:635-642.

Paily, K. P., and K. Balarman. 2000. Susceptibility of ten species of mosquito larvae to the parasitic nematode *Romanomermis iyengari* and its development. Medical and Veterinary Entomology 14:426-429.

Park, T. 1948. Experimental studies of interspecies competition: Competition between populations of the flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. Ecological Monographs 18:265-308.

Petersen, J. J. 1972. Factor affecting sex-ratios of a mermithid parasite of mosquitos. Journal of Nematology 4:83-&.

Petersen, J.J. 1975a. Development and fecundity of *Reesimermis nielseni*, a nematode parasite of mosquitoes. Journal of Nematology 7: 211-214.

Petersen, J.J. 1975b. Penetration and development of mermithid nematode *Reesimermis nielseni* in 18 species of mosquitos. Journal of Nematology 7:207-210.

Petersen, J. J. 1984. Nematode Parasites of Moquitoes, Pages 797-820 *in* W. R. Nickle, ed. Plant and Insect Nematodes. New York, Marcel Dekker Inc.

Petersen, J. J. 1985. Nematode Parasite, Pages 110-122 *in* H. C. Chapman, ed. Biological Control of Mosquitoes. Fresno, American Mosquito Control Association Inc.

Petersen, J. J., H. C. Chapman, and D. B. Woodard. 1967. Preliminary observations on the incidence and biology of a mermithid nematode of *Aedes sollicitans* (Walker) in Louisiana. Mosquito News 27:493-498.

Petersen, J. J., and O. R. Willis. 1970. Some factors affecting parasitism by mermithid nematodes in southern house mosquito larvae. Journal of Economic Entomology 63:175-&.

Petersen, J. J., and O. R. Willis. 1972. Results of preliminary field applications of *Reesimermis nielseni* (Mermithidae-Nematoda) to control mosquito larvae. Mosquito News 32:312-&.

Petersen, J. J., and O. R. Willis. 1974a. Experimental release of a mermithid nematode to control *Anopheles* mosquitos in Louisiana. Mosquito News 34:316-319.

Petersen, J. J., and O. R. Willis. 1974b. *Diximermis peterseni* (Nematoda - Mermithidae) - Potential biocontrol agent of *Anopheles* mosquito larvae. Journal of Invertebrate Pathology 24:20-23.

Petersen, J. J., and O. R. Willis. 1975. Establishment and recycling of a mermithid nematode for control of mosquito larvae. Mosquito News 35:526-532.

Petersen, J. J., and H. C. Chapman. 1979. Checklist of mosquito species tested against the nematode parasite *Romanomermis culicivorax*. Journal of Medical Entomology 15:468-471.

Platzer, E. G. 1981. Biological control of mosquitoes with mermithids. Journal of Nematology 13: 257-262

Poinar, G. O. 1983. Nematode parasites of invertebrates, Pages 161-202 *in* G. O. J. Poinar, ed. The Natural History of Nematodes. Englewood Cliffs, NJ, Prentice-Hall Inc.

Poulin, R. 1992. Altered behavior in parasitized bumblebees – parasite manipulation or adaptive suicide. Animal Behaviour 44:174-176.

Poulin, R. 1995. "Adaptive" changes in the behaviour of parasitized animals: A critical review. International Journal for Parasitology 25:1371-1383.

Poulin, R., J. Brodeur, and J. Moore. 1994. Parasite manipulation of host behavior - Should hosts always lose? Oikos 70:479-484.

Reardon, T. P., and S. R. Lunt. 1989. Parasitism of adult *Aedes vexans* by a mermithid (Nematoda, Mermithidae) in Iowa. Journal of the American Mosquito Control Association 5:446-447.

Ross, J. F., and S. M. Smith. 1976. A review of the mermithd parasites (Nematoda: Mermithidae) described from North American mosquitoes (Diptera: Culicidae) with descriptions of three new species. Canadian Journal of Zoology 54: 1085-1102

Rouault, J. 1979. Influence of entomophagous parasties in the competition between *Drosophila* sibling species - experimental approach. Comptes Rendus Hebdomadaires Des Seances De L. Academie Des Sciences Serie D 289:643-646.

Shamseldean, M. M., and E. G. Platzer. 1989. *Romanomermis culicivorax* - Penetration of larval mosquitos. Journal of Invertebrate Pathology 54:191-199.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434-441.

Sjöström, P. 1985. Hunting behaviour of perlid stonefly nymph *Dinocras cephalotes* (Plecoptera) under different light conditions. Animal Behaviour 33:534-540.

Slansky, F. J. 1986. Nutritional ecology of endoparasitic insects and their hosts: An overview. Journal of Insect Physiology 32:255-261.

Stamp, N. E. 1982. Behavioral interactions of parasitoids and Baltimore checkerspot caterpillars (*Euphydryas phaeton*). Environmental Entomology 11:100-104.

Steiner, G. 1924. Remarks on a mermithid found parasitic in the adult mosquito (*Aedes vexans* Meigan) in B. C. The Canadian Entomologist:161-164.

Thompson, S. N. 1990. Physiological alterations during parasitism and their effects on host behaviour, Pages 64-94 *in* C. J. Barnard, and J. M. Behnke, eds. Parasitism and Host Behaviour. London, Taylor & Francis Ltd.

Tompkins, D. M., R. A. H. Draycott, and P. Hudson. 2000. Field evidence of apparent competition mediated via the shared parasites of two game bird species. Ecology Letters 3:10-14.

Trpiš, M., W. O. Haufe, and Shemanch.Ja. 1968. Mermithid parasites of mosquito *Aedes vexans* Meigen in British Columbia. Canadian Journal of Zoology 46:1077-&.

Tsai, Y. H., and A. W., Grundmann. 1969. *Reesimermis nielseni* gen. et. Sp. n. (Nematoda - Mermithidae) parasitizing mosquitoes in Wyoming. Proceedings of the Helminthological Society of Washington 36:61-&.

Vance, S. A. 1996a. The effect of the mermithid parasite *Gasteromermis* sp (Nematoda: Mermithidae) on the drift behaviour of its mayfly host, *Baetis bicaudatus* (Ephemeroptera: Baetidae): A trade off between avoiding predators and locating food. Canadian Journal of Zoology 74:1907-1913.

Vance, S. A. 1996b. Morphological and behavioural sex reversal in mermithid-infected mayflies. Proceedings of the Royal Society of London Series B-Biological Sciences 263:907-912.

Vance, S. A., and B. L. Peckarsky. 1997. The effect of mermithid parasitism on predation of nymphal *Baetis bicaudatus* (Ephemeroptera) by invertebrates. Oecologia 110:147-152.

Vinson, S. B., and G. F. Iwantsch. 1980. Host regulation by insect parasitoids. The Quarterly Review of Biology 55:143-165.

Walker, E. D., and R. W. Merritt. 1991. Behavior of larval *Aedes triseriatus* (Diptera, Culicidae). Journal of Medical Entomology 28:581-589.

Washburn, J. O., D. R. Mercer, J. R. Anderson 1991. Regulatory role of parasites - impact on host population shifts with resource availability. Science 253:185-188.

Watson, D. W., B. A. Mullens, and J. J. Petersen. 1993. Behavioral fever response of *Musca domestica* (Diptera, Muscidae) to infection by *Entomophthora muscae* (Zygomycetes, Entomophthorales). Journal of Invertebrate Pathology 61:10-16.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987a. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the susceptibility of *Aedes aegypti* larvae to predation by guppies (*Poecilia reticulata*) and meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology 65:2346-2348.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987b. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the behavior of *Aedes aegypti* Larvae. Canadian Journal of Zoology 65:1340-1342.

Welch, H. E. 1960. *Hydromermis churchillensis* n. sp. (Nematoda: Mermithidae) a parasite of *Aedes communis* (DeG.) from Churchill, Manitoba with observation of its incidence and bionomics. Canadian Journal of Zoology 38:466-474.

Wellnitz, T., L. Giari, B. Maynard, and B. S. Dezfuli. 2003. A parasite spatially structures its host population. Oikos 100:263-268.

Williams, J. K., C. R. Townsend, and R. Poulin. 2001. Mermithid nematode infections and drift in the mayfly *Deleatidium* spp. (Ephemeroptera). Journal of Parasitology 87:1225-1227.

Woodard, D. B., and T. Fukuda. 1977. Laboratory resistance of mosquito *Anopheles quadrimaculatus* to mermithid nematode *Diximermis peterseni*. Mosquito News 37:192-195.

Yan, G. Y. 1996. Parasite-mediated competition: A model of directly transmitted macroparasites. American Naturalist 148:1089-1112.

Yan, G. Y., and L. Stevens. 1995. Selection by parasites on components of fitness in Tribolium beetles - the effect of intraspecific competition. American Naturalist 146:795-813.

Yan, G. Y., L. Stevens, C. J. Goodnight, and J. J. Schall. 1998. Effects of a tapeworm parasite on the competition of *Tribolium* beetles. Ecology 79:1093-1103.

Yasuda, H. 1996. Abundance and age structure of the predatory mosquito larva, *Toxorhynchites towadensis* (Dipt, Culicidae) in relation to prey community structure. Journal of Applied Entomology 120:291-295.

Yasuda, H., and T. Mitsui. 1992. Laboratory observations of prey behavior for prey acquisition by a predator among 3 larval mosquitos. Ecological Research 7:179-182.

Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2004. Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Annals of the Entomological Society of America 97:720-728.

PARASITOID-INDUCED BEHAVIORAL ALTERATIONS OF *AEDES AEGYPTI* MOSQUITO LARVAE INFECTED WITH MERMITHID NEMATODES (NEMATODA: MERMITHIDAE).

ABSTRACT

A wide range of parasites are known to cause behavioral changes in their hosts and parasitized insects are especially amenable to the study of such changes. The majority of studies addressing parasite-induced behavioral alterations have focused on parasites with complex life cycles and the adaptive nature of such changes. Behavioral changes caused by parasitoids, single-host parasites that kill their host upon emergence, have been studied less and the adaptive nature of these changes is likely to be different. I investigated behavioral alterations in *Aedes aegypti* mosquito larvae infected with parasitoid nematodes (family Mermithidae). I conducted several experiments in which I tested the following hypotheses: 1) Mermithid nematodes induce behavioral changes in mosquito larvae and the degree to which they do depends on intensity of infection. 2) Different species of mermithid nematodes induce similar changes in mosquito larvae behavior. 3) Behavioral alterations vary with mermithid developmental stage. 4) Mosquito larvae infected with mermithid nematodes behave similarly to uninfected food-deprived mosquito larvae. I found that *Ae. aegypti* infected with *Romanomermis culicivorax* or *Strelkovimermis spiculatus* exhibited resting behaviors significantly more than uninfected controls but that intensity of infection did not affect activity levels.

The difference in behaviors between infected and uninfected *Ae. aegypti* were more pronounced when larvae were infected with *S. spiculatus*. Early in development, infected mosquito larvae were more active than uninfected controls in some behaviors associated with feeding. There was no significant difference between infected and uninfected food-deprived mosquito behavior in nine of the ten behaviors observed. In addition, both infected and uninfected food-deprived larvae differed significantly from well-fed uninfected larvae in resting and filter-feeding behaviors. The decrease in activity of infected *Ae. aegypti* at specific stages in parasitoid development indicates that these behavioral alterations may be parasitoid adaptations that reduce the risk of predation and thus increase host survival. The increase in feeding activity early in development as well as the lack of difference between food-deprived and infected *Ae. aegypti* behavior may also indicate that these behaviors are parasitoid adaptations that increase host feeding behaviors in order to acquire sufficient nutrients for successful parasitoid development. However, without definitive evidence of increased fitness, it is difficult to distinguish between an adaptive explanation for these changes and on that indicates these behaviors may be an accidental alteration with no adaptive value.

INTRODUCTION

Parasite-induced behavioral alterations in hosts are ubiquitous. Parasitized insects are especially amenable to the study of such changes, which may include alterations in behaviors as varied as thermal preference, mate choice, geotaxis, phototaxis, and predator avoidance. The majority of studies which investigate parasite-induced behavioral alterations in insect hosts have been conducted on parasites that have complex life cycles (see Moore 2002 for review). Far fewer studies have investigated behavioral alterations induced by parasitoids; however, parasitoid-induced behavioral alterations have been reported (Vinson and Iwantsch 1980, Fritz 1982, Slansky 1986, Brodeur and McNeil 1989, Schmid-Hempel and Müller 1991, Müller 1994, Vance 1996, Chow and Mackauer 1999). Parasitoids have both a parasitic and free-living stage. They develop within a single host which they usually kill as they emerge and become free-living. Because of inevitable host death upon parasitoid emergence, parasitoid-host relationships offer an interesting perspective in the study of parasite-induced behavioral alterations.

Nematodes in the family Mermithidae are parasitoids, some of which infect many species of mosquito larvae. *Romanomermis culicivorax*, the most well studied mermithid species, is a mosquito host generalist, naturally infecting 17 species, including several species of *Aedes, Anopheles*, and *Culex*. *Romanomermis culicivorax* is also able to infect an additional 78 species when exposed in the laboratory or field (Petersen 1984, Blackmore et al. 1993). *Strelkovimermis spiculatus,* another host general has been found naturally in mosquitoes of the genera *Aedes* and *Culex* (Becnel and Johnson 1998). Both mermithid species infect *Aedes aegypti* mosquito larvae in the laboratory but have not been isolated from natural populations. The life cycles of both *R. culicivorax* and *S.*

spiculatus in mosquito larvae involve pre-parasitic non-feeding juveniles that hatch from eggs laid in the substratum of an aquatic environment. The pre-parasitic juveniles locate and burrow into a first instar mosquito where they become parasitic and develop in synchrony with the mosquito larva. The parasitic stage is the only stage in which the mermithid feeds. Over a period of six to eight days, the mermithid goes through several molts within the mosquito larva; after this time, a post-parasitic nematode emerges, molts several more times, mates, then deposits eggs (Poinar 1983).

Mermithid-induced behavioral alterations in hosts other than mosquitoes have been studied. These studies address behavioral alterations as either adaptive (Maeyama et al. 1994, Vance 1996, Vance and Peckarsky 1996, 1997, Poulin and Latham 2002) or non-adaptive side-effects of infection (Benton and Pritchard 1990, Williams et al 2001). Maeyama and others (1994) found that ants infected with mermithids committed suicide by drowning. The authors hypothesized that the ant behavior was manipulated by the mermithids in order to bring them to the appropriate habitat for emergence. Mermithid-induced behavioral alterations in larval mayflies alter predation risk (Benton and Pritchard 1990, Vance 1996, Vance and Peckarsky 1997, Williams et al. 2001); however, only one (Vance and Peckarsky 1996) supported the hypothesis that parasitoids should alter behaviors in ways that reduce predation risk (Fritz 1982).

Although parasite-induced behavioral changes in adult mosquito feeding have been and continue to be investigated (Koella and Packer 1996, Koella et al. 1998, 2002, Anderson et al. 1999, Hurd 2003, Ferguson and Read 2004), very few studies have addressed parasite-induced behavioral alterations in larval mosquitoes (but see Welch 1969, Webber et al. 1987 a, b). Mermithid studies mirror this imbalance. Behavioral changes induced by mermithid species that develop in adult mosquitoes have been investigated (Steiner 1924, Petersen et al. 1967, Blackmore 1994), with the majority of studies showing that mermithid parasitism rarely affects adult behavior (Petersen et al. 1967, Tripiš et al. 1968, Harlos et al. 1980, Ewing et al. 1989, Reardon and Lunt 1989, but see Doucet et al. 1979, Blackmore 1994). Behavioral studies on mosquito larvae infected with mermithid nematodes are less abundant (Welch 1960, Gaugler et al. 1984, Shamseldean and Platzer 1989). In the only study that compared infected and uninfected larvae, Welch (1960) presented limited quantitative evidence that *Ae. aegypti* larvae infected with *Romanomermis neilseni* were half as active as uninfected larvae. Welch (1960) used the number of times the anal siphon of the mosquito larvae broke the surface of the water as an indicator of larval activity. The study I present here is the second study to quantitatively assess the behavioral effects of mermithids on mosquito larvae, and the first to use multiple indicators, such as frequency and duration of more than five behaviors.

Two broad hypotheses concerning the origin of parasite-induced behavioral alterations exist: 1) Parasite-induced behavioral alterations may have arisen as adaptations via natural selection where either the host or the parasite benefits, or 2) the alterations arose from pathology or stress associated with the infection (Minchella 1985; Horton and Moore 1993). As Moore (2002) points out, these hypotheses are not mutually exclusive. The former hypothesis, as it applies to parasite transmission, has been the most appealing to investigators and evidence from parasites with complex life cycles supports it (Bethel and Holmes 1973, 1977, Moore 1983, 1984). In these cases, parasites alter the behavior of their intermediate hosts in ways that increase predation by the definitive host, thus ensuring completion of the parasite's life cycle. By contrast, adaptive behavioral modifications in which predation on the host is increased are not likely to evolve in parasitoid-host relationships (but see Poulin 1992). The host does not survive the infection and in non-social insects, where kin-selected suicide is unlikely, has little influence on the evolution of the relationship other than providing food and shelter for the developing parasitoid (Vinson and Iwantsch 1980, but see Poulin 1992). Thus, if parasitoid-induced behavioral alterations are adaptive, parasitoids should alter host behavior in ways that enhance parasitoid development and survival (Fritz 1982, Poulin et al. 1994, Brodeur and Boivin 2004), or ensure delivery to appropriate microhabitats for emergence (Maeyema et al. 1994, Vance 1996).

Although parasite-induced behavioral alterations as adaptations are the most evolutionarily intriguing, without definitive evidence (see Poulin 1995), behavioral alterations as accidental side-effects with no adaptive value cannot be discarded as an explanation (Holmes and Zohar 1990, Thompson 1990, Horton and Moore 1993). Some pathological effects of parasite/parasitoid infection of invertebrates that could cause a change in host behavior include organ malfunction due to direct tissue damage, modification of the neuroendocrine control system, or impaired nutrition (Holmes and Zohar 1990). In host-parasitoid systems pathological effects are likely due to the physiological stress (namely nutritional) parasitoids place on their hosts (Slansky 1986). Pathological effects on host behavior can be viewed in two ways. Pathology can be considered a mechanism of adaptation if it occurs routinely and has adaptive value or it can be considered an accidental alteration if there is no adaptive value. In the case of host-parasitoid interactions, this dichotomy is not easy to separate. Because parasitoids kill their host, changes in host behavior as a result of pathology are intuitively thought to be accidental side effects of a lethal infection, especially if the behavioral alterations occur close to the time of host death. However, the same pathological effects that eventually cause host death may result in altered behaviors that promote the survival of the parasitoid. Even with the knowledge that modified host behavior is a result of pathology, the adaptive nature of the behaviors cannot be determined without experimental evidence of increased fitness of the parasite.

In the study of parasite-induced behavioral alterations one should consider addressing behavioral modifications as adaptations and as side-effects of infection. Likewise, one should take into account the nature of the host-parasite relationship when determining how behavioral modifications might be adaptive. I investigated parasitoid-induced behavioral alterations in *Ae. aegypti* mosquito larvae infected with mermithid nematodes. Specifically, I tested the following hypotheses: 1) Mermithid nematodes induce behavioral changes in mosquito larvae and the degree to which they do depends on infection intensity. 2) Different mermithid species induce similar changes in mosquito larvae behavior. 3) Behavioral alterations vary with mermithid developmental stage. 4) Mosquito larvae infected with mermithid nematodes behave similarly to uninfected food-deprived mosquito larvae. I then discuss possible underlying mechanisms of the behavioral alterations that I observed and their potential adaptive significance.

MATERIALS AND METHODS

Mosquito Rearing

A laboratory strain of *Ae. aegypti* mosquitoes was provided by Dr. William Black IV, Department of Microbiology, Immunology and Pathology, Colorado State University.

The strain was established by Ballinger-Crabtree et al. (1992) from a wild population collected in Ogbomosho, Nigeria, and had been maintained in culture for 15 generations prior to arriving in Dr. Black's laboratory. All subsequent generations of *Ae. aegypti* mosquitoes were reared and maintained under a 12:12 LD photoperiod at 27°C ± 2°C. Larvae were hatched under anoxic conditions in plastic shoeboxes (28cm x 15cm) filled with 2 L of tap water (larval rearing containers). Upon hatching, first instar larvae were removed and placed into separate larval rearing containers at densities of 150-200 larvae/container. Larvae were fed ground Tetra-min® Fish Food *ad libitum*. Pupae were removed and allowed to emerge in adult rearing cages (150 pupae / adult rearing cage: 28cm x 15cm x 22cm plastic box with a screen top). Adult mosquitoes were provided with sugar cubes and allowed to feed once a week on anesthetized rats (Grant number: 1 R01 AI 45573-01 Project Start Date: 06/01/99 Project End Date:03/30/04 ACUC protocol number shown on original protocol approval: 98-221A-04). After the first blood meal an oviposition site was provided. Eggs laid on filter papers were collected twice between feedings and placed in plastic bags to be used for later experiments.

Infection Procedures

All infection procedures were initiated seven days prior to behavioral experiments. *Romanomermis culicivorax* eggs were provided by Dr. Edward Platzer, University of California – Riverside and *Strelkovimermis spiculatus* eggs were provided by Dr. James J. Becnel, USDA, ARS, CMAVE, Gainesville, Florida. All mermithid eggs were stored in plastic bags of moist sand at room temperature. Nematode hatching was initiated by flooding approximately four tablespoons of egg/sand mixture with 100 mL of deionized water. After approximately four hours, the water was sampled for pre-parasitic nematodes and the number of nematodes per mL was calculated. Approximately 200 first instar (< 24 hours of age) *Ae. aegypti* mosquito larvae were transferred into each of two 7cm x 7cm round plastic containers filled with 50 mL deionized water. Approximately 1,000 pre-parasitic nematodes were then added to one of the two containers (5:1 nematode:mosquito) and the total volume of deionized water in each container was brought up to 100 mL. Both groups of *Ae. aegypti* larvae (exposed to mermithids and unexposed) were held in these containers for 12-18 hours without food and then transferred into separate larval rearing containers, where they were maintained in a manner similar to that used with stock cultures (see above). This procedure resulted in 50-60% prevalence with approximately one to three mermithids per mosquito larva. Infection was determined by observing mermithid nematodes through the cuticle of fourth instar mosquito larvae with the use of a dissecting microscope. Only *R. culicivorax* was used in the Experiment II and in Experiment III because the supplier from which *S. spiculatus* were obtained had a mass die-off of their colonies.

Experiment I – General Behaviors

I tested whether infection and intensity of infection with *R. culicivorax* influenced *Ae. aegypti* behavior. Four trials using *Ae. aegypti* mosquito larvae and *R. culicivorax* mermithid nematodes and five trials using *Ae. aegypti* larvae and *S. spiculatus* mermithid nematodes were conducted approximately five days post infection for a total of nine trials and approximately 180 mosquitoes. Each trial consisted of ten pairs of fourth instar *Ae. aegypti* mosquito larva (one infected, one uninfected), each pair in its own 4cm x 4cm plastic container of 40mL tap water and a pinch of ground Tetra-min® fish food. Prior to the addition of mosquitoes to the observation containers, infection status of each larva was determined by observing the presence or absence of a mermithid nematode through the larval cuticle. Mosquito larvae were allowed to acclimate for 20 minutes. In order to reduce the influence of overhead light on larval behavior, a black cardboard square was placed above each container.

The behavior of a single *Ae. aegypti* larva in the pair was observed at eye level for five minutes. Mosquitoes were observed consecutively, for a total observation period of ten minutes per mosquito pair. The observer did not know the infection status of the larvae due to the fact that infection cannot be seen with the naked eye. The behaviors were recorded on a hand-held tape recorder in real time. One occurrence of a behavior was recorded for every five seconds or less that the behavior was exhibited by the mosquito. If the same behavior was exhibited by the mosquito for more than five consecutive seconds, each five second interval of that behavior was recorded as an additional occurrence. The duration of time spent in a single behavior could later be assessed by counting the number of 5-second occurrences in a row.

A single observer recorded behaviors in all trials in order to maximize consistency. The behaviors that were recorded were modifications of those described by Walker and Merritt (1991; Table 1, Figure 1). Immediately after both mosquito larvae in the observation chamber had been observed, each was placed into a separate holding container for later dissection. Four to five hours after observations, each larva was dissected and infection status determined. If a larva had pupated or died by the time of dissection, the behavioral data for that larva were not used. Data were analyzed using an analysis of variance split-plot model to account for trial, container, and mermithid species effects.

Experiment II – Behaviors During Parasitoid-Host Development

I tested the hypothesis that infection with *R. culicivorax* affected the behavior of *Ae. aegypti* mosquito larva differently as the two developed. Two trials using second, third, and fourth instar *Ae. aegypti* and *R. culicivorax* mermithid nematodes were conducted two, three, and four days post infection (PI). Each trial consisted of ten pairs of *Ae. aegypti* larvae (one exposed/IN, one unexposed/UN) per day PI (total 30 per trial). At day four PI, the infection status was determined by observing the presence or absence of a mermithid nematode through the cuticle of the mosquito larvae. However, at days two and three PI, mermithid nematodes could not be seen through the cuticle; thus, IN larvae were selected randomly from the group of exposed *Ae. aegypti* and were reared to fourth instars after the test to determine infection status. The mosquito larvae were allowed to acclimate for 20 minutes. A 4 cm x 4 cm black cardboard square was placed on top of each observation container. No mosquito was observed more than once. Behavioral observations were carried out in a similar manner to those described in Experiment I with the exception of one behavioral designation. Due to the small size of the mosquito larvae at days two and three PI, mouthparts were difficult to observe, making US (underwater-still) and FS (suspension feed) indistinguishable. Therefore, a new designation, FS/US, was used when the mosquito larvae were motionless and the movement of mouthparts was not visible (Table 1).

After both mosquito larvae in the observation chamber had been observed, each was placed into a separate holding container for later dissection. Larvae observed two and three days PI were allowed to develop in their separate holding containers for two to three more days in order for the infection status to be determined. Larvae observed four days PI were dissected four to five hours after the behavioral observations had been recorded. If a larva had pupated or died by the time of dissection (in the four day PI groups), the behavioral data for that larva were not used. Data were analyzed using an analysis of variance split-plot model to account for trial, container, and day PI.

Experiment III – Food Deprivation Behaviors

In this set of experiments, I tested the hypothesis that *Ae. aegypti* larvae infected with *R. culicivorax* behaved similarly to uninfected food-deprived *Ae. aegypti*; thus, indirectly addressing nutrition as a possible underlying mechanism of parasite-induced behavioral alterations. Three trials were conducted using infected (IN), uninfected (UN), and uninfected food-deprived (FD) fourth instar *Ae. aegypti* mosquito larvae. Each trial consisted of ten observation containers, four with a pair of UN mosquitoes and six with a pair of IN and FD mosquitoes. UN and FD were not paired because they were indistinguishable. Infection and the determination of infection status were carried out as described in Experiment I.

For each trial within Experiment III, the establishment of the IN, UN and FD groups of mosquito differed in the feeding regime and the day the mosquito eggs were set to hatch. Because depriving *Ae. aegypti* mosquito larvae of adequate food delays development (Nguyen et al. 2002, personal observation), mosquito larvae used in the FD group were hatched one day prior to those in the IN and UN groups, insuring that at the time of behavior observations, all mosquitoes would be in the same instar. After the parasite exposure period, 100 larvae were removed from each group (IN, UN, and FD) and placed into larval rearing containers according to previously described methods. UN and IN mosquitoes were fed Tetra-min® fish food *ad libitum* whereas the FD mosquitoes were fed at a rate of 0.03, 0.04, 0.06, 0.06, 0.06 mg/larva for five days respectively. Measurements on larval growth, head capsule width and total length, were recorded for each group on the day of behavior observations to determine larval instar and sufficient food deprivation respectively (Christophers 1960). Mosquitoes were place between two slides and measured using a compound microscope with an ocular micrometer. Behavior observations were carried out as described in Experiment I. An analysis of variance was performed to determine if there was a significant difference in behaviors observed among IN, UN, and FD mosquito larvae.

RESULTS

Experiment I – General Behaviors

There was no trial or container effect; thus, data were analyzed treating both trial and container within trial as random variables. Infection with *R. culicivorax* significantly affected only bottom feeding behaviors of *Ae. aegypti* larvae (Figure 2a); the occurrence of bottom feeding was significantly lower in IN larvae than in UN larvae (IN mean = 1.46 times, UN mean = 4.16 times, $P \leq 0.05$) (Figure 2a). In contrast, *Ae. aegypti* larvae infected with *S. spiculatus* did exhibit significantly different behaviors from uninfected controls. The occurrences of wriggle swim (IN mean = 21.17 times, UN mean = 30.75 times), suspension feeding (IN mean = 7.69 times, UN mean = 11.79 times), bottom feeding (IN mean = 2.52 times, UN mean = 6.42 times), chewing substrate (IN mean = 0.76 times, UN mean = 1.98 times), and diving (IN mean = 2.72, UN mean = 6.21 times) were significantly less in IN than in UN larvae ($P \leq 0.05$) (Figure 2b). The occurrence of remaining still (IN mean = 30.0 times, UN mean = 12.06 times) was significantly greater in the IN than in the UN larvae ($P \leq 0.01$; Figure 2b).

Behaviors of *Ae. aegypti* infected with *R. culicivorax* did not significantly differ from behaviors of *Ae. aegypti* infected with *S. spiculatus* (Figure 2c). Therefore, data were pooled over mermithid species (IN = *R. culicivorax* + *S. spiculatus*). By analyzing the data in this manner, I was able to increase power and thus reduce type II errors. These data showed a significant behavioral difference between IN and UN larvae (Figure 2d). Filter feeding (IN mean = 9.91 times, UN mean = 11.32 times, $P \leq 0.05$), bottom feeding (IN mean = 2.02 4times, UN mean = 5.6 times, $P \leq 0.01$), chewing substrate (IN = mean 0.65 times, UN mean = 1.49 times, $P \leq 0.01$), and diving (IN mean = 4.27 times, UN mean = 5 .77 times, $P \leq 0.05$) all occurred significantly more often in UN larvae than in IN larvae. IN larvae remained motionless significantly more than UN larvae (IN mean = 26.73 times, UN mean = 17.21 times, $P \leq 0.01$; Figure 2d).

In order to determine the effect of parasite intensity on behaviors, only *Ae. aegypti* infected with *R. culicivorax* were analyzed due to the low numbers of *Ae. aegypti* infected with more than one *S. spiculatus*. I analyzed three different infection intensities; one mermithid, two mermithids, and three or more mermithids. There was no significance difference in behaviors among the infection intensities (Figure 3).

Experiment II – Behaviors during parasitoid-host development

On day two PI there was no significant difference in behaviors between IN and UN *Ae. aegypti* larvae with the exception of brushing wall (IN mean = 34.5 times, UN mean = 19 times, $P \leq 0.05$; Figure 4a). On day three PI, IN larvae exhibited significantly more wriggle swimming (IN mean = 37.73 time, UN mean = 30.64 times, $P \leq 0.05$), bottom feeding (IN mean = 5.18 times, UN mean = 2 times, $P \leq 0.01$), and diving behaviors (IN mean = 5.09 times, UN mean = 3.36 times, $P \leq 0.05$) than UN larvae

(Figure 4b). On day four PI, IN larvae exhibited significantly more wriggle swimming (IN mean = 43.92 times, UN mean = 32 times, $P \leq 0.01$) and diving (IN mean = 8.16 times, UN = 4.12 times, $P \leq 0.01$) behaviors than UN larvae (Figure 4c).

Within each infection group (IN and UN), significant differences in behaviors occurred over the course of mosquito and mermithid development (Figures 4 and 5). IN larvae on day two PI exhibited significantly less interfacial feeding, wriggle swimming, still, suspension feeding, and diving behaviors than they did on days three, four, and five PI ($P \leq 0.01$; Figure 5). UN larvae were similar in that they exhibited significantly less still, suspension feeding, and diving behaviors on day two PI than on days three, four, and five PI. However, unlike their IN counterparts, they exhibited significantly more interfacial feeding than they did on days three, four, and five days PI ($P \leq 0.01$; Figure 6).

Experiment III – Food Deprivation Behaviors

In trials one and three, FD larvae were significantly shorter (Trial 1: n=5, mean=2.9mm, range=3.5-4.5mm; Trial 3: n=5, mean=3.9mm, range=3.5-4.25mm) than IN (Trial 1: n=5, mean=5.75mm, range=5-6mm; Trial 3: n=5, mean=4.55, range=4-5mm) or UN larvae (Trial 1: n=5, mean=6.25mm, range=5.25-7mm; Trial 3: n=5, mean=4.75mm, range=4.5-5mm) ($P \leq 0.05$). In trial two, the mean total lengths of FD (n=5, mean=4.55mm, range=3.75-5mm) and UN larvae (n=5, mean=5.6mm, range=4-5mm) were not significantly different, but both were significantly shorter than IN larvae (n=5, mean=5.25, range=4.5-5.75mm) ($P \leq 0.05$). According to Christophers (1960), late third instar and early fourth instar *Ae. aegypti* head capsule width should be approximately 0.74 mm and 0.98 mm respectively. The head capsule widths of all three treatment groups at time of behavior trials fell within the range indicative of larvae that were late third instar to early fourth instar (UN: n=15, mean=0.83 mm, range = 0.55-1.0 mm; IN: n=15, mean=0.85 mm, range = 0.78-0.93 mm; FD: n=15, mean=0.82 mm, range = 0.55-0.93).

No significant differences in occurrences of wriggle swimming, interfacial feeding, brushing wall, and bottom feeding behaviors among the three treatment groups were detected (ANOVA - $\alpha \leq 0.05$; Figure 7). In four of the five behaviors where a difference in occurrence did occur (still, suspension feed, underwater suspension feed, and dive), IN and FD were not significantly different (Figure 7). IN and FD mosquito larvae exhibited significantly more still behaviors (IN mean = 11.71 times, FD = 7.19 times, $P \leq 0.05$) than did UN larvae (UN mean = 2.25 times). UN mosquito larvae exhibited significantly more suspension feeding behavior (UN mean = 17.94 times) than either IN or FD larvae (IN mean = 9.62 times, FD mean = 8.76 times, $P \leq 0.05$; Figure 7). The occurrence of underwater suspension feeding and chewing substrate was significantly higher in UN (UN means = 1.69 and 1.44 times, respectively) than in IN larvae (IN means = 0.38 and 0.1 times, respectively), but was not significantly higher than in FD larvae (FD means = 1.38 and 0.8 times, respectively; $P \leq 0.05$). Diving occurred significantly more often in UN larvae (UN mean = 7.44 times) than in FD larvae (FD mean = 4.95 times), but not significantly more than IN larvae (IN mean = 6.33 times; $P \leq 0.05$; Figure 7).

DISCUSSION

General Behaviors

Aedes aegypti infected with mermithid nematodes were less active than the uninfected controls. Infected mosquito larvae spent significantly more time motionless and significantly less time in higher activity behaviors (filter feeding, bottom feeding, chewing substrate, and diving) than did the uninfected larvae (Figure 2d). These results are consistent with Welch's (1960) conclusions that mosquito activity is hindered by mermithid infection. Parasite intensity did not have an effect on host behaviors (Figure 3). However, because of the low numbers of mosquitoes infected with higher densities of mermithids, a significant difference may not have been detected in these groups (Type II error). Several other studies have shown that parasitoid intensity has a differential impact on host behavior (Vinson and Iwantsch 1980, Slansky 1986) and Webber et al. (1987 a,b) demonstrated that *Ae. aegypti* infected with more than three *Plagiorchis noblei* metacercaria were less active than those infected with fewer than three.

There are two main hypotheses as to how parasite-induced behavioral alterations arise: 1) they may arise via natural selection where the changes in host behavior benefit either the host or the parasite or 2) they could be a result of pathology or stress caused by the parasite (Minchella 1985, Horton and Moore 1993); however, this does not resolve the problem that pathological effects on host behavior can also be considered a mechanism of adaptation if it occurs routinely and has adaptive value (Moore 2002). Either of these scenarios, including pathology as an adaptation, could explain the behavioral alterations seen in the mosquito/mermithid system. If these behavioral alterations arose through natural selection, how might parasites benefit? It is generally accepted that parasites can benefit from altered host behavior by enhancing host development and survival, thus promoting their own, or by enhancing transmission when the original host is no longer needed (Poulin et al. 1994). Given the mermithid lifecycle if alterations of mosquito larvae are adaptive, then the behavioral changes most likely to benefit the parasitoid are those that enhance host development or survival.

Adaptive Nature of Behavioral Modifications

In this study, mermithids did not retard *Ae. aegypti* growth or development. This contrasts with previous studies that show mermithid infection decreases host size and increases development time (Bailey and Gordon 1973, Gordon 1981, Gordon et al. 1981, Galloway and Brust 1985). The failure of infection to influence mosquito growth and development may be the result of different rearing methods and/or altered behavior early in development that increases nutritional gain.

Mermithid nematodes nutritionally stress their hosts (Bailey and Gordon 1973, Gordon, 1981, Gordon et al. 1981, Galloway and Brust 1985). Thus, in order for adequate development of the larval host to occur, it would be important that mermithids alter the behaviors of their host in ways that counteract nutritional stress. This is consistent with the idea presented by Thompson (1990) that parasites that compete with their host for nutrients should do so in a way that does not prevent the host from developing and therefore allows the parasite to continue its own development. One of the ways in which mermithid nematodes could do this is by increasing host feeding. I found that early in development (two and three days PI), IN larvae were significantly more active than UN larvae in two feeding behaviors (brushing wall and bottom feeding) as well as in two behaviors associated with foraging (diving and wriggle swimming) (Figure 4 a and b). However, later in development IN and UN larvae did not exhibit significant differences in feeding behaviors (Figure 4). Because the nutritional demands that mermithids place on their mosquito host do not occur until four days PI (Gordon et al. 1974), it is possible that by increasing foraging early in development, mermithids allow their host to acquire sufficient nutrients and thus develop adequate fat body to support their later development. In addition, this gain may then enable the parasite to reduce normally higher predation risk in later instars (Fincke 1997) by reducing activity. This hypothesis remains to be tested. Meanwhile, behaviors of both IN and UN *Ae. aegypti* change in similar ways as they develop (Figure 5 and 5), signifying that mosquito development itself also influences behavior.

A shift in behavior from IN being more active than UN larvae at two and three days PI (Figure 4 a and b) to less active than UN larvae at day five PI (Figure 2d) was seen. These data suggest that mermithid nematodes alter the behavior of their late-instar hosts in ways that reduce activity levels. Activity level of mosquito larvae is correlated with predation rate. Species of mosquito larvae that are considered highly active are at a higher risk of predation than less active species (Sih 1986, Juliano and Reminger 1992, Yasuda and Mitsui 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavarju and Juliano 2004). Fritz (1982) hypothesized that parasitoids should alter behaviors of their host in ways that decrease the risk of predation, thereby increasing the parasitoid's chance of survival to their free-living stage. Therefore, once feeding requirements are met, it is possible that mermithid nematodes reduce the activity levels of their mosquito hosts and thus reduce predation risk. Moreover, if early instar larvae are at a lower predation risk than later instars (Fincke 1997), then the increase in activity in early instars and a decrease in activity in later instars further supports the hypothesis that parasitoids alter behaviors of their hosts in ways that decrease predation risk.

Poulin et al. (1994) indicated that the timing of parasite-induced behavioral alterations affects how parasites might benefit. They suggested that once infection occurred, the parasite could benefit by 1) immediately altering host behavior, 2) not altering host behavior, or 3) by altering host behaviors later in development. If an early increase in feeding behavior sustains later development, then the parasitoid could benefit from reduced activity levels and reduced predation risk later in development. Evidence for adaptive stage-specific behavioral modifications has been shown in adult mosquitoes infected with *Plasmodium* (Koella et al. 2002).

Behavioral Modifications as a Consequence of Pathology and/or Stress

Although behavioral modifications of *Ae. aegypti* by mermithid nematodes could be adaptive in nature (increased foraging to meet nutritional demands or avoidance of predators), the possibility exists that these modifications are a consequence of pathology and/or stress due to infection. Pathology can be considered a mechanism of adaptation if it occurs routinely and has adaptive value. However, because mermithids kill their mosquito host, changes in host behavior as a result of pathology are often thought to be accidental side effects of a lethal infection, especially if the behavioral alterations occur close to the time of host death. However, the same pathological effects that eventually cause host death could also promote parasitoid survival.

Holmes and Zohar (1990) identified several pathological effects of infection that could result in changes in host behavior: organ malfunction due to direct tissue damage, modulation of endocrine control systems, and impaired nutrition. Organ malfunction due to direct tissue damage is most likely not a mechanism of altered host behavior in this system because *R. culicivorax* is not located within host tissues; rather it develops within the haemocoel of the mosquito larvae (Poinar 1983). Behavioral alterations via modification of endocrine control systems also seem unlikely because there has been no evidence that mermithids alter hormone levels (Gordon 1981). Impaired nutrition however, could cause behavioral alterations in *Ae. aegypti* infected with mermithid nematodes. Mermithid nematodes, like many other parasitoids, place nutritional demand on their hosts (Bailey and Gordon 1973, Gordon, 1981, Gordon et al. 1981). The fact that *Aedes vexans* infected with *R. culicivorax* resemble starved uninfected larvae in their development, growth, and failure to develop significant fat body (Galloway and Brust 1985) provides further evidence for a nutritional explanation for the behavioral changes reported here.

In order to determine if nutritional demands play a role in behavior, I compared IN larvae with FD and UN larvae. There was significant difference in five of the nine behaviors among the three treatment group. In four of the five behaviors (still, suspension feed, underwater suspension feed, and dive), IN and FD were not significantly different (Figure 7). Specifically, both IN and FD larvae exhibited significantly more still behaviors and significantly fewer suspension feeding behaviors than UN larvae. Only one of the two treatment groups differed significantly from UN in suspension feeding and diving (Figure 7). Although no significant difference in the occurrences of wriggle swimming (WS), interfacial feeding (FI), brushing wall (BW), and bottom feeding (BF) behaviors among the three treatment groups was detected, IN and FD larvae appear to be more similar than either are to UN (Figure 7). These data support the notion that impaired nutrition due to mermithid parasitism may be the underlying mechanism for behavioral modifications. Whether these behavioral modifications are adaptive remains to be tested.

Conclusion

According to Poulin (1995), four criteria should be considered when determining if parasite-induced behavioral modifications are adaptive. First, the complexity of behavioral alterations may indicate the adaptive nature. However, the complexity of behavioral changes is highly subjective and behaviors that are considered simple may be the result of complex physiological mechanisms (Poulin 1995). Therefore, it is possible that simple behaviors can also be parasite adaptations. The major trend seen in the results presented here is that the activity level of infected mosquito larvae is altered. Because activity levels of uninfected mosquito larvae are shown to decrease in response to predation (Sih 1986, Juliano and Gravel 2002) and increase in response to hunger (Juliano et al. 1993), it can be argued that changes in activity level of mosquito larvae are complex and thus may indicate adaptation.

Second, Poulin (1995) states that behavioral alterations should have a "purposive design". Increased feeding activity of the host may provide the necessary nutrients needed for successful development of the mermithid while at the same time increasing predation risk. A decrease in activity level on the other hand may reduce the risk of predation on the host at the cost of inadequate nutrient acquisition for the development of the mermithid. Both of these scenarios indicate that parasite-induced changes in mosquito larvae activity may have an adaptive function. However, without predation experiments or further food deprivation experiments it is difficult to understand if and how these changes might be adaptive.

Third, Poulin (1995) states that parasite-induced behavioral alterations are more likely to be adaptations if they have arisen independently in several lineages of host or parasite. However, this does not exclude parasite-induced behavioral alterations that have arise within a single lineage (Poulin 1995). Although mermithid-induced behavioral alterations have not been analyzed in light of phylogenetic relationships, it is possible that convergent evolution has occurred. Various behavioral alterations, ranging from habitat shift to changes in activity levels, have been caused by several species of mermithid nematodes in both the larvae and adults of several mosquito species (Shamseldean and Platzer 1989, Blackmore and Nielsen 1990, Blackmore 1994) as well as by mermithids of other insects (Benton and Pritchard 1990, Maeyama et al. 1994, Vance 1996, Vance and Peckarsky 1996,1997, Williams et al. 2001, Poulin and Latham 2002).

Finally, Poulin (1995) states that parasite-induced behavioral alterations should lead to an increase in fitness of either the host or the parasite. Because mermithid nematodes render their host incapable of reproducing, behavioral alterations should affect only mermithid fitness. Fitness of mermithids could be enhanced by increased mosquito feeding behavior when food resources are low. Gordon et al. (1981) showed that mermithid nematodes that developed within food-deprived mosquitoes were smaller and more often male than mermithids that developed within well-fed mosquito larvae. In order to accurately assess whether changes in feeding behavior affect the fitness of mermithid nematodes, further studies which address the effect of food deprivation on the behavior of infected mosquito larvae and the resulting fitness consequences would need to be conducted.

The behavioral change that occurred most consistently throughout the study was that of decreased activity in late instar infected mosquito larvae compared to uninfected controls (Figure 2, Figure 4c, Figure 7). The decrease in activity could enhance the fitness of mermithids by decreasing predation on their mosquito hosts. Mosquito larvae activity is correlated with predation rate (Sih 1986, Juliano and Reminger 1992, Yasuda and Mitsui 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavarju and Juliano 2004) and Fritz (1982) provided examples of several host-parasitoid systems in which parasitoid-induced altered behavior decreased predation risk. Additional studies that incorporate a predator are needed before one can assess the effect of mermithid-induced activity reduction on parasite survival.

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REFERENCES CITED

Anderson, R. A., J. C. Koella, and H. Hurd. 1999. The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. Proceedings of the Royal Society of London Series B-Biological Sciences 266:1729-1733.

Bailey, C. H., and R. Gordon. 1973. Histopathology of *Aedes aegypti* (Diptera-Culicidae) larvae parasitized by *Reesimermis nielseni* (Nematoda-Mermithidae). Journal of Invertebrate Pathology 22:435-441.

Becnel J. J., and M. A. Johnson. 1998. Pathogenicity tests on nine mosquito species and several non-target organisms with *Strelkovimermis spiculatus* (Nemata Mermithidae). Journal of Nematology 30: 411-414.

Ballinger-Crabtree, M. E., W. C. Black IV, and B. R. Miller. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. American Journal of Tropical Medicine and Hygiene 47:893-901.

Benton, M. J., and G. Pritchard. 1990. Mayfly locomotory responses to endoparasitic infection and predator presence - the effects on predator encounter rate. Freshwater Biology 23:363-371.

Bethel, W. M., and J. C. Holmes. 1973. Altered evasive behavior and responses to light in ampipods 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.

Blackmore, M. S. 1994. Mermithid parasitism of adult mosquitoes in Sweden. American Midland Naturalist 132:192-198.

Blackmore, M. S., and L. T. Nielsen. 1990. Observations on the biology of *Romanomermis* Sp (Nematoda, Mermithidae) parasites of *Aedes* in Western Wyoming. Journal of the American Mosquito Control Association 6:229-234.

Blackmore, M. S., R. L. Berry, W. A. Foster, E. D. Walker, T. R. Wilmot, and G. B. Craig. 1993. Records of mosquito-parasitic mermithid nematodes in the North-Central United-States. Journal of the American Mosquito Control Association 9:338-343.

Brodeur, J., and J. N. McNeil. 1989. Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behavior. Science 244:226-228.

Brodeur, J., and G. Boivin. 2004. Functional ecology of immature parasitoids. Annual Review of Entomology 49:27-49.

Chow, A., and M. MacKauer. 1999. Altered dispersal behaviour in parasitised aphids: parasitoid-mediated or pathology? Ecological Entomology 24:276-283.

Christophers, S. R. 1960, *Aedes aegypti* (L.) the yellow fever mosquito. Cambridge, Cambridge University Press.

Doucet, M. M., C. Laumond, and O. Bain. 1979. *Empidomermis riouxi* N Sp (Nematoda, Mermithidae). Annales De Parasitologie Humaine Et Comparee 54:341-351.

Ewing, S. A., J. J. Peterson, and J. S. Afolabi. 1989. Prevalence of parasitism of adult *Aedes vexans* by a mermithid (Nematoda, Mermithidae) in Oklahoma. Journal of the American Mosquito Control Association 5:106-108.

Ferguson, H. M., and A. F. Read. 2004. Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts. Malaria Journal 3.

Fincke, O. M., S. P. Yanoviak, and R. D. Hanschu. 1997. Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. Oecologia 112:244-253.

Fritz, R. S. 1982. Selection for host modification by insect parasitoids. Evolution 36:283-288.

Galloway, T. D., and R. A. Brust. 1985. The effects of parasitism by *Romanomermis-culicivorax* (Nematoda, Mermithidae) on growth and development of *Aedes vexans* (Diptera, Culicidae) in laboratory and field-tests. Canadian Journal of Zoology 63:2437-2442.

Gaugler, R., S. Wraight, and D. Molloy. 1984. Bionomics of a mermithid parasitizing snow-pool *Aedes* spp mosquitos. Canadian Journal of Zoology 62:670-674.

Gordon, R. 1981. Mermithid nematodes - Physiological relationships with their insect hosts. Journal of Nematology 13:266-274.

Gordon, R., C. H. Bailey, and J. M. Barber. 1974. Parasitic development of mermithid nematode *Reesimermis nielseni* in larval mosquito *Aedes aegypti*. Canadian Journal of Zoology 52:1293-&.

Gordon, R., J. M. Squires, S. J. Babie, and I. R. Burford. 1981. Effects of host diet on *Romanomermis culicivorax*, a mermithid parasite of mosquitos. Journal of Nematology 13:285-290.

Grill, C. P., and S. A. Juliano. 1996. Predicting species interactions based on behaviour: Predation and competition in container-dwelling mosquitoes. Journal of Animal Ecology 65:63-76.

Harlos, J., R. A. Brust, and T. D. Galloway. 1980. Observations on a nematode parasite of *Aedes vexans* (Diptera, Culicidae) in Manitoba. Canadian Journal of Zoology 58:215-220.

Holmes, J. C., and S. Zohar. 1990. Pathology and host behaviour, Pages 35-63 *in* C. J. a. B. Barnard, J. M., ed. Parasitism and Host Behaviour. London, Taylor & Francis Ltd.

Horton, D. R., and J. K. Moore. 1993. Behavioral effects of parasites and pathogens in insect hosts, Pages 107-124 Parasites and Pathogens of Insects.

Hurd, H. 2003. Manipulation of medically important insect vectors by their parasites. Annual Review of Entomology 48:141-161.

Juliano, S. A., and L. Reminger. 1992. The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. Oikos 63:465-476.

Juliano, S. A., and M. E. Gravel. 2002. Predation and the evolution of prey behavior: an experiment with tree hole mosquitoes. Behavioral Ecology 13:301-311.

Kesavaraju, B., and S. A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. Annals of the Entomological Society of America 97:194-201.

Koella, J. C., and M. J. Packer. 1996. Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. Parasitology 113:105-109.

Koella, J. C., F. L. Sorensen, and R. A. Anderson. 1998. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. Proceedings of the Royal Society of London Series B-Biological Sciences 265:763-768.

Koella, J. C., L. Rieu, and R. E. L. Paul. 2002. Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. Behavioral Ecology 13:816-820.

Maeyama, T., M. Terayama, and T. Matsumoto. 1994. The abnormal behavior of *Colobopsis* sp. (Hymenoptera: Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. Sociobiology 24:115-119.

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

Moore, J. 1983. Responses of an avian predator and its isopod prey to an acanthocephalan parasite. Ecology 64:1000-1015.

Moore, J. 1984. Altered behavioral responses in intermediate hosts - an acanthocephalan parasite strategy. American Naturalist 123:572-577.

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

Müller, C. B. 1994. Parasitoid induced digging behavior in bumblebee workers. Animal Behaviour 48:961-966.

Nguyen, D., P. Dutilleul, and M. E. Rau. 2002. Impact of nutrition and exposure to the parasite *Plagiorchis elegans* (Trematoda : Plagiorchiidae) on the development of *Aedes aegypti* (Diptera : Culicidae): Analysis by time-dependent transition probabilities. Environmental Entomology 31:54-64.

Petersen, J. J. 1984. Nematode parasites of moquitoes, Pages 797-820 *in* W. R. Nickle, ed. Plant and Insect Nematodes. New York, Marcel Dekker Inc.

Petersen, J. J., H. C. Chapman, and D. B. Woodard. 1967. Preliminary observations on the incidence and biology of a mermithid nematode of *Aedes sollicitans* (Walker) in Louisiana. Mosquito News 27:493-498.

Poinar, G. O. 1983. Nematode parasites of invertebrates, Pages 161-202 *in* G. O. J. Poinar, ed. The Natural History of Nematodes. Englewood Cliffs, NJ, Prentice-Hall Inc.

Poulin, R. 1992. Altered behavior in parasitized bumblebees - Parasite manipulation or adaptive suicide. Animal Behaviour 44:174-176.

Poulin, R. 1995. "Adaptive" changes in the behaviour of parasitized animals: A critical review. International Journal for Parasitology 25:1371-1383.

Poulin, R., J. Brodeur, and J. Moore. 1994. Parasite manipulation of host behavior - Should hosts always lose? Oikos 70:479-484.

Poulin, R., and D. M. Latham. 2002. Parasitism and the burrowing depth of the beach hopper *Talorchestia quoyana* (Amphipoda : Talitridae). Animal Behaviour 63:269-275.

Reardon, T. P., and S. R. Lunt. 1989. Parasitism of Adult *Aedes vexans* by a mermithid (Nematoda, Mermithidae) in Iowa. Journal of the American Mosquito Control Association 5:446-447.

Schmid-Hempel, R., and C. B. Müller. 1991. Do parasitized bumblebees forage for their colony?. Animal Behaviour 41:910-912.

Shamseldean, M. M., and E. G. Platzer. 1989. *Romanomermis culicivorax* - Penetration of Larval Mosquitos. Journal of Invertebrate Pathology 54:191-199.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434-441.

Slansky, F. J. 1986. Nutritional ecology of endoparasitic insects and their hosts: An overview. Journal of Insect Physiology 32:255-261.

Steiner, G. 1924. Remarks on a mermithid found parasitic in the adult mosquito (*Aedes vexans* Meigan) in B. C. The Canadian Entomologist:161-164.

Thompson, S. N. 1990. Physiological alterations during parasitism and their effects on host behaviour, Pages 64-94 *in* C. J. Barnard, and J. M. Behnke, eds. Parasitism and Host Behaviour. London, Taylor & Francis Ltd.

Trpiš, M., W. O. Haufe, and Shemanchuck, J. A. 1968. Mermithid parasites of the mosquito *Aedes vexans* Meigen in British Columbia. Canadian Journal of Zoology 46:1077

Vance, S. A. 1996. Morphological and behavioural sex reversal in mermithid-infected mayflies. Proceedings of the Royal Society of London Series B-Biological Sciences 263:907-912.

Vance, S. A., and B. L. Peckarsky. 1996. The infection of nymphal *Baetis bicaudatus* by the mermithid nematode *Gasteromermis* sp. Ecological Entomology 21:377-381.

Vance, S. A., and B. L. Peckarsky. 1997. The effect of mermithid parasitism on predation of nymphal *Baetis bicaudatus* (Ephemeroptera) by invertebrates. Oecologia 110:147-152.

Vinson, S. B., and G. F. Iwantsch. 1980. Host regulation by insect parasitoids. The Quarterly Review of Biology 55:143-165.

Walker, E. D., and R. W. Merritt. 1991. Behavior of larval *Aedes triseriatus* (Diptera, Culicidae). Journal of Medical Entomology 28:581-589.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987a. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the susceptibility of *Aedes aegypti* larvae to predation by guppies (*Poecilia reticulata*) and meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology 65:2346-2348.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987b. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the behavior of *Aedes aegypti* larvae. Canadian Journal of Zoology 65:1340-1342.

Welch, H. E. 1960. *Hydromermis churchillensis* n. sp. (Nematoda: Mermithidae) a parasite of *Aedes communis* (DeG.) from Churchill, Manitoba with observation of its incidence and bionomics. Canadian Journal of Zoology 38:466-474.

Williams, J. K., C. R. Townsend, and R. Poulin. 2001. Mermithid nematode infections and drift in the mayfly *Deleatidium* spp. (Ephemeroptera). Journal of Parasitology 87:1225-1227.

Yasuda, H., and T. Mitsui. 1992. Laboratory observations of prey behavior for prey acquisition by a predator among 3 larval mosquitoes. Ecological Research 7:179-182.

Behavior	Abbreviation	Description
Wriggle-swim	WS	Mosquito thrashes back and Forth
Still	US	Still, no movement of mouthparts
Suspension Feed	FS	Mosquito suspended by anal siphon from the surface of the container and mouthparts are moving. With or without movement of larva across surface
Suspension Feed / Still	FS/US	Either FS or US – larvae too small to determine whether mouthparts were moving. Used in development experiments.
Interfacial Feed	FI	Mosquito suspended by anal siphon from the surface of the container, body is bent with mouthparts brushing surface of the water
Underwater Suspension Feed	UF	Mosquito within the water column, mouth parts moving
Brush Wall	BW	Mosquito brushes wall of container with mouth parts
Bottom Feed	BF	Mosqutio brushes bottom of container with mouth parts
Chew Substrate	CS	Mosquito manipulates food particles with mouth parts
Dive	D	Mosquito actively dives to bottom of the container

Table 1. Behaviors of *Aedes aegypti* mosquito larvae (adapted from Walker and Merrit (1991)).

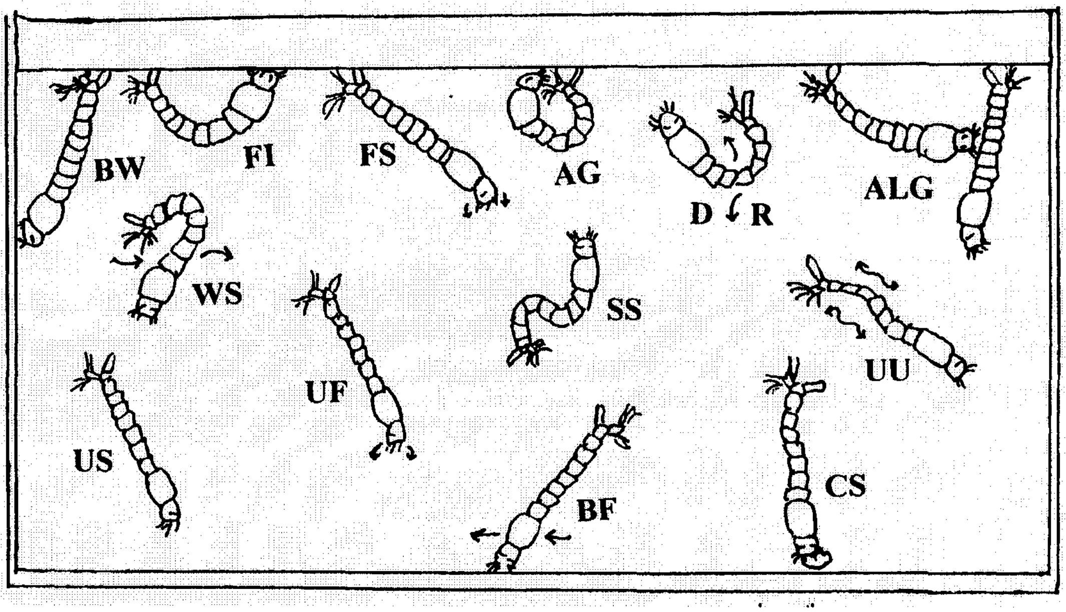
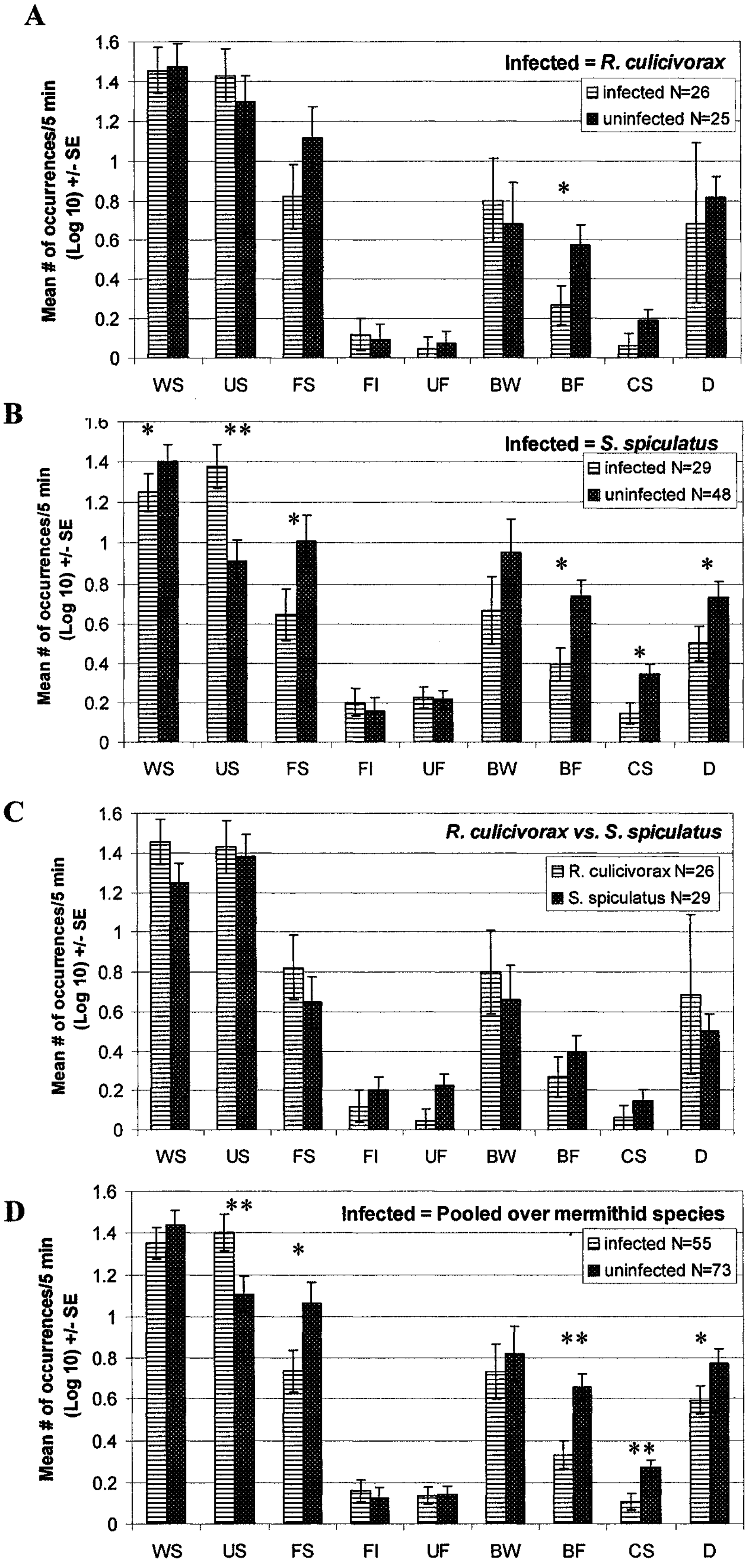


Figure 1. Schematic representation of *Ae. aegypti* mosquito larvae behavior. Adapted from Walker and Merrit 1991

Figure 2. Mean number of occurrences (Log 10 transformed) of behaviors in infected and uninfected *Aedes aegypti* mosquito larvae over the course of five minutes at five days PI. A) *Ae. aegypti* infected with *Romanomermis culicivorax*, B) *Ae. aegypti* infected with *Strelkovimermis spiculatus*, C) Comparison between *Ae. aegypti* infected with *R. culicivorax* and larvae infected with *S. spiculatus*, D) Data combined over species. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. * = $P \leq 0.05$, ** = $P \leq 0.01$.


A
Infected = *R. culicivorax*
infected N=26
uninfected N=25
Mean # of occurrences/5 min (Log 10) +/- SE
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
*
WS
US
FS
FI
UF
BW
BF
CS
D
B
Infected = *S. spiculatus*
infected N=29
uninfected N=48
Mean # of occurrences/5 min (Log 10) +/- SE
*
**
*
*
*
*
WS
US
FS
FI
UF
BW
BF
CS
D
C
R. culicivorax vs. S. spiculatus
R. culicivorax N=26
S. spiculatus N=29
Mean # of occurrences/5 min (Log 10) +/- SE
WS
US
FS
FI
UF
BW
BF
CS
D
D
Infected = Pooled over mermithid species
infected N=55
uninfected N=73
Mean # of occurrences/5 min (Log 10) +/- SE
**
*
**
*
**
WS
US
FS
FI
UF
BW
BF
CS
D

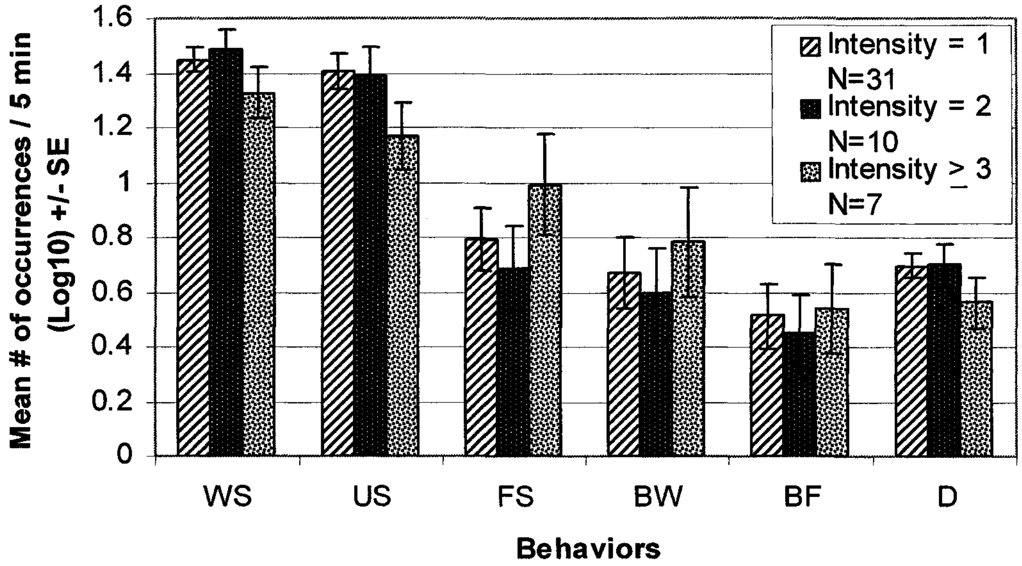


Figure 3. Mean number of occurrences (Log_{10}) of behaviors of *Aedes aegypti* infected with different intensities of *Romanomermis culicivorax* over the course of five minutes. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; BW, brush wall; BF, bottom feed; D, dive.

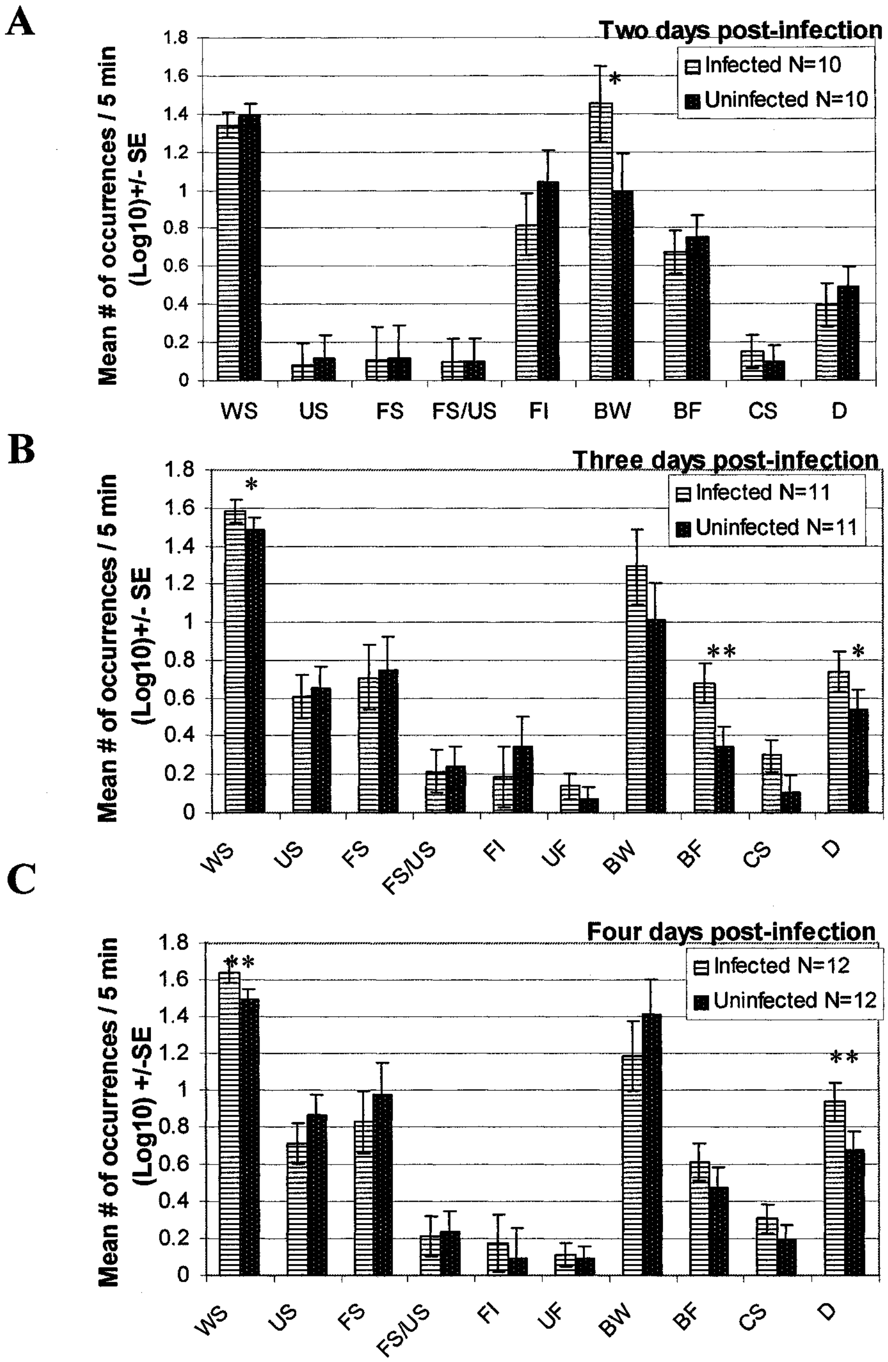


Figure 4. Mean number of occurrences (Log$_{10}$) of behaviors in infected and uninfected *Aedes aegypti* mosquito larvae over the course of five minutes at two, three, and four days PI. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FS/US, suspension feed/still (indistinguishable); FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive (note: UF was removed from the two day PI (A) due to the inability to distinguish between UF and FS/US). Within each behavior * = $P \leq 0.05$, ** = $P \leq 0.01$

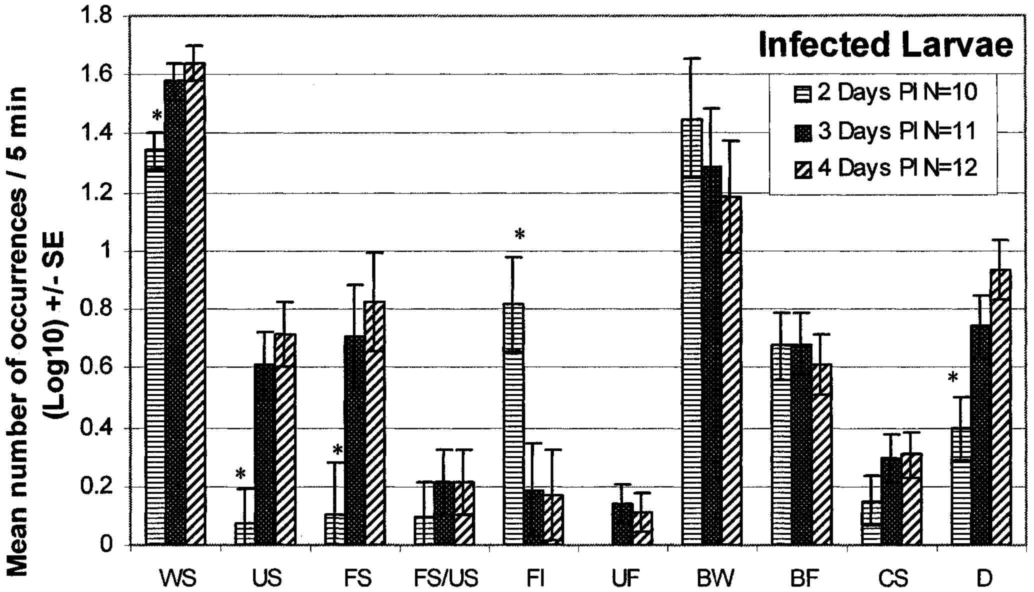


Figure 5. Mean number of occurrences (Log_{10}) of behaviors in **infected** *Aedes aegypti* mosquito larvae over the course of five minutes at 2, 3, and 4 days post-infection. Mosquito behavior codes are:WS, wriggle swim; US, underwater still; FS, suspension feed; FS/US, suspension feed/still (indistinguishable); FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive (note: UF was removed from the 2 days PI observations due to the inability to distinguish between UF and FS/US). Within each behavior, * = $P \leq 0.01$

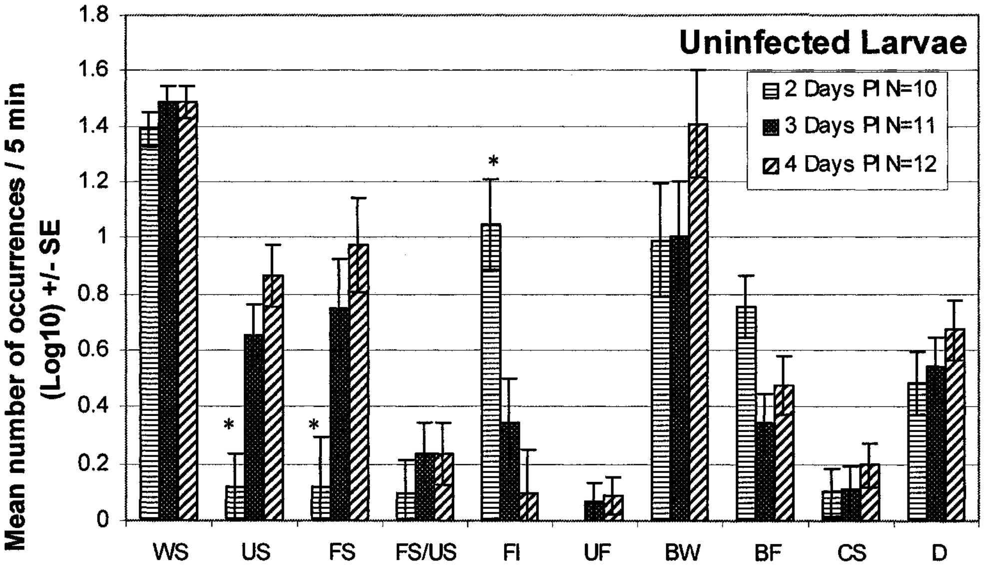


Figure 6. Mean number of occurrences (Log_{10}) of behaviors in **uninfected** *Aedes aegypti* mosquito larvae over the course of five minutes at 2, 3, and 4 days post-infection. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FS/US, suspension feed/still (indistinguishable); FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive (note: UF was removed from the 2 days PI observations due to the inability to distinguish between UF and FS/US). Within each behavior, * = $P \leq 0.01$

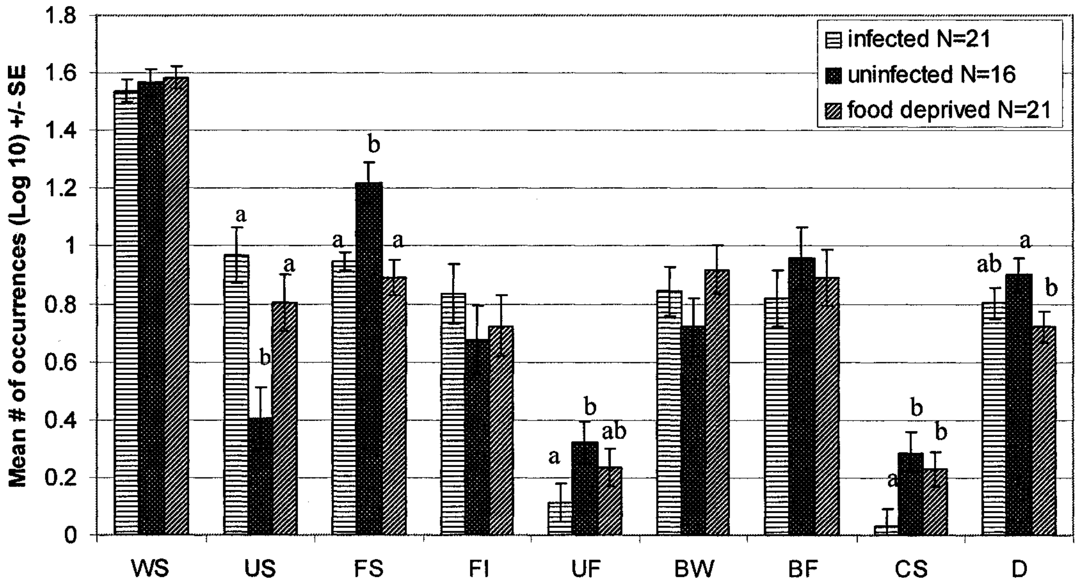


Figure 7. Mean number of occurrences (Log_{10}) of behaviors of *Aedes aegypti* infected with *Romanomeris culicivorax*, uninfected *Ae. aegypti*, and food deprived uninfected *Ae. aegypti* over the course of five minutes. Mosquito behavior codes are: WS, wriggle swim; US, underwater still; FS, suspension feed; FI, interfacial feed; UF, underwater suspension feed; BW, brush wall; BF, bottom feed; CS, chew substrate; D, dive. Within each behavior, means sharing the same letter are not significantly different ($\alpha \leq 0.05$).

PREDATOR AVOIDANCE BEHAVIOR OF *AEDES AEGYPTI* MOSQUITO LARVAE INFECTED WITH MERMITHID NEMATODES (NEMATODA:MERMITHIDAE)

ABSTRACT

Parasite-induced behavioral alterations in hosts are ubiquitous. Parasitized insects are especially amenable to the study of such changes, which may include alterations in behaviors as varied as thermal preference, mate choice, geotaxis, phototaxis, and predator avoidance. Parasitoids are parasites that have a direct life cycle and kill their host upon emergence. Based on this life cycle parasitoids should alter host behaviors in ways that decrease predation risk, thus, increasing parasitoid survival. Infection with mermithid parasitoids can alter host activity levels; these changes may affect host predation rates by predators that use activity levels to locate prey. In this paper I will address how parasitoids alter host behavior and if these behavioral alterations increase parasitoid survivorship. Specifically, I hypothesized that *Romanomermis culiciovorax* (Nematoda: Mermithidae) alter *Aedes aegypti* larval behavior in ways that reduce predation by the predator, *Toxorhynchites rutilus*. I compared the behaviors of infected and uninfected *Ae. aegypti* in the presence and absence *Tx. rutilus*. Studies have shown that differences in activity levels between species of mosquito larvae can lead to differential predation rates by *Tx. rutilus*. The following questions were addressed in this study: 1) Does the recovery time after an artificial alarm stimulus differ between infected and uninfected

Ae. aegypti larvae? 2) Do *Ae. aegypti* larvae switch to low-risk behaviors in the presence of *Tx. rutilus* and if so, does infection influence the shift? 3) Does infection with *R. culicivorax* affect predation on *Ae. aegypti* by *Tx. rutilus*? Infected *Ae. aegypti* did not facultatively switch their behaviors in the presence of *Tx. rutilus* whereas uninfected controls did. The lack of a shift in behaviors of infected *Ae. aegypti* is most likely due to the low activity levels already seen in the absence of *Tx. rutilus*. When the behaviors of infected and uninfected larvae were compared in the presence of *Tx. rutilus*, their behaviors did not differ, indicating that both groups maintain low levels of activity in the presence of a predator. These results were consistent with the results of the experiment on prey choice; there was no difference in predation on infected or uninfected *Ae. aegypti* by *Tx. rutilus*. Based on these results, I found no evidence that mermithid nematodes alter the predator avoidance behavior of *Ae. aegypti* mosquito larvae in ways that decrease predation risk.

INTRODUCTION

Parasite-induced behavioral alterations in hosts are ubiquitous. Parasitized insects are especially amenable to the study of such changes, which may include alterations in behaviors as varied as thermal preference, mate choice, geotaxis, phototaxis, and predator avoidance. In so doing, these changes can have far-reaching effects on trophic interactions and community structure (Thomas et al. 1998). Many studies on parasite-induced behavioral alterations have hypothesized that changes in host behaviors are adaptations of parasites with complex life cycles that increase transmission to the next host (see Moore 2002 for review). However, few studies have addressed whether increased transmission actually occurs (Poulin 1995). In this paper I will address how parasitoids, single-host parasites that have a free-living stage and kill their host upon emergence, alter host behavior and if these behavioral alterations increase parasitoid survivorship.

Fritz (1982) hypothesized that parasitoids should alter behaviors of hosts in ways that decrease the host's risk of predation, thereby increasing the parasitoid's chance of survival. Host behaviors that may be targeted by the parasitoid include activity levels, microhabitat selection, and developmental growth rates (Fritz 1982). An alternative hypothesis is that the behavioral changes of parasitoid infected hosts are side effects of infection with no adaptive value. Because most parasitoids use host nutrition, this nutrient depletion could alter host activity level in one of at least two ways: The host could feed more actively in response to parasite-induced starvation (Gordon 1981, Galloway and Brust 1985, Benton and Pritchard 1990, Thompson 1990), or the host could become lethargic due to decreased energy reserves or interference with sensory capabilities (Gordon 1981, Vance and Peckarsky 1997, Williams et al. 2001).

Nematodes in the family Mermithidae are parasitoids that infect many species of mosquito larvae as well as other insects. *Romanomermis culicivorax* is a mosquito host generalist (Petersen 1984, Blackmore 1993) and readily infects *Aedes aegypti*, an artificial container dwelling mosquito, in the laboratory and in field releases (Petersen and Chapman 1979). However, *Ae. aegypti* has not been found to be naturally infected with *R. culicivorax*. The life cycle of *R. culicivorax* in mosquito larvae involves pre-parasitic non-feeding juveniles that hatch from eggs laid in the substratum of an aquatic environment. The pre-parasitic juveniles locate and burrow into a first instar mosquito where they become parasitic and develop in synchrony with the mosquito larva. The parasitic stage is the only stage during which the mermithid feeds. Over a period of six to eight days, the mermithid molts several times within the mosquito larva; thereafter a post-parasitic nematode emerges, molts several more times, mates, then deposits eggs (Poinar 1983).

Mermithid-induced behavioral alterations have been studied in hosts other than mosquitoes (Benton and Pritchard 1990, Maeyama et al. 1994, Vance 1996, Vance and Peckarsky 1997, Williams et al. 2001, Poulin and Latham 2002). Several studies have addressed behavioral alterations as parasitoid adaptations. Maeyama and others (1994) found that ants infected with mermithids committed suicide by drowning. The authors hypothesized that the mermithid manipulated ant behavior in order to bring them to the appropriate habitat for emergence. Mermithid-induced behavioral alterations in larval mayflies have been shown to alter predation risk in a variety of ways (Benton and

Pritchard 1990, Vance 1996, Vance and Peckarsky 1997, Williams et al. 2001). Some studies indicate that infected mayflies exhibited more risky behaviors than uninfected conspecifics by drifting more (Williams et al. 2001), being more active (Benton and Pritchard 1990) or by failing to escape a predator (Vance and Peckarsky 1997), while another study showed that infected mayflies drifted less and thus reduced their exposure to fish predators (Vance 1996). Of these studies, only that by Vance (1996) is consistent with Fritz's hypothesis (1982) that parasitoid-induced behavioral alterations reduce predation risk. However, the remainder of the studies do not necessarily indicate that the behavioral alterations were non-adaptive. One possible explanation for increased drift (i.e., increased risk) is that a trade-off exists between predation risk and feeding. Because mermithid nematodes feed only during their parasitic stage, host nutrition plays an important role in mermithid development (Gordon et al. 1974, Galloway and Brust 1985). Therefore, it is possible that the need for nutrition obtained by drifting outweighs predation risk. Another explanation involves a potential balance between risks from different predators. For example, drifting is a risky behavior when visual/swimming predators are present (e.g. fish). In constrast, drifting as an escape from sit and wait ambush predators is beneficial to larval mayflies (Williams et al. 2001).

Although parasite-induced behavioral changes in adult mosquitoes have been and continue to be investigated (Koella and Packer 1996, Koella et al. 1998, 2002, Anderson et al. 1999, Hurd 2003, Ferguson and Read 2004), few studies have addressed parasite-induced behavioral alterations in larval mosquitoes (but see Webber et al. 1987 a,b). The same disparity is reflected in mermithid-mosquito associations (Steiner 1924, Petersen et al. 1967, Blackmore 1994): only one study has quantitatively addressed mermithid-induced behavioral alterations in larvae. In that study, *Ae. aegypti* larvae infected with *R. culicivorax* spent more time motionless and filter fed less than uninfected *Ae. aegypti* (Wise de Valdez in submission, but see Welch 1960). These behavioral differences between infected and uninfected A. *aegypti* larvae may influence predation risk by a natural predator, *Toxorhynchites rutilus.*

In the presence of a predator, mosquito larvae are known to facultatively switch from high-risk to low-risk behaviors (Sih 1986, Juliano and Gravel 2002, Kesavarju and Juliano 2004). Because *Toxorhynchites* sp. is an ambush predator, high-risk behaviors of mosquito larvae are those that involve high activity in general, but especially activity near the bottom of a container. Resting and other low-activity behaviors near the top of a container are considered low-risk behaviors (Juliano and Reminger 1992). The activity levels of mosquito larvae directly impact predation by *Toxorhynchites* sp.; prey species that are considered active, such as *Ae. aegypti* and *Ae. albopictus,* are preyed upon more readily than less active species such as *Orthopodomyia* sp. (Yasuda and Mitsui 1992, Grill and Juliano 1996). Interspecific differences in predator avoidance behaviors that result in differential predation affect mosquito population and community structure in ways that are only beginning to be understood (Yasuda 1996, Juliano and Gravel 2002, Spencer et al. 2002, Juliano et al. 2003).

In this paper I address how mermithid nematodes alter the behavior of *Aedes aegypti* mosquito larvae and if these alterations increase parasitoid survivorship. Following Fritz (1982), I hypothesized that *R. culicivorax* alter *Ae. aegypti* larval behavior in ways that reduce predation by *Tx. rutilus*, specifically by altering host activity levels. The following questions were addressed in this study: 1) Does the recovery time after an artificial alarm stimulus differ between infected and uninfected *Ae. aegypti* larvae? 2) Do *Ae. aegypti* larvae switch to low-risk behaviors in the presence of *Tx. rutilus* and if so, does infection influence the shift? 3) Does infection with *R. culicivorax* affect predation on *Ae. aegypti* by *Tx. rutilus*?

MATERIALS AND METHODS

Mosquito Rearing

A laboratory strain of *Ae. aegypti* mosquitoes was provided by Dr. William Black IV, Department of Microbiology, Immunology and Pathology, Colorado State University. The strain was established by Ballinger-Crabtree et al. (1992) from a wild population collected in Ogbomosho, Nigeria, and had been maintained in culture for 15 generations prior to arriving in Dr. Black's laboratory. All subsequent generations of *Ae. aegypti* mosquitoes were reared and maintained under a 12:12 LD photoperiod at 27°C ± 2°C. Eggs were hatched under anoxic conditions in plastic shoeboxes (28cm x 15cm) filled with 2 L of tap water (larval rearing containers). Upon hatching, first instar larvae were removed and placed into separate larval rearing containers at densities of 150-200 larvae/container. Larvae were fed ground Tetra-min® Fish Food *ad libitum*. Pupae were removed and allowed to emerge in adult rearing cages (150 pupae / adult rearing cage). The adult mosquitoes were maintained in 28cm x 15cm x 22cm plastic boxes with a screen top, provided with sugar cubes, and allowed to feed once a week on anesthetized rats. After the first blood meal an oviposition site was provided. Eggs laid on filter papers were collected twice between feedings and placed in plastic bags to be used for later experiments.

A laboratory strain of *Toxorhynchites rutilus rutilus,* a natural predator of *Ae. aegypti* larvae, was provided by Dr. Roxanne Rutledge from the University of Florida Medical Entomology Laboratory, Vero Beach, Florida. The laboratory colony of *Tx. rutilus* was maintained in a 12:12 LD photoperiod at 27°C ± 2°C. *Toxorhynchites rutilus* larvae were reared individually in 4cm x 4cm open-topped plastic specimen jars filled with deionized water and fed a daily ration of ten *Ae. aegypti* larvae of the same instar as *Tx. rutilus.*

Infection Procedures

All infection procedures were initiated seven days prior to behavioral experiments. *Romanomermis culicivorax* eggs were provided by Dr. Edward Platzer, University of California – Riverside and *Strelkovimermis spiculatus* eggs were provided by Dr. James J. Becnel, USDA, ARS, CMAVE. All mermithid eggs were stored in plastic bags of moist sand at room temperature. Nematode hatching was initiated by flooding four to five tablespoons of egg/sand mixture with 100 mL of deionized water. After three to five hours, the water was sampled for pre-parasitic nematodes and the number of nematodes per mL was calculated. Approximately 200 first instar (< 24 hours of age) *Ae. aegypti* mosquito larvae were transferred into each of two 7cm x 7cm round plastic containers filled with 50 mL deionized water. Approximately 1,000 pre-parasitic nematodes were then added to one of the two containers (5:1 nematode:mosquito) and the total volume of deionized water in each container was brought up to 100 mL. Both groups of *Ae. aegypti* larvae (exposed to mermithids and unexposed) were held in these containers for 12-18 hours without food and then transferred into separate larval rearing containers, where they were maintained in a manner similar to that used with stock cultures (see above). This procedure resulted in 50-60% prevalence with intensities of one to three mermithids per mosquito larva. Infection was determined by observing mermithid nematodes through the cuticle of fourth instar mosquito larvae with the use of a dissecting microscope.

Experiment 1 – Alarm Stimulus

The purpose of this experiment was to determine the effect of mermithid infection on the response of *Ae. aegypti* mosquito larvae to an artificial alarm stimulus. A total of three trials was carried out. Each trial consisted of three 10cm x 10cm containers of tap water sprinkled with ground Tetra-min® fish food; each container held ten fourth instar *Ae. aegypti* larvae (container 1, larvae infected with *R. culicivorax*; container 2, larvae infected with *S. spiculatus*; container 3, uninfected larvae). Mosquitoes were allowed to acclimate for 20 minutes. The alarm stimulus, a black cardboard square attached to a wand and passed over each container, was applied after the acclimation period and after all larvae were present at the surface of the water. After the stimulus, the time (seconds) that it took for half of the mosquitoes (5) to return to the surface of the water was recorded. The mosquitoes were then allowed to recover (approximately 15 minutes) and the stimulus was applied again. The alarm stimulus was applied a total of four times per trial to each container.

A one-way ANOVA was run to determine if a significant difference in recovery time post stimulus existed among the three treatment groups (infected with *R. culicivorax,* infected with *S. spiculatus*, and uninfected). Because an interaction between trial and treatment group was detected, the three trials, were analyzed individually. There was no effect of replication on behavioral response to the stimulus.

Experiment 2 – Predator Avoidance Behaviors

I asked if the predatory mosquito *T. rutilus* influenced larval *Ae. aegypti* behavior. I also asked if the mermithid *R. culicivorax* influenced the response to *Tx. rutilus*. The experiment was carried out in a split-plot design, with a total of four trials. Each trial consisted of ten pairs of seven-day-old *Ae. aegypti* mosquito larva (one infected, one uninfected), each pair in its own 10cm x 10cm container with a pinch of ground Tetra-min® fish food. These ten containers were divided into two treatment groups: 1) five containers of conditioned water (water in which 1 *Tx. rutilus* larva had been feeding for 10 days) and one well-fed *Tx. rutilus* larva (i.e., "Tox"), and 2) five containers of tap water without a predator (i.e., "NoTox"). Tox-treatment *Ae. aegypti* larvae were allowed to acclimate in conditioned water for 20 minutes prior to the addition of one *Tx. rutilus* larva; all three mosquito larvae were then allowed to acclimate for an additional 20 minutes. NoTox-treatment *Ae. aegypti* were allowed to acclimate for 40 minutes. A black cardboard square was placed on top of each observation container to minimize the effect of overhead light.

The behavior of a single *Ae. aegypti* larva in the pair was observed at eye level for five minutes. I did not know the infection status of the larvae. Behaviors were tape recorded in real time and later transcribed. One occurrence of a behavior was recorded for every five seconds or less that the behavior was exhibited by the mosquito. If the same behavior was exhibited by the mosquito for more than five consecutive seconds, each five second interval of that behavior was recorded as an additional occurrence. The duration of time spent in a single behavior could later be assessed by counting the number of five-second occurrences in a row. The behaviors that were recorded were modifications of those described by Walker and Merritt (1991; Table 1, Figure 1). For analysis of behaviors I then combined recorded behaviors into three categories; low risk, moderate risk, and high risk behavior. Behaviors that brought *Ae. aegypti* larvae into direct contact with the *Tx. rutilus* or those that attracted the attention of *Tx. rutilus* were considered high risk (Table 1). Moderate risk behaviors were those where there was a potential for contact and a moderate activity level (Table 1). In low risk behaviors *Ae. aegypti* larvae were far from the predator and relatively inactive, with a reduced chance of attracting the predator's attention (Table 1).

Immediately after both mosquito larvae in the observation chamber had been observed, each was placed into a separate holding container for later dissection. Four to five hours after the behaviors of all 20 *Ae. aegypti* larvae had been recorded, each larva was dissected and infection status determined. If a larva had pupated or died by the time of dissection, the behavioral data for that larva were not included in the analysis.

The number of occurrences of each behavior (low, moderate, and high risk) within each treatment group (Tox/Inf, Tox/Un, No Tox/Inf, No Tox/Un) was log transformed to normalize the data and then averaged over trials. Data were analyzed using a two-factor analysis of variance of a split-plot model.

Experiment 3 – Prey Choice

I asked if *Tx. rutilus* preys more readily on infected or uninfected *Ae. aegypti* when given the choice. *Toxorhynchites rutilus* larvae were reared individually in 10cm x 10cm open-topped plastic containers filled with deionized water under the same conditions as *Ae. aegypti* larvae. Each *Tx. rutilus* larva was fed 20 *Ae. aegypti* larvae of the same instar as *Tx. rutilus* every two days.

The experiment consisted of five trials. Four trials were replicated three times and one trial, twice. Each replicate involved one *Tx. rutilus* and 20 fourth instar *Ae. aegypti* larvae (10 infected and 10 uninfected). *Aedes aegypti* larvae were placed into a 10cm x 10cm container of conditioned water. After a 20 minute acclimation period, one *Tx. rutilus* larva was gently pipetted into the center of the container. The number of remaining *Ae. aegypti* larvae was recorded at three-hour intervals until 50% of the mosquitoes remained. Any *Ae. aegypti* larva that died or pupated was removed and not included. After each trial, a chi-squared analysis was performed.

RESULTS

Experiment 1 – Alarm Stimulus

In trials one and two, *Ae. aegypti* infected with *S. spiculatus* took significantly longer (means = 49 and 85 sec respectively) than uninfected *Ae. aegypti* larvae (means = 34 and 49 sec respectively) to recover after an artificial stimulus ($P \leq 0.05$; Figure 2). In these trials there was no significant effect of infection with *R. culicivorax* on recovery time (means = 43 and 42 sec respectively, $P \leq 0.05$; Figure 2). In trial three however, *R. culicivorax* infected *Ae. aegypti* took significantly longer (mean = 62 sec) to recover than either *S. spiculatus* infected larvae (mean = 45 sec) or uninfected larvae (44 sec) while the latter two did not significantly differ ($P \leq 0.05$; Figure 2).

Experiment 2 – Predator Avoidance Behaviors

The occurrence of low-risk behaviors of infected *Ae. aegypti* in the presence versus absence of Tx. rutilus was not significantly different (Tox mean = 36 times, NoTox mean = 33 times; $P \leq 0.05$; Figure 3). Likewise, the presence of *Tx. rutilus* did not affect the occurrence of moderate and high risk behaviors of infected *Ae. aegypti* (Tox means = 9 and 25 times respectively; NoTox means = 10 and 33 times respectively; $P \leq 0.05$; Figure 3). However, when comparing high to low risk behaviors, the occurrence of low-risk behaviors was significantly higher than the occurrence of high risk behaviors in the presence of *Tx. rutilus* ($P \leq 0.05$).

The occurrence of low-risk behaviors of uninfected *Ae. aegypti* tended to be higher when *Tx. rutilus* was present (Tox mean = 35.18, NoTox mean = 25.93); however, this difference was not significant ($P \leq 0.05$; Figure 3). The presence of *Tx. rutilus* significantly reduced the occurrence of high risk behaviors (Tox mean = 28.04, NoTox mean = 41.21; $P \leq 0.05$; Figure 3). No shifts in moderate-risk behaviors were seen. In the presence of *Tx. rutilus*, low risk behaviors occurred significantly more than high risk behaviors ($P \leq 0.05$; Figure 3).

In the absence of *Tx. rutilus*, there was no effect of infection on *Ae. aegypti* behavior (Figure 3). Although infection had no effect on low- and high-risk behaviors, infected larvae tended to spend more time in low-risk and less in high-risk behaviors than the uninfected larvae (Figure 3). In the presence of *Tx. rutilus*, there was no effect of infection on *Ae. aegypti* behavior, nor was there a trend indicating a possible difference (Figure 3).

Experiment 3 – Prey Choice

Infection status did not influence prey choice. In four of five trials, infection status did not affect consumption (Table 1). When combined over all trials, infected mosquitoes were 0.78 times more likely to be consumed by *Tx. rutilus* than uninfected; this was not significant (Table 3).

DISCUSSION

Mermithid nematodes do not alter the behaviors of fourth instar *Ae. aegypti* mosquito larvae in ways that reduce the risk of *Tx. rutilus* predation. Although the initial experiment using an artificial alarm stimulus indicated a difference in behavioral responses between the infected and uninfected *Ae. aegypti* larvae, subsequent experiments using *Tx. rutilus* did not indicate behavioral or predation risk differences between infected and uninfected *Ae. aegypti.*

The alarm response of *Aedes* mosquito larvae is to swim actively to the bottom and, upon recovery, float passively to the surface (Mellanby 1958). Both infected and uninfected larvae exhibited alarm response to overhead disturbance (Expt. 1); however, in each of three trials comparing the alarm reaction between the two groups, infected larvae took significantly longer to resurface than did uninfected larvae (Figure 2). This difference in alarm response did not translate into a difference in predator avoidance success when confronted by *Tx. rutilus* (Table 2). One explanation for this discrepancy might be the fact that the artificial alarm stimulus was initiated from above whereas the predator, *Tx. rutilus*, attacked from below. The effect of infection on nutritional status might also play a role. Olsson and Klowden (1998) found that *Ae. aegypti* larvae that were nutritionally deprived dived less in response to an artificial alarm stimulus (light from above) than did well-fed controls. When nutritionally deprived larvae did dive, they spent more time below the surface than the well-fed controls. The authors suggested that because diving is energetically expensive, food-deprived larvae conserved energy by diving less and by not resurfacing as quickly when they did dive. Mermithid nematodes place nutritional demands on their mosquito hosts that cause the infected larvae to exhibit symptoms of starvation (Gordon 1981, Galloway and Brust 1985, Wise de Valdez in submission). Thus, the difference between infected and uninfected larvae in response to an alarm stimulus may reflect nutritional differences rather than a direct manipulation of anti-predator behaviors by mermthid nematodes.

Toxorhynchites rutilus had no effect on low-risk behavior of infected larvae (Figure 3), perhaps because larval behavior in the absence of *Tx. rutilus* is already biased towards those that are low-risk. In addition, the presence of *Tx. rutilus* did not significantly alter high-risk behaviors of infected larvae (Figure 3). However, *Tx. rutilus* had an effect on the occurrence of low-risk behaviors compared to high-risk behaviors; infected larvae spent significantly less time in high-risk behaviors than in low-risk behaviors (Figure 3). The effects of *Tx. rutilus* on behaviors of uninfected larvae were more pronounced; uninfected larvae shifted their behaviors. Uninfected larvae significantly reduced their high-risk behaviors and tended to increase their low-risk behaviors in the presence of *Tx. rutilus* versus absence of *Tx. rutilus* (Figure 3). *Toxorhynchites rutilus* also affected the occurrence of low-risk behaviors compared to high-risk behaviors; uninfected larvae spent significantly less time in high-risk behaviors than in low-risk behaviors (Figure 3).

When the behaviors of uninfected larvae and infected larvae were directly compared in the presence and absence of *Tx. rutilus*, no significant behavioral differences existed between the two infection groups in either the presence or absence of *Tx. rutilus* (Figure 3). In the absence of *Tx. rutilus* however, infected larvae appear to be less active than uninfected larvae. This trend, although not significant, indicates that infected larvae maintain a low-level of activity regardless of the presence of *Tx. rutilus*, whereas uninfected larvae tend to switch to low activity levels (similar to those of the infected larvae). These results are consistent with previous studies (Wise de Valdez in submission) that showed that *Ae. aegypti* larvae infected with either *R. culicivorax* or *S. spiculatus* exhibited significantly more resting behaviors than did uninfected *Ae. aegypti.*

The mechanism of behavioral shifts of mosquito larvae in the presence of *Tx. rutilus* is not completely understood. Shifts in behavior can occur in response to chemical, visual, and tactile cues (Sih 1986, Juliano and Gravel 2002) and can differ depending on the type of predator present and the evolutionary history between predator and prey (Sih 1986). In addition, hunger can play a role in the degree to which mosquito larvae shift their behaviors (Juliano et al. 1993). It is therefore likely that infection with mermithid nematodes may also alter the degree to which mosquito larvae shift their behaviors in the presence of *Tx. rutilus* via nutritional depletion, direct manipulation, or both.

Because infected *Ae. aegypti* larvae did not shift their behaviors in the presence of *Tx. rutilus* nor did their behaviors differ significantly from uninfected mosquito larvae in the presence of *Tx. rutilus*, it is possible that reducing high-risk behaviors such as those associated with feeding is beneficial to host and parasitoid up to a certain point. For example, food acquisition is necessary for mosquito development and thus influences their behavior (Juliano et al. 1993). Host nutrition also plays a vital role in parasite development (Gordon et al. 1981, Thompson 1990). If mermithid nematodes alter the nutritional status of the host (Gordon 1981), one might expect altered feeding behavior that benefits the parasitoid. The risk of predation by *Tx. rutilus* probably does not outweigh nutritional need.

The risk of predation can also play a role in how a parasitoid affects behavior. Thus, mermthid nematodes face a trade-off between avoiding predators and obtaining sufficient nutrients for development. Therefore, if mermithid nematodes alter the behaviors of *Ae. aegypti* larvae in ways that significantly reduce food acquisition, they not only risk delaying their own development, but the development of their host as well. Fritz (1982) predicted that the degree of host modification that benefits the parasitoid should be proportional to the intensity of predation on the host. It is likely then, that the intensity of predation risk by *Tx. rutilus* influences the degree to which *Ae. aegypti* infected with mermithid nematodes alter their behavior in the presence of *Tx. rutilus*. The trade-off between predation risk and feeding is well established in aquatic systems and has also been studied in light of mermithid parasitism. Vance (1996) hypothesized that larval mayflies infected with mermithid nematodes decreased drifting behavior in order to avoid predators at the cost of reduced food location, indicating that locating food is less important than the risk of predation. In contrast, Benton and Pritchard (1990) found that infected mayfly larvae increased their activity in the presence of a predator compared to uninfected controls, indicating that increased foraging due to mermithid-induced nutritional deficit is more important than eliciting predator avoidance behaviors. In my experiments mermithid-infected *Ae. aegypti* larvae did not increase activity; however, the fact that their behaviors were not altered beyond that of the uninfected controls in the presence of *Tx. rutilus* indicated that feeding remained of high importance.

The fact that mermithid nematodes did not reduce the activity levels below those of uninfected controls in the presence of *Tx. rutilus* suggests that there may be a threshold of decreased activity beyond which mosquito larvae, regardless of infection, do not go.

For example, *Plasmodium* spp. oocysts require host survival; they also decrease mosquito host feeding persistence and thus reduce the risk of death (Anderson et al. 1999, Koella et al. 2002). However, oocysts do not cause the mosquito to cease feeding altogether because some nutrition is necessary for host survival and oocyst development to sporozoites (the transmittable stage) (Schwartz and Koella 2001).

Another possible explanation for why my data do not support Fritz's (1982) hypothesis is that the evolutionary relationship among *Ae. aegypti*, mermithid nematodes, and *Tx. rutilus* may be relatively recent. Although *Ae. aegypti* should have had time to evolve defensive behaviors against *Tx. rutilus*, there is no evidence that indicates the rate at which parasitoids, such as *R. culicivorax*, evolve tactics to manipulate their host defensive behavior. Behavioral responses of mosquito larvae to predators can indicate their evolutionary relationship (Sih 1986, Juliano and Gravel 2002, Kesavarju and Juliano 2004). *Aedes albopictus*, recently introduced into the United States, fails to alter its behavior in the presence of *Tx. rutilus*, indicating that *Ae. albopictus* has not undergone strong selection pressure for predator avoidance from *Tx. rutilus* (Kesavarju and Juliano 2004). However, under selection pressure by *Tx. rutilus* in the laboratory, the facultative behavioral responses of *Ochlertatus triseriatus* larvae can quickly evolve (Juliano and Gravel 2002). Although the evolutionary relationships among mosquitoes and their predators can be seen and even measured by behavioral responses, the evolutionary relationships among mermithid parasitoids, their hosts, and the predators of their hosts are more difficult to assess. The evolution of adaptive parasite-induced behavioral alterations has been studied to a greater extent than the speed at which parasites evolve the manipulative effort has not (but see Moore and Gotelli 1996).

In Experiment 3, I compared *Tx. rutilus* predation on infected and uninfected *Ae. aegypti* larvae. In four out of five trials there was no significant effect of infection (Table 2). In fact, predation on both groups of *Ae. aegypti* appeared to be random with more uninfected larvae consumed in two of five trials (Table 2). When all five trials were included, infected larvae were 0.78 times more likely to be eaten than uninfected larvae but this value was not significant (Table 2). Because differences in predation rates on mosquito species are usually due to differences in predator avoidance behaviors (Sih 1986, Yasuda and Mitsui 1992, Grill and Juliano 1996, Yasuda 1996), the absence of differential predation on infected and uninfected *Ae. aegypti* by *Tx. rutilus* is consistent with the absence of differences in predator avoidance behaviors between the two groups.

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REFERENCES CITED

Anderson, R. A., J. C. Koella, and H. Hurd. 1999. The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. Proceedings of the Royal Society of London Series B-Biological Sciences 266:1729-1733.

Ballinger-Crabtree, M. E., W. C. Black IV, and B. R. Miller. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. American Journal of Tropical Medicine and Hygiene 47:893-901.

Benton, M. J., and G. Pritchard. 1990. Mayfly locomotory responses to endoparasitic infection and predator presence - the effects on predator encounter rate. Freshwater Biology 23:363-371.

Blackmore, M. S. 1993. Ecology of mosquito-parasitic mermithid nematodes. Vector Control Bulletin 2:5-7.

Blackmore, M. S. 1994. Mermithid parasitism of adult mosquitoes in Sweden. American Midland Naturalist 132:192-198.

Ferguson, H. M., and A. F. Read. 2004. Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts. Malaria Journal 3.

Fritz, R. S. 1982. Selection for host modification by insect parasitoids. Evolution 36:283-288.

Galloway, T. D., and R. A. Brust. 1985. The effects of parasitism by *Romanomermis culicivorax* (Nematoda, Mermithidae) on growth and development of *Aedes vexans* (Diptera, Culicidae) in laboratory and field-tests. Can. J. of Zool. 63:2437-2442.

Gordon, R. 1981. Mermithid nematodes - physiological relationships with their insect hosts. J. Nematology 13:266-274.

Gordon, R., C. H. Bailey, and J. M. Barber. 1974. Parasitic development of mermithid nematode *Reesimermis nielseni* in larval mosquito *Aedes aegypti*. Canadian Journal of Zoology 52:1293-&.

Gordon, R., J. M. Squires, S. J. Babie, and I. R. Burford. 1981. Effects of host diet on *Romanomermis culicivorax*, a mermithid parasite of mosquitoes. Journal of Nematology 13:285-290.

Grill, C. P., and S. A. Juliano. 1996. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. Journal of Animal Ecology 65:63-76.

Hurd, H. 2003. Manipulation of medically important insect vectors by their parasites. Annual Review of Entomology. 48:141-161.

Juliano, S. A. 1996. Geographic variation in *Aedes triseriatus* (Diptera: Culicidae): Temperature-dependent effects of a predator on survival of larvae. Environmental Entomology 25:624-631.

Juliano, S. A., and L. Reminger. 1992. The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. Oikos 63:465-476.

Juliano, S. A., L. J. Hechtel, and J. R. Waters. 1993. Behavior and risk of predation in larval treehole mosquitoes: effects of hunger and population history of predation. Oikos 68:229-241.

Juliano, S. A., and M. E. Gravel. 2002. Predation and the evolution of prey behavior: an experiment with treehole mosquitoes. Behavioral Ecology 13:301-311.

Kesavaraju, B., and S. A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. Annals of the Entomological Society of America 97:194-201.

Koella, J. C., and M. J. Packer. 1996. Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. Parasitology 113:105-109.

Koella, J. C., F. L. Sorensen, and R. A. Anderson. 1998. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. Proceedings of the Royal Society of London Series B-265:763-768.

Koella, J. C., L. Rieu, and R. E. L. Paul. 2002. Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. Behavioral Ecology. 13:816-820.

Maeyama, T., M. Terayama, and T. Matsumoto. 1994. The abnormal behavior of *Colobopsis* sp. (Hymenoptera: Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. Sociobiology 24:115-119.

Mellanby, K. 1958. The alarm reaction of mosquito larvae. Entomological Experimentation and Application 1:153-160.

Moore, J. 2002, Parasites and the behavior of animals: Ecology and Evolution. New York, Oxford University Press.

Moore, J., and N. J. Gotelli. 1996. Evolutionary patterns of altered behavior and susceptibility in parasitized hosts. Evolution 50:807-819.

Olsson, B., and M. J. Klowden. 1998. Larval diet affects the alarm repsonse of *Aedes aegypti* (L.) mosquitoes (Diptera: Culicidae). Journal of Insect Behavior. 11: 593-596.

Petersen, J. J. 1984. Nematode Parasites of Moquitoes, Pages 797-820 *in* W. R. Nickle, ed. Plant and Insect Nematodes. New York, Marcel Dekker Inc.

Petersen, J. J., and H. C. Chapman. 1979. Checklist of mosquito species tested against the nematode parasite *Romanomermis culicivorax*. Journal of Medical Entomology 15:468-471.

Poinar, G. O. 1983. Nematode parasites of invertebrates, Pages 161-202 *in* G. O. J. Poinar, ed. The Natural History of Nematodes. Englewood Cliffs, NJ, Prentice-Hall Inc.

Poulin, R. 1995. "Adaptive" changes in the behaviour of parasitized animals: A critical review. International Journal for Parasitology 25:1371-1383.

Poulin, R., and D. M. Latham. 2002. Parasitism and the burrowing depth of the beach hopper *Talorchestia quoyana* (Amphipoda : Talitridae). Animal Behavior 63:269-275.

Schwartz, A., and J. C. Koella. 2001. Trade-offs, conflicts of interest and manipulation in Plasmodium-mosquito interactions. Trends in Parasitology 17:189-194.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434-441.

Spencer, M., L. Blaustein, and J. E. Cohen. 2002. Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. Ecology 83:669-679.

Steiner, G. 1924. Remarks on a mermithid found parasitic in the adult mosquito (*Aedes vexans* Meigan) in B. C. The Canadian Entomologist:161-164.

Thomas, F., F. Renaud, T. de Meeus, and R. Poulin. 1998. Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? Proceedings of the Royal Society of London Series B 265:1091-1096.

Thompson, S. N. 1990. Physiological alterations during parasitism and their effects on host behaviour, Pages 64-94 *in* C. J. Barnard, and J. M. Behnke, eds. Parasitism and Host Behaviour. London, Taylor and Francis Ltd.

Vance, S. A. 1996. The effect of the mermithid parasite *Gasteromermis* sp (Nematoda: Mermithidae) on the drift behaviour of its mayfly host, *Baetis bicaudatus* (Ephemeroptera: Baetidae): A trade off between avoiding predators and locating food. Canadian Journal of Zoology 74:1907-1913.

Vance, S. A., and B. L. Peckarsky. 1997. The effect of mermithid parasitism on predation of nymphal *Baetis bicaudatus* (Ephemeroptera) by invertebrates. Oecologia 110:147-152.

Walker, E. D., and R. W. Merritt. 1991. Behavior of larval *Aedes triseriatus* (Diptera, Culicidae). Journal of Medical Entomology 28:581-589.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987a. The Effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the behavior of *Aedes aegypti* larvae. Canadian Journal of Zoology 65:1340-1342.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987b. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the susceptibility of *Aedes aegypti* larvae to predation by guppies (*Poecilia reticulata*) and meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology 65:2346-2348.

Welch, H. E. 1960. *Hydromermis churchillensis* n. sp. (Nematoda: Mermithidae) a parasite of *Aedes communis* (DeG.) from Churchill, Manitoba with observation of its incidence and bionomics. Canadian Journal of Zoology 38:466-474.

Williams, J. K., C. R. Townsend, and R. Poulin. 2001. Mermithid nematode infections and drift in the mayfly *Deleatidium* spp. (Ephemeroptera). Journal of Parasitology 87:1225-1227.

Wise de Valdez, M. R. In submission. The behavior of *Aedes aegypti* larvae infected with mermithid nematodes (Nematoda: Mermithidae).

Yasuda, H., and T. Mitsui. 1992. Laboratory observations of prey behavior for prey acquisition by a predator among 3 larval mosquitoes. Ecological Research 7:179-182.

Yasuda, H. 1996. Abundance and age structure of the predatory mosquito larva, *Toxorhynchites towadensis* (Dipt, Culicidae) in relation to prey community structure. Journal of Applied Entomology 120:291-295.

Behavior	Abbreviation	Description	Location or movement	Risk
Wriggle-swim	WS	Mosquito thrashes back and forth	Top Middle Bottom	Moderate High High
Still	US	Mosquito is still, no movement of mouthparts	Top Middle Bottom	Low Low Moderate
Suspension Feed	FS	Mosquito suspended by anal siphon from the surface of the container and mouthparts are moving. With or without movement of larva across surface	Without With	Low Moderate
Brush Wall	BW	Mosquito brushes wall of container with mouth parts	Top Middle Bottom	Moderate Moderate High
Bottom Feed	BF	Mosquito brushes bottom of container with mouth parts	Bottom	High
Dive	D	Mosquito actively dives to bottom of the container	N/A	Moderate

Table 1. Ethogram of behaviors of *Aedes aegypti* mosquito larvae adapted from Walker and Merrit (1991) including risk of behavior adapted from Juliano and Reminger (1992).

		Infected	Uninfected	X^2	Odds Ratio	P value
Trial 1	Consumed	10	6	3.11	3.33	0.10
	Not Consumed	7	14			
Trial 2	Consumed	8	9	0.06	1.16	1.0
	Not Consumed	13	17			
Trial 3	Consumed	6	11	1.43	0.47	0.36
	Not Consumed	15	13			
Trial 4	Consumed	4	3	4.79	7.56	0.04
	Not Consumed	3	17			
Trial 5	Consumed	7	16	2.82	0.39	0.11
	Not Consumed	18	16			
All Trials	***Consumed***	***37***	***45***	***0.73***	***0.78***	***0.46***
	Not Consumed	***56***	***53***			

Table 2. Chi-Squared table of consumption of infected and uninfected *Aedes aegypti* mosquito larvae by the predator *Toxorhynchites rutilus*. Odds ratios indicate the likelihood of infected animals vs. uninfected being consumed.

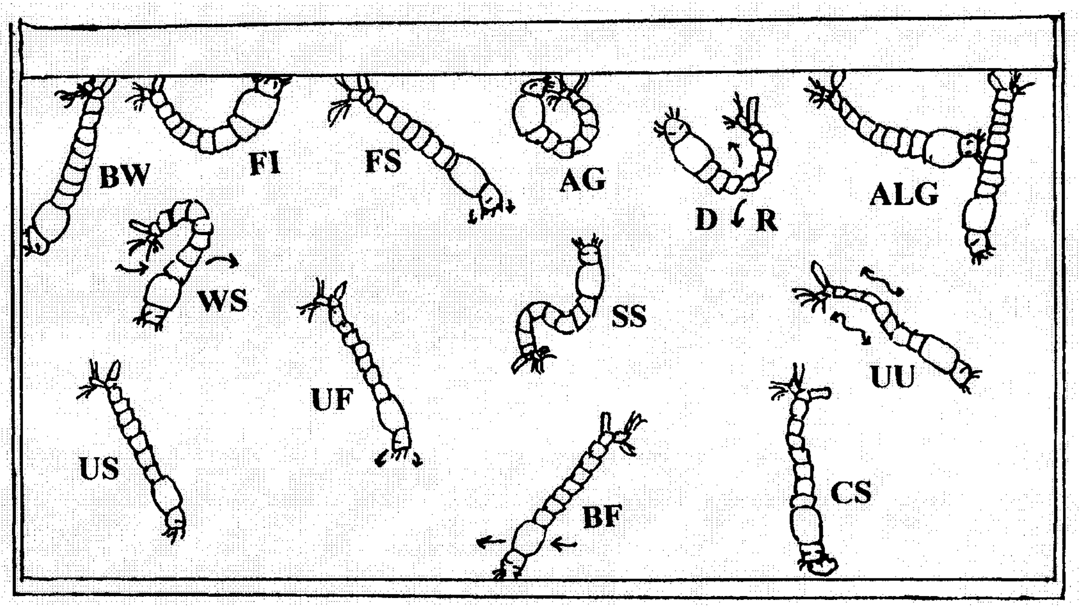

Figure 1. Schematic representation of *Ae. aegypti* mosquito larvae behavior. Adapted from Walker and Merrit 1991

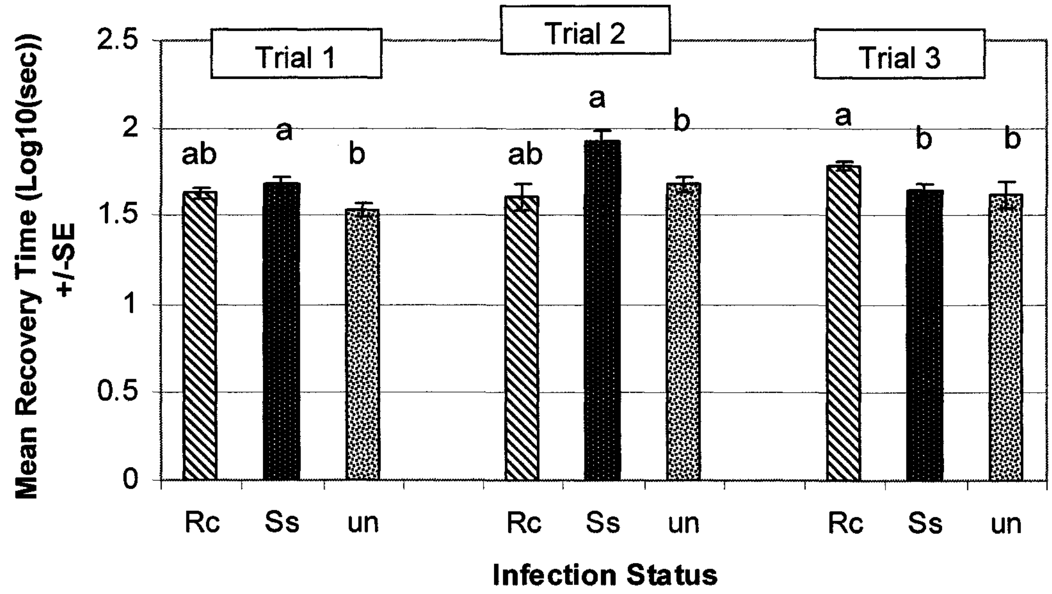

Figure 2. The mean recovery times ($\log_{10}$ transformed ±SE) of infected and uninfected fourth instar *Aedes aegypti* after an artificial alarm stimulus in 3 trials. Recovery time: time from stimulus application to time when 50% of the mosquitoes return to water surface. Rc: mosquitoes infected with *Romanomermis culicivorax* (n = 10/trial), Ss: mosquitoes infected with *Strelkovimermis spiculatus* (n = 10/trial), un: uninfected mosquitoes (n = 10/trial). Means sharing the same letter within each trial are not significantly different ($P \leq 0.05$).

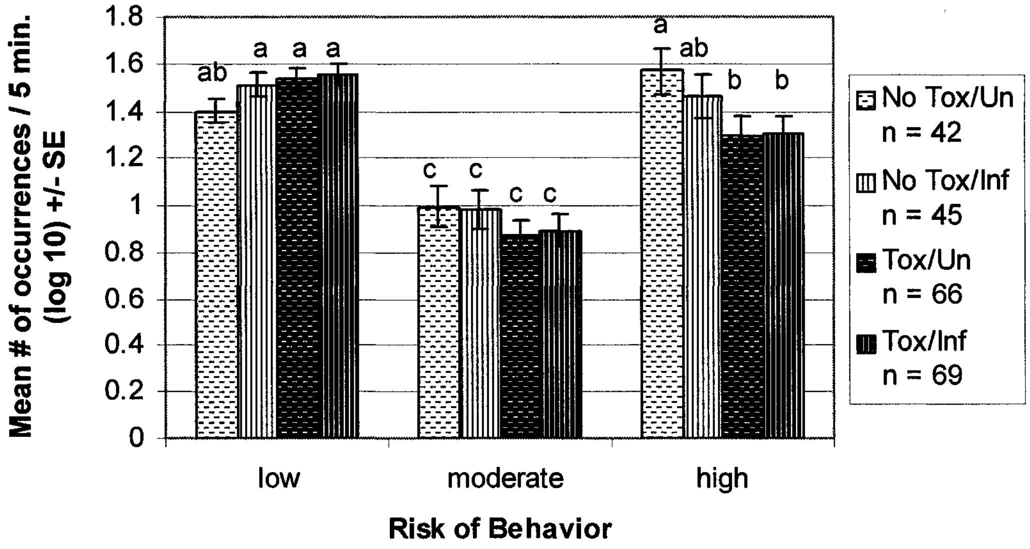

Figure 3. Mean number of occurrences (Log_{10} transformed ± SE) of low, moderate, and high risk behaviors of fourth instar *Aedes aegypti* mosquito larvae infected with *Romanomermis culicivorax* (IN) or uninfected (UN) with (Tox) and without (NoTox) the presence of the predator *Toxorhynchites rutilus*. Means with the same letter are not significantly different (P<0.05).

POTENTIAL ROLE OF PARASITES IN STRUCTURING COMMUNITIES OF CONTAINER-DWELLING MOSQUITO LARVAE

ABSTRACT

Parasites have the potential to alter community composition and structure. Likewise, they may play a role in ecosystem stability, biodiversity, food web patterns, and species invasions. The mechanisms by which parasites can influence components of host ecology are varied and can directly or indirectly alter competitive interactions, predator-prey relationships, or change host demography. Unfortunately, experimental evidence that addresses the roles of parasites in structuring communities is limited. One of the limiting factors is the level of community complexity that exists in most host-parasite systems. Therefore, in order to adequately examine the impact of parasitism at the community level, host-parasite systems in simple communities are needed. Container dwelling mosquitoes and their parasites provide such a system. These habitats contain small, well-defined communities, have discrete boundaries, and are naturally replicated in the field. In this paper I summarize the main features of container communities as well as give a brief review of the literature addressing container dwelling mosquito ecology. I summarize the parasites and pathogens of mosquitoes found in these habitats and review the known effects they have on their mosquito hosts. Finally, I synthesize this information in a discussion about the potential impact of parasites on container dwelling mosquito community structure.

INTRODUCTION

Parasites have the potential to alter community composition and structure (Minchella and Scott 1991). Likewise, they may play a role in ecosystem stability, biodiversity (Combes 1996), food web patterns (Marcogliese and Cone 1997), and species invasions (Minchella and Scott 1991, Prenter et al. 2004). The influences parasites have on these aspects of ecological interactions are varied and can be most easily classified into direct and indirect effects (Poulin 1999). Direct effects involve decreasing the abundance of a particular host species via pathology (Mouritsen and Poulin 2002). For example, parasites can directly alter birth and death rate of their hosts (Minchella and Scott 1991). The resultant change in population size can affect intra- or interspecific interactions, trophic level interactions, and host demography (Dobson and Hudson 1986, Hochberg 1991, Michella and Scott 1991, Morand and Gonzalez 1997, Poulin 1999, Mouritsen and Poulin 2002). Competitive interactions can also be altered if one host in a two-host system is more susceptible or incurs a greater cost due to parasitism than the other. For example, if a normally superior competitor is more susceptible or has a higher death rate after infection, the inferior competitor is released from competition and thus both species are able to coexist (Park 1948). Alternatively, differential effects can further debilitate an inferior competitor and lead to local extinction (Combes 1996).

Altered behaviors or host life history traits can *indirectly* modify the functional importance of hosts in their community or ecosystem (Poulin 1999, Thomas et al. 1999, 2000). It has been well established that parasites alter the behavior of their hosts (Moore 2002). Parasite-induced changes in activity (Bethel and Holmes 1973), feeding

(Anderson et al. 2000), or habitat choice (Moore 1983) can lead to increased predation. Depending on whether predation was additive (a reduction in survival) or compensatory (no change in survival), the resulting changes in population size could alter competition and trophic interactions. Parasite-induced changes in dispersal capabilities can modify spatial distribution of hosts (Thomas et al. 2000, Mouritsen and Poulin 2002), which could then alter community structure.

Life history characteristics such as fecundity, development, growth and reproduction are important determinants of species coexistence. Parasite-induced alterations in any of these traits can alter intra- and interspecific species interactions (Thomas et al. 2000). For example, changes in development or growth can modify the temporal segregation among species and differences in host fecundity could result in reduced or increased population size and thus indirectly alter community structure (Thomas et al. 2000).

Unfortunately, assessing the roles parasites play in structuring communities is a difficult task. Most communities are highly complex, making a survey of all community components and interactions virtually intractable. A survey of the parasites alone could be a substantial undertaking, not to mention determining the effects of parasitism on individual hosts. It may be for these reasons that many researchers have chosen to develop theoretical models to understand the consequences of parasitism on community structure (Hochberg 1991, Holt and Lawton 1993, Yan 1996, Morand and Gonzalez 1997, Hudson and Greenman 1998, Bonsall and Hassel 1999, Bonsall 2004).

Experimental studies on the influence of parasites on community structure can be conducted if the system is manageable. A manageable system is one in which species interactions are easily examined and one that is amenable to manipulation. Simple communities, as defined in this paper, are those in which food webs can be easily defined and examined, and those that support easy manipulation and determination of species interactions. Parasites of mosquitoes that develop in phytotelmata (water filled treeholes, bamboo stumps, flower bracts, and pitcher plants) and artificial containers are ideal for experimentally addressing the influence of parasites on communities. These habitats have simple and well-defined communities, are small in size, have discrete boundaries, and are naturally replicated in the field (Washburn 1995, Barrera 1996a, Kitching 2000). Phytotelmata and container habitats allow both observational and experimental field studies to be conducted. Because of the small size and simplicity of the communities, components of the natural habitat can be manipulated for experimental field studies or can be artificially created in the field to exercise more control over natural variation. In addition, these habitats can be simulated in the laboratory for additional experimental control.

There is a large body of work that has addressed various aspects of the larval ecology of mosquitoes developing in phytotelmata and artificial containers (Table 1). With *a priori* knowledge of species interactions, community composition and structure, and population dynamics of these mosquitoes, we can begin to address the impact of their parasites on aspects of their ecology. However, it is important to first determine which parasites infect mosquitoes in these communities and their effects on individual hosts. There are a number of studies that have addressed the effects of parasites on survival, reproduction, growth, and behavior of their mosquito hosts (Table 2). With this information, a suitable mosquito-parasite system for investigation of the impact of parasites on community structure could be chosen. A few studies have already experimentally addressed how parasites affect competitive interactions (Juliano 1998, Aliabadi and Juliano 2002) and population dynamics (Washburn et al. 1991, Nguyen et al. 2002, Schwab et al. 2003, Tseng 2004) of container dwelling mosquito larvae.

Understanding the ecology of mosquitoes that develop in phytotelmata and artificial container habitats could prove to be medically and economically important. These mosquitoes are vectors of diseases such as malaria, dengue, yellow fever, and West Nile, all of which have a tremendous social impact. With an increased knowledge of vector ecology, we can identify patterns of disease transmission, more effectively target control efforts, implement effective biological control, and perhaps predict invasions of novel disease vectors. Parasites and pathogens of mosquitoes alter various aspects of mosquito life history and because these types of alterations have been shown to impact host ecology, future studies on mosquito population and community ecology should incorporate parasites.

In this paper, I will give a brief summary of the main features of phytotelmata and artificial containers with an emphasis on their mosquito ecology. I will then give an overview of the parasites and pathogens of mosquitoes found in these habitats with summaries of the effects they have on their mosquito hosts. Finally, I will discuss possible ways in which these effects of parasitic infection may alter mosquito community structure.

LARVAL MOSQUITO SYSTEMS

I will consider phytotelmata, artificial containers and temporary pools. Phytotelmata and artificial containers are larval mosquito habitats with unique characteristics that allow community structure to be easily investigated. Phytotelmata is the general term used to describe small bodies of aquatic habitats that are formed in plant-based containers (Kitching 2000). They include bromeliad "tanks", pitcher plants, treeholes, bamboo internodes, and axil waters (Kitching 2000). Although phytotelmata are found within larger ecosystems such as forests, woodlands, or swamps, their communities are relatively simple with species numbering from one to 30 and ranging from protozoans to amphibians (Kitching 2000). The most widely studied communities of phytotelmata are those that occur in treeholes (Table 1) and can be found wherever there is a mature stand of hardwood trees. Their range extends from the subartic to the equator (Bradshaw and Holzapfel 1983, 1992). The geographic variation of treehole habitats leads to variation in species composition and community complexity (Bradshaw and Holzapfel 1983, Barrera 1996b, Juliano 1996, Finke et al. 1997, Yanoviak 2001).

Artificial container habitats of mosquitoes are artificial vessels that contain water, such as discarded tires, cemetery vases, discarded appliances, cans, bottles, and open water tanks. These habitats are most often located near human habitation, increasing the medical and economic importance of mosquitoes found in these areas. Artificial container habitats are similar to those of phytotelmata in that they are small in size, have discrete boundaries, are detritus-based with little to no primary productivity, and their communities are simple. Artificial and natural containers are often grouped together into one habitat type because of their ecological similarities (Washburn 1995), and I will combine them in this paper.

Although container habitats are the most manageable habitats with which to study the impact of parasites on community structure, I include some analyses of temporary pools as they have also been successfully investigated (Morrison and Andreadis 1992, Blaustein and Margalit 1994, Schneider et al. 2000, Eitam et al. 2002) and several parasites infecting these mosquitoes have been studied in detail. In addition, some potential effects of parasites on temporary pool mosquito communities could be applicable to container mosquito communities. Temporary pools are bodies of water that go through seasonal or annual cycles of dry and wet periods. The size of these pools can vary from small rock holes and depressions left by hoof-prints to larger snow-melt and over-flow pools. On average, temporary pools are larger than container habitats and thus the community composition is likely to contain more species and trophic levels (Washburn 1995), making assessment of these communities more difficult. In addition, temporary ground pools will usually exceed container habitats' primary productivity (Washburn 1995). The differences between container habitats and temporary pools can lead to differences in how their communities are regulated by natural enemies (Wasburn 1995). Therefore, when studying the impact of parasites on mosquito communities, the characteristics of the habitat, as well as the life histories of the species that inhabit it, should be considered.

According to Kitching (2000), because container habitats have detritus based communities, species can be categorized into three main groups: saprophages, predators, and top predators. The saprophagous species are further divided into filter feeders (rotifers, copepods, anopheline and aedine mosquitoes, mites) fine detritus feeders (nematodes, ostracods, culicine mosquitoes, chironomids, ceratopogonids, scritid beetles, mites), and macrosaprophages (oligochaete worms, tipulid larvae, sarcophagid larvae, tadpoles). Predators, too, can be further divided into detritus-based predators (planarians, chaoborid midges, predatory chironomids, predatory mites), fixed substrate predators (cecidomyiid larvae, muscid fly larvae), surface predators (hemipterans), and free-swimming predators (damselfly larvae, some *Culex* and *Anopheles* mosquito larvae, periscelid fly larvae, dytiscid beetle larvae, predatory mites). Among the top predators, there are free-swimming predators (*Toxorhynchites* mosquito larvae, carnivorous tadpoles), sit and wait predators (*Toxorhynchites* mosquito larvae, dragonfly larvae), and semi-terrestrial predators (carabid beetles, ants). Mosquitoes are almost always present in these communities and are often top-level consumers (Kaufman et al. 1999); they often have been the focus of container habitat ecological studies (Table 1). Authors have studied how competitive interactions, predator-prey interactions, behaviors, and life history traits of mosquitoes influence population and community structure and species coexistence.

Studies of mosquito communities

Composition and structure of mosquito communities are affected by several factors. Intra- and interspecific competitive interactions among mosquitoes affect species coexistence and thus community structure by altering the balance among species (Schneider et al. 2000). Competition is affected by mosquito life history traits, changes in mosquito population densities, and the presence and absence of predators. Predation, too, can affect community structure by differentially removing some species or cohorts of a population and not others. Species-specific behaviors of larval mosquitoes can influence differential predation as well as alter competitive interactions, thus affecting community structure. In addition, species-specific ovipositing behavior can affect dispersal patterns and thus affect community composition.

Competitive interactions among mosquitoes are influenced by mosquito life history traits such as development time, egg characteristics, and behaviors. Sunahara and Mogi (1997) showed that differences in development rate of two container dwelling mosquito species affected which would become predominant over short- versus long-term interspecific competition. They found that in unstable habitats (short-term interspecific competition) species that developed more quickly became the predominant species, but in stable habitats (long-term interspecific competition) the more slowly developing species predominated. Desiccation resistance of mosquito eggs can also influence which species are likely to colonize certain containers. Species of mosquitoes with drought resistant eggs colonize habitats regardless of their permanence whereas other species with less drought resistant eggs are more likely to oviposit in permanent habitats (Bradshaw and Holzapfel 1983). Interspecific differences in feeding behavior can result in a competitive advantage. *Aedes albopictus* are more efficient foragers than *Aedes aegypti,* and this difference in foraging is proposed as one of the mechanisms by which *Ae. albopictus* have replaced *Ae. aegypti* in many areas of the southeast United States (Yee et al. 2004). Parasite-induced alterations in life history traits such as these could result in a shift in outcomes of intra- or interspecific competition and thus in community structure.

Competitive interactions are also influenced by the differential responses of mosquitoes to intra- versus interspecific changes in population density. For example, Schneider et al. (2000) found that survival of *Anopheles gambiae* did not decrease in response to an increase in single-species or mixed-species populations whereas *Anopheles arabiensis* experienced high mortality in both settings. *Anopheles gambiae* therefore had a competitive advantage over *An. arabiensis* in most habitats. Similarly, *Ochlerotatus triseriatus* were more sensitive to an increase in their own density than to an increase in the density of *Orthopodomyia signifiera* (Chambers 1985). Thus, intraspecific competition was a stronger regulatory force than interspecific competition for *Oc. triseriatus* populations. The addition of a predator to the system, however, reduced survivorship of *Oc. triseriatus,* regardless of the density of either conspecifics or *Or. signifera. Orthopodomyia signifera* by contrast was negatively affected only by intraspecific increases in population density and not by the presence of a predator or by increases in *Oc. triseriatus* density (Chambers 1985). The presence of a parasite that reduced the density of a species could therefore alter intra- or interspecific interactions and thus influence community structure.

Predator-prey interactions that affect community structure are often due to changes in competition among prey species in the presence versus absence of a predator (eg. the "Paine effect"; see Paine 1966). Mogi and Chan (1996) found that a predator in nepthine pitcher plants removed the superior competitor and allowed more species to coexist. *Toxorhynchites* sp., a larval mosquito predator, reversed the competitive interactions between *Orthopodomyia anopheloides* and *Ae. albopictus* (Yasuda 1996). *Aedes albopictus* are the superior competitors in the absence of a predator, however, when a predator is present, *Or. anopheloides* become the predominant mosquito species (see also Grill and Juliano 1996). These switches in competitive interactions are usually due to one species of mosquitoes being more vulnerable to predation.

Both behavior and size influence predation risk. *Aedes albopictus* is a highly active species compared to *Or. anopheloides*. High activity levels are known to attract predators such as *Toxorhynchites* (Sih 1986, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavarju and Juliano 2004). Therefore, *Ae. albopictus* is preyed upon more readily than *Or. anopheloides* (Yasuda and Mitsui 1992). The size of prey can also impact predation rates (Finke et al. 1997, Blaustein 1998, Yanoviak 2001), with predators feeding preferentially on some species and size classes of mosquitoes. For example, notonectid predators can organize community structure by selectively preying on large invertebrates (late instar mosquitoes), and not affecting the densities of small or benthic species (Blaustein 1998). To the extent that parasites modify these traits, they alter predator-prey encounters.

Mosquitoes themselves can serve as top-level consumers (Kaufman et al. 1999). The presence or absence of mosquitoes has effects on community structure similar to those exerted by predators. Mosquitoes eliminate protozoa, microinvertebrates, and other mosquitoes (Maguire et al. 1968). They can also alter the microbial community by feeding and thus reducing microbial densities (Kaufman et al. 1999). In addition, some species of mosquitoes have a greater impact on the lower trophic levels than do other mosquito species (Edgerly et al. 1999).

Oviposition behaviors play a role in structuring communities as well. Species-specific egg dispersal patterns can affect species distribution and abundance. Edgerly et al. (1998) showed that *Oc. triseriatus* altered its oviposition behavior based on the potential threat of competition and on potential habitat stability. Early in the season, when eggs were likely to hatch and develop, *Oc. triseriatus* avoided treeholes that contained more than 15 larvae. However, later in the season, when eggs were more likely to enter diapause, *Oc. triseriatus* deposited eggs in habitats with high larval densities.

High larval densities indicate high habitat productivity; therefore, *Oc. triseriatus* ensure that overwintering eggs would hatch in a suitable environment. Mosquitoes also avoid containers that hold predators or parasites (Blaustein 1998, Zahiri et al. 1997a,b, Zahiri and Rau 1998, Eitam et al. 2002, Spencer et al. 2002). Some species respond differently than others to oviposition signals (Zahiri et al. 1997a). Species-specific avoidance patterns due to the presence of parasites or predators can alter species distribution and thus alter community structure.

PARASITES AND PATHOGENS OF CONTAINER BREEDING MOSQUITOES

Overview

There are many parasites and pathogens that infect mosquitoes. I will focus on those where the effects on various aspects of their mosquito hosts are known (Table 2). Again, I include parasites of mosquitoes breeding in temporary pools because of the economic and medical importance of these parasite-mosquito systems. Parasites of mosquitoes include those from the phyla Microspora, Apicomplexa, Ciliophora, Platyhelminthes, and Nematoda.

Edhazardia aedis is a microsporan parasite of *Ae. aegypti* larvae and adults. The lifecycle of *E. aedis* can include both vertical and horizontal transmission or can be completed via horizontal transmission only. These differences in life-cycles depend upon life history traits and response to environmental conditions (Koella et al. 1998, Agnew and Koella 1999). Vertical transmission involves transmitting binucleate spores to the eggs. The development of uninuceate spores occurs in the larvae that develop from infected eggs. These larvae rarely survive to adulthood and upon death release uninucleate spores into the environment. Horizontal transmission occurs when larvae ingest uninucleate spores found in the surrounding water. These infected larvae may survive to adulthood resulting in infected adults.

Protozoans of the genus *Ascogregarina* are single-celled semi-virulent apicomplexan parasites of various *Aedes* species. Mosquito larvae become infected when they ingest the free-floating oocyst stage. The parasites then develop in the gut epithelium where they eventually produce more oocysts. During metamorphosis of the mosquito, oocysts are released into the surrounding water where they can then be ingested by uninfected larvae (Chen 1999 as cited in Tseng 2004). Gregarines rarely cause mortality in their mosquito hosts and there is some degree of host specificity (Aliabadi and Juliano 2004 and references therein).

Plasmodium, the causative agent of malaria, is another genus of apicomplexan parasites that has been widely studied because of its medical importance. The basic *Plasmodium* life cycle involves two hosts, an invertebrate intermediate host and a vertebrate definitive host. Vertebrate hosts become infected when an infected female mosquito injects sporozoites into the blood stream; the sporozoites then enter the liver cells, where they undergo asexual reproduction. The resulting merozoite stage is released and infects other liver cells or red blood cells. Within the red blood cells, the merozoites undergo further asexual development; some become gameotocytes that are transmitted to an uninfected mosquito when it takes a blood meal from an infected vertebrate host. Within the midgut of the mosquito, fertilization takes place and the resulting ookinetes invade the gut epithelial cells. After escaping the midgut, the ookinetes develop into oocysts. The oocysts undergo sporogony, resulting in sporozoites that are released into the haemocoel and migrate to the mosquito salivary glands. Once in the salivary glands, the sporozoites are ready to be injected into the next definitive vertebrate host. Mosquitoes in the genera *Anopheles* and *Aedes,* both of which develop in container habitats, can transmit *Plasmodium.*

Lambornella clarki is a parasitic ciliate of treehole mosquito larvae. Its life cycle begins with a free-living stage that undergoes metamorphoses and begins host-seeking behavior. After attaching to first instar mosquito larvae the parasite burrows through the cuticle and establishes in the haemocoel where it reproduces, eventually causing death of the host (Washburn et al. 1991).

Trematodes in the genus *Plagiorchis* and *Haematoloechus* can infect larval mosquitoes. Both genera contain species that use an aquatic snail as the first intermediate host and aquatic insects as the second intermediate host; their definitive hosts range from small rodents and amphibians to birds. Mosquitoes can serve as second intermediate hosts and are infected when the free-swimming cercarial stage, released from the snail, burrows through the cuticle and develops into a metacercaria. Definitive hosts become infected by ingesting the mosquitoes. The definitive hosts release miracidia or eggs into the water, where snails ingest them as they feed.

Nematodes can infect both larval and adult mosquitoes. Those that have been studied in mosquitoes are filarial nematodes that infect adult mosquitoes and mermithid nematodes that can infect both adult and larval mosquitoes. Filarial nematodes, such as *Wuchereria bancrofti, Brugia malayi*, and *Dirofilaria immitis*, are carried by mosquitoes and are of medical importance because they cause several diseases of humans and domestic animals. The basic life cycle of filarial nematodes involves two hosts, an arthropod vector and a mammalian definitive host. Mosquitoes acquire the microfilaria when they feed on an infected vertebrate host. Within the mosquito, the juvenile nematodes undergo a series of developmental stages, and then migrate to the head and mouthparts and await the next blood meal. The juvenile worms are not injected directly into the vertebrate host, rather, they are released onto the skin. The juveniles penetrate the skin and migrate to species-specific locations where they mature into adult forms. Sexual reproduction in the vertebrate host results in microfilariae that are released into the circulatory system where they can be ingested by the next mosquito vector (Roberts and Janovy, 2005).

Mermithid nematodes of mosquitoes are parasitoids (parasites that kill their host upon emergence). They exhibit two types of life cycles: development within the larvae and development within the adult mosquito. In both, the nematode eggs are released into an aquatic environment. After hatching, the free-living juvenile seeks out and penetrates a first instar mosquito larva (Petersen 1985). At this point, the two types of life cycles diverge. Mermithids that develop in mosquito larvae migrate to the thoracic region and initiate development. After the initial growth period, the parasite emerges from the fourth instar mosquito, killing it, and burrows into the soil where it matures and mates (Petersen 1984). Mermithids that develop in the adult mosquito migrate to the head capsule of the larva and do not develop further until host eclosion. Mermithids then migrate to the abdomen of the newly emerged adult mosquito where they develop (Blackmore 1993). Mermithids of adult mosquitoes emerge when the host engages in oviposition behavior.

Arboviruses (Arthropod-Borne Viruses) are common pathogens of mosquitoes and are of great medical and veterinary importance. Arboviruses that affect mosquito hosts include Eastern and Western Equine Encephalitis viruses (EEE, WEE), La Crosse Encephalitis virus (LAC), and Dengue virus (DEN). Both EEE and WEE viruses are alphaviruses and have a two-host life cycle that involves mosquitoes and birds; these viruses can be transmitted to humans and horses by bridging vectors. The primary mosquito vector of EEE virus is *Culiseta melanura,* which is usually found in swampy areas of the United States. The bridging vectors of EEE virus are *Coquillettidia perturbans, Aedes sollicitans, Aedes vexans*, and *Culex nigripalpus* (Calisher 1994). Some aspects of the life cycle have yet to be determined. WEE virus, found mostly in the western United States, is transmitted primarily by *Culex tarsalis*; other vectors include *Aedes melanimon, Aedes dorsalis*, and *Aedes campestris* (Calisher 1994). LAC virus is a bunyavirus and is transmitted by *Oc. triseriatus* to squirrels and chipmunks, which are the primary amplifying hosts (Calisher 1994). The life cycle is usually maintained within deciduous forests. Female *Oc. triseriatus* can transovarially transmit the virus. Finally, DEN is a virus found within tropical and subtropical regions, transmitted primarily by *Ae. aegypti* and *Ae. albopictus*. Its amplifying hosts are humans. Once infected, mosquitoes can transmit the virus to humans for the rest of the mosquito life and can also transovarially transmit the virus.

Known effects - Survival

Survival of mosquitoes can be directly affected by the parasites and pathogens that infect them. Representatives from each group of parasite or pathogen reduce mosquito host survivorship (Table 2). Yee and Anderson (1995b) found that adult male and female *Aedes sierrensis* infected with the ciliate *L. clarki* died earlier than uninfected controls. They proposed that the parasite negatively affected fat body resources needed for survival. *Plagiorchis noblei* metacercariae increased mortality in *Ae. aegypti* by interfering with pupation and emergence of adults (Dempster et al. 1986). Mosquito larvae infected with the mermithid nematode *Romanomermis culicivorax* never survive to adulthood (Petersen 1984).

The effects of *Plasmodium* on adult mosquito survival are less clear (Ferguson and Read 2002). Some combinations of mosquito species and *Plasmodium* indicate reduced survival while other combinations show no change. In a meta-analysis, Ferguson and Read (2002) found that reduced survival associated with *Plasmodium* is most often seen in unnatural vector-parasite combinations. Chege and Beier (1990) studied *An. gambiae* and *Anopheles funestus* naturally infected with *P. falciparum*. Within-species effects of infection were absent, but infected *An. gambiae* had a higher daily mortality rates than did infected *An. funestus*. Moncayo et al. (2000) also found that among *Ae. albopictus*, *Anopheles quadrimaculatus*, and *Cq. perturbans*, three bridge vectors of EEE virus, only *Cq. perturbans* experienced increased mortality.

Environmental differences can also influence the effects of parasites on mosquito survival. For example, the microsporidian parasite, *E. aedis*, has a wide range of effects on *Ae. aegypti* larval survival depending upon environmental conditions (Agnew and Koella 1999). Low food resources decrease mosquito larvae growth rate and increase time for *E. aedis* spore production. The higher the spore load, the more likely the larva is to die before emergence. Because females develop more slowly than males, they accumulate more spores and thus incur a greater mortality rate than males. This results in a male-based sex ratio (Agnew and Koella 1999). Likewise, size differences within a population of mosquito hosts can result in differential parasite-induced mortality. Lyimo and Koella (1992) found that the largest *An. gambiae* adults had the highest load of *P. falciparum* oocysts and the intermediate sized *An. gambiae* had the highest load of sporozoites. They hypothesized that intermediate sized *An. gambiae* contained the most sporozoites because high oocyst loads in the large mosquitoes decreased their survivorship prior to sporozoite development.

Indirect effects can decrease survival rates of infected mosquito hosts. Parasite-induced behavioral changes are examples of indirect effects that affect survival rates. For example, *An. gambiae* infected with *P. falciparum* sporozoites experienced higher feeding associated mortality than did uninfected controls (Anderson et al. 2000). Infected mosquitoes spent more time probing and probed more often than uninfected mosquitoes (Rossignol et al. 1984), and were at a higher risk of dying due to vertebrate host defenses.

Known effects – Fecundity

Although parasites and pathogens that affect mosquito fecundity are usually found in adult mosquitoes, it is possible for infected larvae to produce less fecund adults. In a food-deprived environment, as the mean age at pupation of *Ae. aegypti* larvae infected with *E. aedis* increased, adult fecundity decreased (Koella et al. 1998). This correlation was not seen in uninfected larvae. Another study, which does not specifically address fecundity reduction as a result of parasitism, shows that a reduction in overall population fecundity is probable. Dempster et al. (1986) found that 62% of *Ae. aegypti* adults emerging from *P. noblei* infected larvae were malformed. Only 4% of infected *Ae. aegypti* larvae developed into functional adults while 82% of the uninfected larval population did (Dempster et al. 1986).

The majority of studies that have shown definitive evidence of parasite-induced fecundity reduction in mosquito hosts have used *Plasmodium*-mosquito parasite-host systems (Table 2). *Anopheles gambiae, Anopheles stephensi*, and *Ae. aegypti*, are less fecund when infected with *Plasmodium* spp. (Table 2), a condition that initially was attributed to feeding difficulty (Rossignol et al. 1986). However, Hogg and Hurd (1995b) found that blood meal size was not the reason for decreased egg production in infected females. They suggested that *Plasmodium* reduced vitellogenesis. *Plasmodium* can also decrease mosquito fertility (number of eggs that hatch into viable offspring); *An. stephensi* infected with *Plasmodium yoelli nigeriensis,* experienced fertility reduction by 38 – 61% (Jahan and Hurd 1997, Ahmed et al. 1999).

The inability to take a complete blood meal and the cost of an immune response have been implicated as contributing to reduced fecundity in mosquitoes infected with the filarial nematodes (Courtney et al. 1985, Ferdig et al. 1993). Ferdig et al. (1993) showed that mounting an energetically costly immune response to infection took needed resources from other biological functions such as ovarian development. *Armigeres subalbatus* that mounted an immune response to *B. malayi* had altered vitelline accumulation, delayed ovary development, and delayed oviposition compared to uninfected controls (Ferdig et al.1993). In addition, reduction of mosquito fecundity by filarial nematodes was intensity-dependent (Ferdig et al. 1993). Likewise, the mean output of eggs by *Ae. aegypti* was inversely related to an increase in the number of *Brugia pahangi* larvae (Javadian and Macdonald 1974).

Several mosquito arboviruses affect mosquito fecundity. Scott and Lorenz (1998) found that *Cs. melanura* infected with EEE virus had reduced fecundity. The virus did not affect the rate of oogenesis but rather the number of oocytes that developed (see also Maiimood et al. 2004). McGaw et al. (1998) found that the overwintering success of *Oc. triseriatus* eggs infected with LAC virus was significantly decreased compared to uninfected eggs.

Parasite-induced changes in host life history traits, such as a reduction in fecundity or fertility, can affect population sizes of larvae or adults. Again, changes in population size of any component of a community can alter its structure.

Known effects – Behavior

Behaviors such as feeding, flight, oviposition, and predator avoidance can be altered by parasites and pathogens of mosquitoes (Table 2). Given its medical importance, the feeding behavior of adult mosquitoes has been studied extensively and along with it, the impact of parasites and pathogens. While most studies have shown that infection increases various feeding behaviors (Grimstad et al. 1980, Koella and Packer 1996, Rivero and Ferguson 2003, Ferguson and Read 2004), in two cases, parasite development may be influential (Koella and Agnew 1997, Anderson et al. 1999, Koella et al. 2002). The feeding success of *Aedes aegypti* infected with *E. aedis* binucleate spores (vertically transmitted) was unchanged compared to decreased feeding in those infected with uninucleate spores (horizontally transmitted) (Koella and Agnew 1997). In the case of *Plasmodium gallinaceum*, *Ae. aegypti* decreased its feeding activity when infected with the oocyst stage (not infective to vertebrate host) and increased its feeding activity when infected with the sporozoite stage (infective to definitive host) (Anderson et al. 1999, Koella et al. 2002). Both of these studies indicate that stage-specific behavioral alterations likely arose as a parasite adaptation that increased parasite transmission to the appropriate host. At a proximate level, increased probing may be the result of an inability to acquire a sufficient blood meal (Grimstad et al. 1980, Rossignol et al. 1984 Rossignol et al. 1986, Koella and Packer 1996, Platt et al. 1997, but see Hogg and Hurd 1995b).

The flight of adult mosquitoes is affected by several different parasites and pathogens. Microfilarial nematodes, *B. pahangi* and *D. immitis*, impair the flight of *Ae. aegypti* (Townson 1970, Hockmeyer et al. 1975, Berry et al. 1987). However, *D. immitis* stimulate flight activity of *Aedes trivittatus* (Berry et al. 1988). Berry et al. (1988) proposed that the difference in flight capabilities between *Ae. aegypti* and *Ae. trivittatus* infected with *D. immitis* reflects the fact that *Ae. trivittatus* is a natural vector of *D. immitis*, whereas *Ae. aegypti* is an experimental vector. At least one protozoan parasite impairs flight capabilities of mosquitoes. *Lambornella clarki* causes *Ae. sierrensis* to fly for shorter distances than uninfected and gravid females (Yee and Anderson 1995a). Finally, Lee et al. (2000) found that *Cx. tarsalis* infected with WEE virus had decreased spontaneous flight activity compared to uninfected controls. The reduction in spontaneous flight activity was related to the severity of infection. Also, because the virus was concentrated in the head region, it may have affected neural tissue and octopamine, which play a role in flight behavior (Lee et al. 2000).

Oviposition behavior is altered by the presence of parasites. Water containing parasitized larvae is repellent to ovipositing females (see references in Table 2). Larval *Ae. aegypti* infected with metacercarial *P. elegans* rendered oviposition sites unattractive to female conspecifics (Zahiri et al. 1997a) and was most repellent compared to crowded waters or water that held starved larvae (Zahiri and Rau 1998). In addition, *Ae. aegypti* were attracted to waters with uninfected *Aedes atropalpus* but were not attracted to waters that contained infected *Ae. atropalpus* (Zahiri et al. 1997b). *Aedes atropalpus* on the other hand, were repelled by waters that contained infected conspecifics but not to water that contained infected *Ae. aegypti* (Zahiri et al. 1997b), indicating that some mosquito species are more sensitive to infection cues than others. Mosquito species can also respond differently to parasite-specific cues. Reeves (2004) found that ovipositioning *Ae. aegypti* were more attracted to water that held conspecifics infected with either the gregarine, *A. taiwanensis*, or the yeast, *Candida* near *pseudoglaebosa* compared to distilled water or water that held uninfected controls. In contrast, *Ae. aegypti* did not respond to water that held conspecifics infected with the fungus *Smittium morbosum*.

Only two studies have experimentally addressed parasite-induced behavioral changes of mosquitoes that result in changes in predation rates. Webber et al. (1987a) found that *Ae. aegypti* larvae infected with more than three *P. noblei* metacercaria were less active and were found more often at the surface of the water than were uninfected conspecifics. This change in behavior led to an increase in predation by meadow voles, a definitive host of *P. noblei* (Webber et al. 1987a). Wise de Valdez (in preparation) found that although there were differences in activity levels of *Ae. aegypti* larvae infected with the nematode *R. culicivorax*, these did not result in a change in predation rate.

Modifications in behaviors such as these are indirect mechanisms by which parasites can alter community structure. Changes in feeding behaviors can also potentially alter mortality risk and fecundity, both of which determine population size. Parasite-induced alterations in predator avoidance behaviors can increase mortality of some species, directly reducing prey populations. In addition, modifying the predator avoidance behaviors of one species can alter interspecific competition. Changes in oviposition behaviors can modify species dispersal or can alter which species are found in the same habitat. The resulting shifts in population sizes and competitive interactions due to changes in these behaviors can influence community structure.

Known effects – ecology

Several studies have mentioned the implications of parasites for mosquito host ecology (Table 2); however, only six have been designed to experimentally investigate the impact of parasites on host ecology. Competitive interactions are an important component of host ecology and play a large role in species coexistence. Juliano (1998) and Aliabadi and Juliano (2002) investigated the impact of the gregarine parasite, *A. taiwanensis*, on the competitive interactions of *Ae. aegypti* and *Oc. triseriatus* with the invasive species *Ae. albopictus*. *Aedes albopictus* was introduced to North America in the 1980s and since then has experienced success in many habitats. Its interaction with other container dwelling mosquito species has attracted attention because it has led to the decline of *Ae. aegypti* in many areas of the southern United States (Juliano 1998 and references therein). Juliano (1998) found that the competitive advantage of *Ae. albopictus* over *Ae. aegypti* was not a result of apparent competition mediated by *A. taiwanensis* because *Ae. aegypti* was rarely infected with *A. taiwanensis* in the field. In a later study, Aliabadi and Juliano (2002) conducted several laboratory experiments to test the effect of *A. taiwanensis* on interspecific competition between *Ae. albopictus* and *Oc. triseriatus*. In laboratory competition experiments, they found that at a larval density of 30, *Oc. triseriatus* survivorship was reduced when 30 uninfected *Ae. albopictus* were added but not when 30 infected *Ae. albopictus* were added. Because *A. taiwanensis* appears to be specific to *Ae. albopictus* (Aliabadi and Juliano 2002 and references therein), *Ae. albopictus* escapes its parasite when invading new sites. This escape from parasitism may give *Ae. albopictus* an initial advantage over *Oc. triseriatus* thus allowing it to successfully colonize sites inhabited by resident species.

Ascogregarina taiwanensis can also impact the population dynamics and intraspecific competition of *Ae. albopictus* differently in varying environmental conditions. Tseng (2004) conducted a study on sex-specific responses of *Ae. albopictus* at varying densities to infection with *A. taiwanensis.* She found that at both high and low densities, infected males were smaller than uninfected males. In contrast, an effect of infection on female size occurred only at low densities. Tseng (2004) proposed that the differential response was a result of the cost of competition and that each sex altered the phenotypic trait least important to its survival and/or fecundity. This implied that attaining a large size was less important to males than to females (Tseng 2004).

Nguyen et al. (2002) studied the effect of infection with *Plagiorchis elegans* on the population dynamics of *Ae. aegypti* under varying food availability. They found that infection with *P. elegans* differentially removed fourth instar mosquito larvae. By removing the superior competitive instar, the first, second, and third instar larvae were released from intraspecific competition and experienced faster development. Although the earlier instars were released from competition, as they reached fourth instar, they too were killed by the parasite. Because infection with *P. elegans* reduced larval mosquito populations regardless of food availability, Nguyen et al. (2002) proposed that *P. elegans* could serve as a biocontrol agent in habitats with fluctuating food resources.

Population structure of container-breeding larval mosquito populations depends on recruitment of new individuals. Female oviposition patterns largely determine which habitats will experience recruitment (Schwab et al. 2003). Because infection with *Plagiorchis* sp. affects the habitat choice of ovipositing females (Table 2) and because *Plagiorchis* sp. can alter larval development (Nguyen et al. 2002), it is likely that *Plagiorchis* infection affects larval mosquito population dynamics (Schwab et al. 2003). Schwab et al. (2003) investigated the impact of infection with *P. elegans* on larval population dynamics of *Ae. aegypti* in high food availability. They found that infection did not impair larval development but did decrease the number of adults emerging. Interestingly, these authors did not find that water holding infected larvae was repellent to oviposition females; therefore, recruitment into these habitats remained high (but see Lowenberger and Rau 1994, Zahiri et al. 1997 a,b, Zahiri and Rau 1998). Schwab et al. (2003) indicate that retained larval recruitment into infected habitats will enhance the suitability of *P. elegans* as a biocontrol agent. Females that oviposit into these sites would lose their reproductive investment and would be less likely to contribute to the adult population. Zahiri and Rau (1998) suggest that avoidance of *Plagiorchis* spp. infected waters could be used as a different method of biocontrol by shifting oviposition sites away from human dwellings.

Larval mosquito population dynamics were also studied in response to the parasite *L. clarki*. The effect of *L. clarki* infection on *Ae. sierrensis* adult size was influenced by larval food availability (Washburn et al. 1991). Adults that emerged from food-limited microcosms with infected larvae were significantly larger than those that emerged from food-limited microcosms with uninfected larvae. In contrast, adults that emerged from non-limited resource microcosms with infected larvae were similar in size to those that emerged from the uninfected control microcosms. Washburn et al. (1991) concluded that intraspecific competition in low-resource microcosms was relaxed via parasite-induced mortality. This study is an example of how the presence of a parasite does not always negatively impact mosquito populations.

DISCUSSION

Parasites play a role in structuring their host communities (Minchella and Scott 1991, Poulin 1999, Thomas et al. 2000, Mouritsen and Poulin 2002). Poulin (1999) proposes that there are at least three ways in which parasites can do so. (1) Parasites can have differential effects on different host species. Differences in host susceptibility to parasitism can lead to shifts in competitive interactions or predator-prey interactions in the presence or absence of parasites. (2) Pathological effects of parasites on a single-host species can directly alter the functional importance of the host in the community. (3) Parasites can indirectly affect the functional importance of their host species by altering behavioral phenotypes or host life history traits. Either direct or indirect consequences of parasitic infection can affect intra- and interspecific competition in many aquatic insects or invertebrates (Jaenike 1995, Kohler and Wiley 1997, Yan et al. 1998, Kiesecker and Blaustein 1999, Feener 2000) and predator-prey interactions (Latham and Poulin, 2002, MacNeil et al. 2003a,b, Wellnitz et al. 2003). Parasites of mosquitoes may be able to affect mosquito host communities in similar ways. In addition to several studies that have addressed the role of mosquito parasites on species coexistence, competition, and population dynamics (reviewed above), I will synthesize studies on mosquito ecology with those on known effects of parasitism on mosquito hosts in order to suggest potential effects parasites might have on mosquito community structure.

(1) *Differential effects of parasitism on mosquito hosts*

Nonspecific parasites that have differential effects on host species or host-specific parasites that preferentially infect superior competitors can play a role in structuring host communities (Minchella and Scott 1991). *Plasmodium spp.* are nonspecific parasites that may infect some species of mosquitoes more readily than others and may have differential effects on specific host species; therefore, this mosquito-parasite system would be amenable to the study of how differential effects of parasitism affect host communities. For example, *Plasmodium* spp. have greater negative impacts on mosquito mortality in unnatural mosquito-*Plasmodium* combinations than in natural combinations (Ferguson et al. 2002). Within natural combinations, *Plasmodium* can differentially affect host survival; *An. gambiae* had a higher infection rate in the field than *An. funestus* as well as a higher mortality rate (Chege and Beier 1990). Similarly, EEE virus is a nonspecific pathogen of mosquitoes that can have differential effects on mosquito species and could provide another system with which to study the impact of parasites on host communities. For example, among the EEE virus bridge vectors, *Ae. albopictus, An. quadrimaculatus*, and *Cq. perturbans*, only *Cq. perturbans* survival was negatively affected (Moncayo et al. 2000). Because *Plasmodium* and EEE virus cause human disease, their differential effects on different host species may play a role in vectorial capacity as well as in community structure.

The impact of parasite-induced adult mosquito mortality on mosquito community structure may be difficult to study. Not only are adult mosquito populations difficult to track, but inability to assess sources of bias and error can influence population estimates (Smith 1985). In addition, adult communities have rarely been studied because of non-discrete boundaries, the rarity of competition for resources (but see Besansky et al. 2004), and the inability to examine all components of the community. However, it is possible that parasitic infection of adult mosquitoes can influence larval populations and therefore, adult mosquito-parasite systems can be studied in light of the effect on larval community composition and structure. For example, adult mortality directly impacts the number of females ovipositing in a given habitat. If the number of female mosquitoes of a given species is severely reduced, then the larval habitat will receive fewer eggs of that particular species. Larval density of that species would then decrease and may affect intra- or interspecific competition. Several studies have shown that variations in intra- and interspecific larval densities affect competitive interactions and species coexistence (Bradshaw and Holzapfel 1985, Chambers 1985, Edgerly et al. 1993, 1998, Miller et al. 1994, Schneider et al. 2000) and as a result, community structure. In these cases, the presence of parasites in the adult population would indirectly alter larval community structure.

Studying differences in parasite-induced mortality of larval mosquitoes and the effect of these differences on community structure is likely to be more manageable than such a task for adults. Only two studies have addressed the impact of parasites on different species of larval mosquitoes (Juliano 1998, Aliabadi and Juliano 2002). In these studies, *Ae. albopictus* was more susceptible to the gregarine parasite, *A. taiwanensis*, than either *Ae. aegypti* or *Oc. triseriatus*. As a result, the competitive interactions between *Oc. triseriatus* and *Ae. albopictus* were reversed in the presence of the parasite

(Aliabadi and Juliano 2002). Reversal of competitive interactions is not unknown in host-parasite systems (Park, 1948, Jaenike 1995, Kiesecker and Blaustein 1999, Yan 1998) and has been implicated as one of the main roles that parasites play in structuring host communities (Price et al. 1988). Therefore, it is likely that examination of the community structure before and after the presence of *A. taiwanensis* would indicate that *A. taiwanensis* has the potential to alter community structure. Several other nonspecific parasites of mosquito larvae, such as *P. elegans* and *P. noblei* (Trematoda), or *R. culicivorax* and *Strelkovimermis spiculatus* (Nematoda) could be considered as potential candidates for studying how mosquito communities can be structured via differential effects on different host species.

(2) *Direct effects on functional importance of mosquito hosts*

Although probable, there are no documented examples that indicate parasites structure communities by directly altering the functional importance of a particular host species (Poulin 1999). Poulin (1999) uses parasite-induced behavioral alterations of a top-predator as a hypothetical example in which a parasite could alter community structure in this manner. If the predator was debilitated by the parasite in some way that decreased its ability to feed on various prey, then the parasite could directly alter community composition of the prey species. Container dwelling mosquitoes and their parasites provide an excellent host-parasite system with which to experimentally study this phenomenon. Mosquitoes are often the top-level consumers of container habitats (Frank and Lounibos 1983) and can play a role in structuring their communities by controlling populations of organisms on which they feed (Maguire et al. 1968, Edgerly et al. 1999, Kaufman et al. 1999, Eisenberg et al. 2000). There are several species of parasites that can directly alter feeding behaviors of mosquitoes. For example, *P. elegans* induced anorexia in *Ae. aegypti* larvae (Zahiri et al. 1998). The change in feeding patterns of this species could potentially result in altered densities of the microbial and microinvertebrate populations on which *Ae. aegypti* feed, thus altering community structure.

Another aspect of larval mosquitoes that could be directly affected by parasitism is mortality rates. An increase in larval mosquito mortality due to parasitism (Kellen et al. 1965, Petersen 1984, Washburn et al. 1991, Comisky et al. 1999, Aparecida de Carvalho 2002, Nguyen et al. 2002) would reduce the abundance of the top-level consumer and thus impact prey populations. A change in mortality could also alter competitive interactions with other top-level consumers and thus have far reaching effects on trophic interactions (Kohler and Wiley 1997). The relationship among *Oc. triseriatus, Ae. aegypti*, and *Ae. albopictus* is a possible example of an association in which to study this phenomenon. *Ochlerotatus triseriatus* are more predatory and less susceptible to predation than either *Ae. aegypti* or *Ae. albopictus*, and more likely to affect larval populations of *Ae. aegypti* and *Ae. albopictus* than either was likely to affect it (Edgerly et al. 1999). A hypothetical experiment might include the addition of a nonspecific parasite, such as *Ascogregarina* spp., to the system and the study of the resultant competitive interactions of these three top-level consumers. For example, if *Oc. triseriatus* experienced greater mortality when infected with a parasite than the other species, then *Ae. aegypti* and *Ae. albopictus* may be released from competition. Moreover, because *Ae. albopictus* is a more efficient forager than *Ae. aegypti* (Yee et al.

2004) and potentially *Oc. triseriatus*, its release from competition could decrease populations of the microinvertebrates and microbes on which they feed.

(3) *Indirect effects on functional importance of mosquito hosts*

Indirect effects on functional importance of hosts within a community are those that are not a result of pathology due to infection. Parasite-induced changes in behaviors and host life history traits are examples of indirect effects. Parasite-induced behavioral alterations in adult mosquitoes have been well studied (Hurd 2003 and references therein) with the majority of the literature addressing changes in adult feeding behavior. These behaviors are unlikely to have a major effect on mosquito host community structure unless parasite-induced alterations in adult feeding behavior result in changes in larval population size.

A more probable way in which parasite-induced changes in adult mosquito behavior affect larval community structure is via alteration of oviposition behavior. Population structure of container breeding larval mosquitoes depends on recruitment of new individuals and oviposition behavior is one of the factors that affect larval recruitment. When and where adult mosquitoes oviposit is governed by several habitat factors (Edgerly et al. 1998, Spencer et al. 2002, Reiskind and Wilson 2004), the presence of parasites being one of them (Table 2). Adult mosquitoes have been shown to be attracted to (Reeves 2004) or repelled by (Zahiri et al. 1997a, b, Zahiri and Rau 1998) water that held infected larvae. Moreover, some mosquito species are repelled by infected conspecifics but not by other infected species (Zahiri et al. 1997b). Differences in sensitivity to parasites that results in differential ovipostion behavior among mosquito species may further influence community structure. Because studies of ovipostion behavior of adult females have already been carried out in the field (Tables 1, 2), the addition of experimentally infected larvae could be incorporated into these studies in order to address the role of parasites in structuring communities.

Parasite-induced alterations of larval mosquito behaviors have been less studied; however, there is the potential for future studies on the effects of these behavioral alterations on community structure. For example, because parasites can alter behaviors of mosquito larvae in ways that increase predation (Webber et al. 1987 a, b), the mosquito population may be reduced. This reduction in mosquito larvae population size may affect prey community and thus the wider community (Minchella and Scott 1991). It is well known that mosquito larval behavior influences predation rates (Juliano and Reminger 1992, Juliano et al. 1993 , Grill and Juliano 1996, Juliano and Gravel 2002) and species-specific behavior that results in differential predation can influence community composition (Sih 1986, Yasuda and Mitsui 1992, Yasuda 1996, Kesavarju and Juliano 2004). If parasites alter activity levels of their hosts (Webber et al. 1987a,b, Wise de Valdez in preparation), predators may preferentially feed on either the infected or uninfected population (dependent upon how the parasite affect activity levels). Thus, it is possible that as a result of behavioral modification, the presence of a parasite may alter community diversity. With the relatively large amount of information on predator-prey relationships of mosquito larvae and the roles of behavior in that relationship, incorporating parasites would create an ideal system in which to study the indirect effects of parasites in structuring mosquito communities.

Parasite-induced changes in mosquito life history traits, such as size or development, could also indirectly affect community structure. Larval size can influence predator-prey interactions (Amalraj and Das 1996, Finke et al. 1997, Walton 2001, Soumare et al. 2004) as well as competitive interactions (Miller et al. 1994, Blaustein and Margalit 1996, Nguyen et al. 2002) in mosquito communities. Mosquito larvae size can be reduced by infection with mermithid nematodes (Gordon 1981) or by other parasites that impose a nutritional stress on their host. A reduction in larval size may result in changes in patterns of predation by predators that select prey based on size. Predators that preferentially prey on specific size classes of invertebrates within mosquito communities can be considered community structure organizers (Finke et al. 1997, Blaustein 1998). Likewise, because parasites of mosquito larvae kill some size classes more than others (Agnew and Koella 1999, Nguyen et al. 2002), parasites can also act as mosquito community organizers.

According to Thomas et al. (2000) hosts may compensate for the negative effects of infection by altering life history traits. Because life history traits of species are important determinants of species coexistence, parasites that indirectly alter host life history traits have the potential to influence community structure (Thomas et al. 2000). *Edhazardia aedis* selects for early pupation; slowly developing larvae acquire more parasites and are killed (Koella et al. 1998). Development rate is a life history trait of mosquitoes that can confer a competitive advantage in specific habitats. For example, *Ae. albopictus* outcompeted *Tripteroides bambusa* in unstable habitats because it developed faster and therefore was able to exploit food resources prior to *Tr. bambusa* (Sunahara and Mogi 1997). However, in a more stable environment where long-term interactions were more likely and competition for food resources were stronger, the competitive advantage was reversed. *Tripteroides bambusa* had sufficient time to develop and was found to be more resistant to starvation than *Ae. albopictus* (Sunahara and Mogi 1997).

Conclusion

Host-parasite systems have been studied in light of altered competitive interactions, predator-prey relationships, and population dynamics. However, there are few experimental comparative studies designed to investigate the implications for these changes on community structure. Container-dwelling mosquito habitats and their host-parasite associations provide a manageable system that allows such experimentation. Parasites can influence population dynamics (Washburn et al. 1991, Nguyen et al. 2002, Schwab et al. 2003, Tseng 2004) and alter competitive interactions between larval mosquito species (Juliano 1998, Aliabadi and Juliano 2002). Further investigation of these effects on mosquito community structure could be manageably conducted in the field or laboratory by comparing the communities with and without parasites.

In this paper, I have amassed evidence that parasites of mosquitoes can alter population dynamics, predator-prey relationships, and competitive interactions. First, mosquito parasites can have different effects on different mosquito hosts. Differences in susceptibility to or costs of parasitism can lead to shifts in interspecific relationships. Second, mosquito parasites can directly affect the functional importance of mosquito hosts. A reduction in mosquito host abundance or changes in behavior due to the pathological effects of parasitism can also modify interspecific relationships. Third, mosquito parasites can indirectly affect the functional importance of mosquito hosts by modifying host behaviors or life history traits. Changes in these host aspects may also lead to altered population dynamics, predator-prey relationships, and competitive interactions. In addition, given current knowledge of species interactions, species coexistence, community structure, and population dynamics of container dwelling mosquitoes, we can begin to address the impact of their parasites on community structure.

Understanding the role of parasites in the structure and composition of mosquito communities could prove to be medically and economically important. Changes in population dynamics or community structure as a result of parasitism may influence patterns of disease transmission and thus alter the target of control efforts. In addition, because parasites of mosquitoes can serve as biocontrol agents, it is important to understand the ecological consequences of implementation. Finally, it is possible that the presence of parasitism in mosquito communities could alter the ability of novel disease vectors to colonize a particular habitat.

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REFERENCES CITED

Agnew, P., and J. C. Koella. 1999. Constraints on the reproductive value of vertical transmission for a microsporidian parasite and its female-killing behaviour. Journal of Animal Ecology 68:1010-1019.

Ahmed, A. M., R. D. Maingon, P. J. Taylor, and H. Hurd. 1999. The effects of infection with *Plasmodium yoelii nigeriensis* on the reproductive fitness of the mosquito *Anopheles gambiae*. Invertebrate Reproduction & Development 36:217-222.

Aliabadi, B. W., and S. A. Juliano. 2002. Escape from gregarine parasites affects the competitive interactions of an invasive mosquito. Biological Invasions 4:283-297.

Amalraj, D. D., and P. K. Das. 1996. Frequency-dependent prey selection by larvae of *Toxorhynchites splendens* (Diptera: Culicidae). Bulletin of Entomological Research 86:633-639.

Anderson, R. A., J. C. Koella, and H. Hurd. 1999. The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. Proceedings of the Royal Society of London Series B-Biological Sciences 266:1729-1733.

Anderson, R. A., B. G. J. Knols, and J. C. Koella. 2000. *Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae* s.l. Parasitology 120:329-333.

Aparecida de Carvalho, G. A., C. F. S. Andrade, and M. T. Ueta. 2002. Experimental infection of *Aedes albopictus* (Diptera : Culicidae) larvae with the xiphidiocercariae of a hematolechid. Memorias Do Instituto Oswaldo Cruz 97:573-578.

Barrera, R. 1996a. Species concurrence and the structure of a community of aquatic insects in tree holes. Journal of Vector Ecology 21:66-80.

Barrera, R. 1996b. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecological Entomology 21:117-127.

Becnel, J. J., J. J. Garcia, and M. A. Johnson. 1995. *Edhazardia aedis* (Microspora, Culicosporidae) effects on the reproductive capacity of *Aedes aegypti* (Diptera, Culicidae). Journal of Medical Entomology 32:549-553.

Berry, W. J., W. A. Rowley, J. L. Clarke, N. S. Swack, and W. J. Hausler. 1987. Spontaneous flight activity of *Aedes trivittatus* (Diptera, Culicidae) infected with Trivittatus virus (Bunyaviridae, California Serogroup). Journal of Medical Entomology 24:286-289.

Berry, W. J., W. A. Rowley, and B. M. Christensen. 1988. Spontaneous flight activity of *Aedes trivittatus* infected with *Dirofilaria immitis*. Journal of Parasitology 74:970-974.

Besansky, N. J., C. A. Hill, and C. Costantini. 2004. No accounting for taste: host preference in malaria vectors. Trends in Parasitology 20:249-251.

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

Blackmore, M. S. 1993. Ecology of mosquito-parasitic mermithid nematodes. Vector Control Bulletin 2:5-7.

Blaustein, L. 1998. Influence of the predatory backswimmer, *Notonecta maculata*, on invertebrate community structure. Ecological Entomology 23:246-252.

Blaustein, L., and J. Margalit. 1994. Differential vulnerability among mosquito species to predation by the cyclopoid copepod, *Acanthocyclops viridis*. Israel Journal of Zoology 40:55-60.

Blaustein, L., and J. Margalit. 1996. Priority effects in temporary pools: Nature and outcome of mosquito larva toad tadpole interactions depend on order of entrance. Journal of Animal Ecology 65:77-84.

Bonsall, M. B. 2004. The impact of diseases and pathogens on insect population dynamics. Physiological Entomology 29:223-236.

Bonsall, M. B., and M. P. Hassell. 1999. Parasitoid-mediated effects: apparent competition and the persistence of host-parasitoid assemblages. Researches on Population Ecology 41:59-68.

Bradshaw, W. E., and C. M. Holzapfel. 1983. Predator-mediated, non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. Oecologia 57:239-256.

Bradshaw, W. E., and C. M. Holzapfel. 1985. The distribution and abundance of treehole mosquitoes in eastern North America: Perspectives from North Florida, Pages 25-53 *in* L. P. Lounibos, J. H. Frank, and J. R. Rey, eds. Ecology of Mosquitoes. Vero Beach, FL, Florida Medical Entomology Laboratory.

Bradshaw, W. E., and C. M. Holzapfel. 1992. Resource limitation, habitat segregation, and species interactions of british tree-hole mosquitoes in nature. Oecologia 90:227-237.

Braks, M. A. H., N. A. Honorio, L. P. Lounibos, R. Lourenco-De-Oliveira, and S. A. Juliano. 2004. Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera : Culicidae), in Brazil. Annals of the Entomological Society of America 97:130-139.

Calisher, C. H. 1994. Medically important arboviruses of the United States and Canada. Clinical Microbiology Reviews 7: 89-116

Chambers, R. C. 1985. Competition and predation among larvae of three species of treehole-breeding mosquitoes, Pages 25-53 *in* L. P. Lounibos, J. H. Frank, and Rey, eds. Ecology of Mosquitoes. Vero Beach, FL, Florida Medical Entomology Laboratory.

Chege, G. M. M., and J. C. Beier. 1990. Effect of *Plasmodium falciparum* on the survival of naturally infected afrotropical *Anopheles* (Diptera: Culicidae). Journal of Medical Entomology 27:454-458.

Chen, W. J. 1999. The life cycle of *Ascogregarina taiwanensis* (Apicomplexa : Lecudinidae). Parasitology Today 15:153-156.

Christensen, B. M. 1981. Effect of *Dirofilaria immitis* on the fecundity of *Aedes trivitattus*. Mosquito News 41:78-81.

Colton, Y. M., D. D. Chadee, and D. W. Severson. 2003. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. Medical and Veterinary Entomology 17:195-204.

Combes, C. 1996. Parasites, biodiversity and ecosystem stability. Biodiversity and Conservation 5:953-962.

Comiskey, N. M., R. C. J. Lowrie, and D. M. Wesson. 1999. Role of habitat components on the dynamics of *Aedes albopcitus* (Diptera: Culicidae) from New Orleans. Journal of Medical Entomology 36:313-320.

Courtney, C. C., B. M. Christensen, and W. G. Goodman. 1985. Effect of *Dirofilaria immitis* on blood meal size and fecundity in *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology 22:398-400.

Dempster, R. A., M. E. Webber, and D. J. Lewis. 1986. The effects of *Plagiorchis noblei* metacercariae on teh development and survival of *Aedes aegypti* larvae in the laboratory. Journal of Parasitology 72:699-702.

Edgerly, J. S., M. S. Willey, and T. P. Livdahl. 1993. The community ecology of *Aedes* egg hatching - Implications for a mosquito invasion. Ecological Entomology 18:123-128.

Edgerly, J. S., M. McFarland, P. Morgan, and T. Livdahl. 1998. A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito. Journal of Animal Ecology 67:805-818.

Edgerly, J. S., M. S. Willey, and T. Livdahl. 1999. Intraguild predation among larval treehole mosquitoes, *Aedes albopictus*, *Ae. aegypti*, and *Ae. triseriatus* (Diptera : Culicidae), in laboratory microcosms. Journal of Medical Entomology 36:394-399.

Egerter, D. E., J. R. Anderson, and J. O. Washburn. 1986. Dispersal of the parasitic ciliate *Lambornella clarki* - Implications for ciliates in the biological-control of mosquitos. Proceedings of the National Academy of Sciences of the United States of America 83:7335-7339.

Egerter, D. E., and J. R. Anderson. 1989. Blood-feeding drive inhibition of *Aedes sierrensis* (Diptera, Culicidae) induced by the parasite *Lambornella clarki* (Ciliophora, Tetrahymenidae). Journal of Medical Entomology 26:46-54.

Eisenberg, J. N. S., J. O. Washburn, and S. J. Schreiber. 2000. Generalist feeding behaviors of *Aedes sierrensis* larvae and their effects on protozoan populations. Ecology 81:921-935.

Eitam, A., L. Blaustein, and M. Mangel. 2002. Effects of *Anisops sardea* (Hemiptera : Notonectidae) on oviposition habitat selection by mosquitoes and other dipterans and on community structure in artificial pools. Hydrobiologia 485:183-189.

Feener, D. J. 2000. Is the assembly of ant communities mediated by parasitoids? Oikos 90:79-88.

Ferdig, M. T., B. T. Beerntsen, F. J. Spray, J. Y. Li, and B. M. Christensen. 1993. Reproductive costs associated with resistance in a mosquito-filarial worm system. American Journal of Tropical Medicine and Hygiene 49:756-762.

Ferguson, H. M., and A. F. Read. 2002. Why is the effect of malaria parasites on mosquito survival still unresolved? Trends in Parasitology 18:256-261.

Ferguson, H. M., A. Rivero, and A. F. Read. 2003. The influence of malaria parasite genetic diversity and anaemia on mosquito feeding and fecundity. Parasitology 127:9-19.

Ferguson, H. M., and A. F. Read. 2004. Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts. Malaria Journal 3.

Frank, J. H. and L. P. Lounibous. 1983. Phytotelmata: terrestrial plants as host for aquatic insects communities. Plexus, Medford, N. J.

Fincke, O. M., S. P. Yanoviak, and R. D. Hanschu. 1997. Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. Oecologia 112:244-253.

Freier, J. E., and S. Friedman. 1976. Effect of host infection with *Plasmodium gallinaceum* on the reproductive capacity of *Aedes aegypti*. Invertebrate Pathology 28:161-166.

Galloway, T. D., and R. A. Brust. 1985. The effects of parasitism by *Romanomermis culicivorax* (Nematoda, Mermithidae) on growth and development of *Aedes vexans* (Diptera, Culicidae) in laboratory and field-tests. Canadian Journal of Zoology 63:2437-2442.

Gordon, R. 1981. Mermithid nematodes - physiological relationships with their insect hosts. Journal of Nematology 13:266-274.

Grill, C. P., and S. A. Juliano. 1996. Predicting species interactions based on behaviour: Predation and competition in container-dwelling mosquitoes. Journal of Animal Ecology 65:63-76.

Grimstad, P. R., Q. E. Ross, and G. B. Craig. 1980. *Aedes triseriatus* (Diptera, Culicidae) and La Crosse Virus .2. modification of mosquito feeding-behavior by virus-infection. Journal of Medical Entomology 17:1-7.

Hochberg, M. E. 1991. Population-dynamic consequences of the interplay between parasitism and intraspecific competition for host-parasite systems. Oikos 61:297-306.

Hockmeyer, W. T., B. A. Schieffer, B. C. Redington, and B. F. Eldridge. 1975. *Brugia pahangi*: effects upon the flight capability of *Aedes aegypti*. Experimental Parasitology 38: 1-5

Hogg, J. C., and H. Hurd. 1995a. Malaria-induced reduction of fecundity during the first gonotrophic cycle of *Anopheles stephensi* mosquitos. Medical and Veterinary Entomology 9:176-180.

Hogg, J. C., and H. Hurd. 1995b. *Plasmodium yoelii nigeriensis*: The effect of high and low intensity of infection upon the egg production and bloodmeal size of *Anopheles stephensi* during three gonotrophic cycles. Parasitology 111:555-562.

Hogg, J. C., and H. Hurd. 1997. The effects of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae* s. l. in north east Tanzania. Parasitology 114:325-331.

Holt, R. D., and J. H. Lawton. 1993. Apparent competition and enemy-free space in insect host-parasitoid communities. American Naturalist 142:623-645.

Hudson, P., and J. Greenman. 1998. Competition mediated by parasites: biological and theoretical progress. Trends in Ecology & Evolution 13:387-390.

Hurd, H. 2003. Manipulation of medically important insect vectors by their parasites. Annual Review of Entomology 48:141-161.

Jaenike, J. 1995. Interactions between mycophagous *Drosophila* and their nematode parasites - From physiological to community ecology. Oikos 72:235-268.

Jahan, N., and H. Hurd. 1997. The effects of infection with *Plasmodium yoelii nigeriensis* on the reproductive fitness of *Anopheles stephensi*. Annals of Tropical Medicine and Parasitology 91:365-369.

Javadian, E., and W. M. Macdonald. 1974. The effect of infection with *Brugia pahangi* and *Dirofilaria repens* on the egg-production of *Aedes aegypti*. Annals of Tropical Medicine and Parasitology 68:477-481.

Juliano, S. A. 1996. Geographic variation in *Aedes triseriatus* (Diptera: Culicidae): Temperature-dependent effects of a predator on survival of larvae. Environmental Entomology 25:624-631.

Juliano, S. A. 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? Ecology 79:255-268.

Juliano, S. A., and L. Reminger 1992. The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences. Oikos 63:465-476.

Juliano, S. A., L. J. Hechtel, and J. R. Waters. 1993. Behavior and risk of predation in larval tree hole mosquitoes: effects of hunger and population history of predation. Oikos 68:229-241.

Juliano, S. A., and M. E. Gravel. 2002. Predation and the evolution of prey behavior: an experiment with treehole mosquitoes. Behavioral Ecology 13:301-311.

Juliano, S. A., L. P. Lounibos, and G. F. O'Meara. 2004. A field test for competitive effects of *Aedes albopictus* on *A. aegypti* in South Florida: differences between sites of coexistence and exclusion? Oecologia 139:583-593.

Kaufman, M. G., E. D. Walker, T. W. Smith, R. W. Merritt, and M. J. Klug. 1999. Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats? Applied and Environmental Microbiology 65:2661-2673.

Kesavaraju, B., and S. A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container-dwelling mosquitoes. Annals of the Entomological Society of America 97:194-201.

Kiesecker, J. M., and A. R. Blaustein. 1999. Pathogen reverses competition between larval amphibians. Ecology 80:2442-2448.

Kitching, R. L. 2000, Food Webs and Container Habitats The natural history and ecology of phytotelmata. Cambridge, Cambridge University Press.

Koella, J. C., and M. J. Packer. 1996. Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. Parasitology 113:105-109.

Koella, J. C., and P. Agnew. 1997. Blood-feeding success of the mosquito *Aedes aegypti* depends on the transmission route of its parasite *Edhazardia aedis*. Oikos 78:311-316.

Koella, J. C., P. Agnew, and Y. Michalakis. 1998a. Coevolutionary interactions between host life histories and parasite life cycles. Parasitology 116:S47-S55.

Koella, J. C., F. L. Sorensen, and R. A. Anderson. 1998b. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. Proceedings of the Royal Society of London Series B-Biological Sciences 265:763-768.

Koella, J. C., L. Rieu, and R. E. L. Paul. 2002. Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. Behavioral Ecology 13:816-820.

Kohler, S. L., and M. J. Wiley. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. Ecology 78:2164-2176.

Latham, A. D. M., and R. Poulin. 2002. Effect of acanthocephalan parasites on hiding behaviour in two species of shore crabs. Journal of Helminthology 76:323-326.

Lee, J. H., W. A. Rowley, and K. B. Platt. 2000. Longevity and spontaneous flight activity of *Culex tarsalis* (Diptera : Culicidae) infected with western equine encephalomyelitis virus. Journal of Medical Entomology 37:187-193.

Li, X. H., B. Sina, and P. A. Rossignol. 1992. Probing behavior and sporozoite delivery by *Anopheles stephensi* infected with *Plasmodium berghei*. Medical and Veterinary Entomology 6:57-61.

Lowenberger, C. A., and M. E. Rau. 1994. Selective oviposition by *Aedes aegypti* (Diptera, Culicidae) in response to a larval parasite, *Plagiorchis elegans* (Trematoda, Plagiorchiidae). Environmental Entomology 23:1269-1276.

Lyimo, E. O., and J. C. Koella. 1992. Relationship between body size of adult *Anopheles gambiae* Sl and infection with the malaria parasite *Plasmodium falciparum*. Parasitology 104:233-237.

MacNeil, C., N. J. Fielding, J. T. A. Dick, M. Briffa, J. Prenter, M. J. Hatcher, and A. M. Dunn. 2003a. An acanthocephalan parasite mediates intraguild predation between invasive and native freshwater amphipods (Crustacea). Freshwater Biology 48:2085-2093.

MacNeil, C., J. T. A. Dick, M. J. Hatcher, R. S. Terry, J. E. Smith, and A. M. Dunn. 2003b. Parasite-mediated predation between native and invasive amphipods. Proceedings of the Royal Society of London Series B-Biological Sciences 270:1309-1314.

Maguire, B., D. Belk, and G. Wells. 1968. Control of community structure by mosquito larvae. Ecology 49:207-&.

Maiimood, F., W. K. Reisen, R. E. Chiles, and Y. Fang. 2004. Western equine encephalomyelitis virus infection affects the life table characteristics of *Culex tarsalis* (Diptera : Culicidae). Journal of Medical Entomology 41:982-986.

Marcogliese, D. J., and D. K. Cone. 1997. Food webs: A plea for parasites. Trends in Ecology & Evolution 12:320-325.

McGaw, M. M., L. J. Chandler, L. P. Wasieloski, C. D. Blair, and B. J. Beaty. 1998. Effect of La Crosse virus infection on overwintering of *Aedes triseriatus*. American Journal of Tropical Medicine and Hygiene 58:168-175.

Miller, T., D. Cassill, C. Johnson, C. Kindell, J. Leips, D. McInnes, T. Bevis et al. 1994. Intraspecific and interspecific competition of *Wyeomyia smithii* (Coq) (Culicidae) in pitcher plant communities. American Midland Naturalist 131:136-145.

Minchella, D. J., and M. E. Scott. 1991. Parasitism - a cryptic determinant of animal community structure. Trends in Ecology & Evolution 6:250-254.

Mogi, M., and K. L. Chan. 1996. Predatory habits of dipteran larvae inhabiting Nepenthes pitchers. Raffles Bulletin of Zoology 44:233-245.

Mokany, A., and R. Shine. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. Austral Ecology 28:33-37.

Moncayo, A. C., J. D. Edman, and M. J. Turell. 2000. Effect of eastern equine encephalomyelitis virus on the survival of *Aedes albopictus, Anopheles quadrimaculatus*, and *Coquillettidia perturbans* (Diptera : Culicidae). Journal of Medical Entomology 37:701-706.

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: Ecology and Evolution. New York, Oxford University Press.

Morand, S., and E. A. Gonzalez. 1997. Is parasitism a missing ingredient in model ecosystems? Ecological Modeling 95:61-74.

Morrison, A., and T. G. Andreadis. 1992. Larval population-dynamics in a community of nearctic *Aedes* inhabiting a temporary vernal pool. Journal of the American Mosquito Control Association 8:52-57.

Mouritsen, K. N., and R. Poulin. 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124:S101-S117.

Nasci, R. S., K. H. Tang, J. J. Becnel, and T. Fukuda. 1992. Effect of per os *Edhazardia aedis* (Microsporida, Amblyosporidae) infection on *Aedes aegypti* mortality and body size. Journal of the American Mosquito Control Association 8:131-136.

Nguyen, D., P. Dutilleul, and M. E. Rau. 2002. Impact of nutrition and exposure to the parasite *Plagiorchis elegans* (Trematoda : Plagiorchiidae) on the development of *Aedes aegypti* (Diptera : Culicidae): Analysis by time-dependent transition probabilities. Environmental Entomology 31:54-64.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist 100:65-&.

Park, T. 1948. Experimental studies of interspecies competition: Competition between populations of the flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. Ecological Monographs 18:265-308.

Petersen, J. J. 1984. Nematode Parasites of Moquitoes, Pages 797-820 *in* W. R. Nickle, ed. Plant and Insect Nematodes. New York, Marcel Dekker Inc.

Platt, K. B., K. J. Linthicum, K. S. A. Myint, B. L. Innis, K. Lerdthusnee, and D. W. Vaughn. 1997. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene 57:119-125.

Poulin, R. 1999. The functional importance of parasites in animal communities: many roles at many levels? International Journal for Parasitology 29:903-914.

Prenter, J., C. MacNeil, J. T. A. Dick, and A. M. Dunn. 2004. Roles of parasites in animal invasions. Trends in Ecology & Evolution 19:385-390.

Price, P. W., M. Westoby, and B. Rice. 1988. Parasite-mediated competition - some predictions and tests. American Naturalist 131:544-555.

Reeves, W. K. 2004. Oviposition by *Aedes aegypti* (Diptera : Culicidae) in relation to conspecific larvae infected with internal symbiotes. Journal of Vector Ecology 29:159-163.

Reiskind, M. H., and M. L. Wilson. 2004. *Culex restuans* (Diptera : Culicidae) oviposition behavior determined by larval habitat quality and quantity in southeastern Michigan. Journal of Medical Entomology 41:179-186.

Rivero, A., and H. M. Ferguson. 2003. The energetic budget of *Anopheles stephensi* infected with *Plasmodium chabaudi*: is energy depletion a mechanism for virulence? Proceedings of the Royal Society of London Series B-Biological Sciences 270:1365-1371.

Robert, V., J. P. Verhave, and P. Carnevale. 1990. *Plasmodium falciparum* infection does not increase the precocious mortality rate of *Anopheles gambiae*. Transactions of the Royal Society of Tropical Medicine and Hygiene 84:346-347.

Roberts, L., and J. J. Janovy. 2005 Foundations of Parasitology, McGraw-Hill Education.

Rossignol, P. A., J. M. C. Ribeiro, and A. Spielman. 1984. Increased intradermal probing time in sporozoite-infected mosquitos. American Journal of Tropical Medicine and Hygiene 33:17-20.

Rossignol, P. A., J. M. C. Ribeiro, and A. Spielman. 1986. Increased biting rate and reduced fertility in sporozoite-infected mosquitos. American Journal of Tropical Medicine and Hygiene 35:277-279.

Schneider, P., W. Takken, and P. J. McCall. 2000. Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae*. Medical and Veterinary Entomology 14:165-170.

Schwab, A. E., D. J. Lewis, and M. E. Rau. 2003. The impact of selective oviposition and infection with *Plagiorchis elegans* on *Aedes aegypti* pre-imago population dynamics at optimal food availability. Journal of Medical Entomology 40:830-840.

Scott, T. W., and L. H. Lorenz. 1998. Reduction of *Culiseta melanura* fitness by eastern equine encephalomyelitis virus. American Journal of Tropical Medicine and Hygiene 59:341-346.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434-441.

Smith, S. M. 1985. Population Dynamics, Pages 185-194 *in* H. C. Chapman, ed. Biological Control of Mosquitoes. Fresno, American Mosquito Control Association Inc.

Sota, T., M. Mogi, and E. Hayamizu. 1994. Habitat stability and the larval mosquito community in treeholes and other containers on a temperate island. Researches on Population Ecology 36:93-104.

Soumare, M. K. F., J. E. Cilek, and E. T. Schreiber. 2004. Prey and size preference of *Mesocyclops longisetus* (Copepoda) for *Aedes albopictus* and *Culex quinquefasciatus* larvae. Journal of the American Mosquito Control Association 20:305-310.

Spencer, M., L. Blaustein, and J. E. Cohen. 2002. Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. Ecology 83:669-679.

Stav, G., L. Blaustein, and Y. Margalit. 2000. Influence of nymphal *Anax* imperator (Odonata : Aeshnidae) on oviposition by the mosquito *Culiseta longiareolata* (Diptera : Culicidae) and community structure in temporary pools. Journal of Vector Ecology 25:190-202.

Sunahara, T., and M. Mogi. 1997. Can the tortoise beat the hare? A possible mechanism for the coexistence of competing mosquitoes in bamboo groves. Ecological Research 12:63-70.

Sunahara, T., and M. Mogi. 2002a. Priority effects of bamboo-stump mosquito larvae: influences of water exchange and leaf litter input. Ecological Entomology 27:346-354.

Sunahara, T., and M. Mogi. 2002b. Variability of intra- and interspecific competitions of bamboo stump mosquito larvae over small and large spatial scales. Oikos 97:87-96.

Sunahara, T., and M. Mogi. 2004. Searching clusters of community composition along multiple spatial scales: a case study on aquatic invertebrate communities in bamboo stumps in West Timor. Population Ecology 46:149-158.

Taylor, P. J., and H. Hurd. 2001. The influence of host haematocrit on the blood feeding success of *Anopheles stephensi*: implications for enhanced malaria transmission. Parasitology 122:491-496.

Thomas, F., R. Poulin, T. de Meeus, J. F. Guegan, and F. Renaud. 1999. Parasites and ecosystem engineering: what roles could they play? Oikos 84:167-171.

Thomas, F., J. F. Guegan, Y. Michalakis, and F. Renaud. 2000. Parasites and host life history traits: implications for community ecology and species co-existence. International Journal for Parasitology 30:669-674.

Townson, H. 1970. The effect of infection with *Brugia pahangi* on the flight of *Aedes aegypti*. Annals of Tropical Medicine and Parasitology 64: 411-420

Tseng, M. 2004. Sex-specific response of a mosquito to parasites and crowding. Proceedings of the Royal Society of London Series B-Biological Sciences 271:S186-S188.

Tun-Lin, W., T. R. Burkot, and B. H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Medical and Veterinary Entomology 14:31-37.

Van Rhein, S. L. 1999. Parasitism by *Ascogregarina barrette*, habitat drying, and host reproductive success in *Aedes triseriatus*. MS thesis, Department of Biological Sciences, Illinois State University

Walton, W. E. 2001. Effects of *Triops newberryi* (Notostraca : Triopsidae) on aquatic insect communities in ponds in the Colorado Desert of southern California. Israel Journal of Zoology 47:491-511.

Washburn, J. O. 1991. Regulatory role of parasites - impact on host population shifts with resource availability. Science 253:185-188.

Washburn, J. O. 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats - implications for biological-control. Journal of the American Mosquito Control Association 11:279-283.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987a. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the susceptibility of *Aedes aegypti* larvae to predation by guppies (*Poecilia reticulata*) and meadow voles (*Microtus pennsylvanicus*). Canadian Journal of Zoology 65:2346-2348.

Webber, R. A., M. E. Rau, and D. J. Lewis. 1987b. The effects of *Plagiorchis noblei* (Trematoda, Plagiorchiidae) metacercariae on the behavior of of *Aedes aegypti* larvae. Canadian Journal of Zoology 65:1340-1342.

Welch, H. E. 1960. *Hydromermis churchillensis* n. sp. (Nematoda: Mermithidae) a parasite of *Aedes communis* (DeG.) from Churchill, Manitoba with observation of its incidence and bionomics. Canadian Journal of Zoology 38:466-474.

Wellnitz, T., L. Giari, B. Maynard, and B. S. Dezfuli. 2003. A parasite spatially structures its host population. Oikos 100:263-268.

Wise de Valdez, M. R. In preparation. Predator avoidance behavior of *Aedes aegypti* larvae infected with mermithid nematodes (Nematoda: Mermithidae).

Yan, G. Y. 1996. Parasite-mediated competition: A model of directly transmitted macroparasites. American Naturalist 148:1089-1112.

Yan, G. Y., L. Stevens, C. J. Goodnight, and J. J. Schall. 1998. Effects of a tapeworm parasite on the competition of *Tribolium* beetles. Ecology 79:1093-1103.

Yanoviak, S. P. 2001. Predation, resource availability, and community structure in neotropical water-filled tree holes. Oecologia 126:125-133.

Yasuda, H. 1996. Abundance and age structure of the predatory mosquito larva, *Toxorhynchites towadensis* (Dipt, Culicidae) in relation to prey community structure. Journal of Applied Entomology 120:291-295.

Yasuda, H., and T. Mitsui. 1992. Laboratory observations of prey behavior for prey acquisition by a predator among 3 larval mosquitos. Ecological Research 7:179-182.

Yee, W. L., and J. R. Anderson. 1995a. Free-flight of *Lambornella clarki*-infected, blood-fed, and gravid *Aedes sierrensis* (Diptera, Culicidae). Journal of Medical Entomology 32:407-412.

Yee, W. L., and J. R. Anderson. 1995b. Tethered flight capabilities and survival of *Lambornella clarki*-infected, blood-fed, and gravid *Aedes sierrensis* (Diptera, Culicidae). Journal of Medical Entomology 32:153-160.

Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2004. Interspecific differences in feeding behavior and survival under food-limited conditions for larval *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Annals of the Entomological Society of America 97:720-728.

Yeh, M. L., C. S. Chen, and K. C. Liu. 1994. Experimental infection of mosquitoes with two *Ascogregarina* species (Eujgregarinidia: Lecudinidae). Bulletin of Tung Hai University 35:87-106.

Zahiri, N., M. E. Rau, and D. J. Lewis. 1997a. Oviposition responses of *Aedes aegypti* and *Ae. atropalpus* (Diptera: Culicidae) females to waters from conspecific and heterospecific normal larvae and from larvae infected with *Plagiorchis elegans* (Trematoda: Plagiorchiidae). Journal of Medical Entomology 34:565-568.

Zahiri, N., M. E. Rau, D. J. Lewis, and S. Khanizadeh. 1997b. Intensity and site of *Plagiorchis elegans* (Trematoda: Plagiorchiidae) infections in *Aedes aegypti* (Diptera: Culicidae) larvae affect the attractiveness of their waters to ovipositing, conspecific females. Environmental Entomology 26:920-923.

Zahiri, N., and M. E. Rau. 1998. Oviposition attraction and repellency of *Aedes aegypti* (Diptera : Culicidae) to waters from conspecific larvae subjected to crowding, confinement, starvation, or infection. Journal of Medical Entomology 35:782-787.

Table 1. Mosquitoes of phytotelmata, artificial containers, and temporary ground pools that have been studied in light of their larval ecology over the last 25 years; specifically, species interactions and coexistence, and population and community ecology. * not normally found in tree holes, † experimental field study, ¥ observational field study, ‡ both experimental and observational field study, £ laboratory study, § both laboratory and field study, µ theoretical study.

Mosquito	Topic	Habitats	References
Aedes			
canadensis	Population dynamics	snow-melt pools	(Morrison and Andreadis 1992[¥])
cinereus			
excrucians			
stimulans			
albopictus	Pred/prey community, Species coexistence, competition	bamboo stumps/artificial containers	(Yasuda and Mitsui 1992[£], Edgerly et al. 1993[£], 1999[£], Sota et al. 1994[¥], Barrera 1996b[£], Yasuda 1996[§], Sunahara and Mogi 1997[†], 2002, Juliano 1998[‡], Aliabadi and Juliano 2002[‡], Braks et al. 2004[†], Juliano et al. 2004[†], Sunahara and Mogi 2004[¥], Tseng 2004[£], Yee et al. 2004[£])
aegypti	Pred/prey community, Species coexistence, competition, Oviposition behavior, population dynamics		(Edgerly et al. 1993[£], 1999[£], Blaustein and Margalit 1994[£], Barrera 1996a[£], Grill and Juliano 1996[£], Juliano 1998[‡], Tun-Lin et al. 2000[§], Sunahara and Mogi 2002a[£], 2002b[†], Nguyen et al. 2002[£], Colton et al. 2003[£], Schwab et al. 2003[£], Braks et al. 2004[†], Juliano et al. 2004[†] , Yee et al. 2004[£])
anandalei	Community structure	Bamboo stumps	
riversi		tree holes/artificial containers	(Sunahara and Mogi 2004[¥])
flavopictus	Pred/prey community		(Sota et al. 1994[¥])
nipponicus			(Yasuda 1996[§])
geniculatus	Species coexistence		(Yasuda 1996[§])
hendersoni	Community structure		(Bradshaw and Holzapfel 1992[‡])
nipponicus			(Barrera 1996a[¥])
japonicus			(Sota et al. 1994[¥])
sierrensis	Population dynamics	treeholes	(Washburn et al. 1991[§])
	Community structure		(Eisenbert et al. 2000[†µ])
Anopheles			
barberi	Community structure, Species coexistence, Pred/prey community	tree holes	(Bradshaw and Holzapfel 1983[‡], Barrera 1996a[¥], Edgerly et al. 1998[†])
plumbeus	Species coexistence		(Bradshaw and Holzapfel 1992[‡])
eiseni			(Finke et al. 1997[‡])
arabiensis	Competition	temporary ground pools	(Schneider et al. 2000[£])
gambiae			
Corethrella			
appendiculata	Species coexistence	tree holes	(Bradshaw and Holzapfel 1983[‡])

Culex			
halifaxii	Pred/prey community	bamboo stumps	(Yasuda 1996[§])
sasai	Pred/prey community Community structure	bamboo stumps/artificial containers/ tree holes	(Sota et al. 1994[¥],Yasuda 1996[§])
urichii		tree holes	(Finke et al. 1997[‡],Yanoviak 2001[‡])
allostigma			(Finke et al. 1997[‡])
conservator			
corrigani			
mollis			
pipiens	Community Structure	temporary ground pools	(Blaustein and Margalit 1994[£], Eitam et al. 2002[†])
laticinctus			
restuans	Species coexistence Oviposition behavior	tree holes/ artificial containers	(Bradshaw and Holzapfel 1983[‡], Barrera 1996a[¥], Reiskind and Wilson 2004[†])
torrentium	Community structure		(Bradshaw and Holzapfel 1992[‡])
eminentia	Pred/Prey community	Pitcher plant	(Mogi and Chan 1996[¥])
Culiseta			
melanura	Species coexistence	tree holes*/ artificial containers	(Bradshaw and Holzapfel 1983[‡])
longiareolata *sp.*	Pred/prey community, Community structure, Oviposition behavior	temporary ground pools, rock pools	(Blaustein and Margalit 1994[£], 1996[£], Blaustein 1998[‡], Stav et al. 2000[†], Eitam et al. 2002[†], Spencer et al. 2002[µ])
Haemagogus			
	Pred/prey community	tree holes	(Finke et al. 1997[‡])
leucotaeniatus			
lucifer			
Limatus			
durhamii	Pred/prey community	tree holes	(Finke et al. 1997[‡])
Ochlerotatus			
australis	Oviposition behavior	rock pools	(Mokany and Shine 2003[†])
notoscriptus	Community structure	bamboo stumps	(Sunahara and Mogi 2004[¥])
triseriatus	Community structure, Species coexistence, Species interaction, Pred/prey community, Competition, Oviposition behavior	tree holes	(Bradshaw and Holzapfel 1983[‡], Chambers 1985[†], Juliano and Reminger 1992[£], Barrera 1996a[£],b[¥], Grill and Juliano 1996[£], Edgerly et al. 1993[£], 1998[†],1999[£], Kaufman et al. 1999[†] , Aliabadi and Juliano 2002[‡])

Orthopodomyia			
anopheloides	Pred/prey community	bamboo stumps/artificial containers	(Yasuda and Mitsui 1992[£], Yasuda 1996[§])
signifera	Community structure, Species coexistence, Competition	tree holes	(Chambers 1985[†], Bradshaw and Holzafpel 1983[‡], Barrera 1996a[¥])
fascipes	Pred/prey community		(Finke et al. 1997[‡])
sp.	Community structure		(Sunahara and Mogi 2004[¥])
Toxorhynchites			
towadensis	Pred/prey community	artificial containers	(Yasuda and Mitsui 1992[£], Yasuda 1996[§])
theobaldi		tree holes	(Finke et al. 1997[‡], Yanoviak 2001[‡])
terrens.			(Finke et al.1997[‡])
rutilus	Community structure, Species coexistence, Competition		(Bradshaw and Holzapfel 1983[‡], Chambers 1985[†], Juliano and Reminger 1992[£], Barrera 1996[¥], Grill and Juliano 1996[£])
septentrionalis	Community structure		(Barrera 1996a[¥])
Trichoprosopon			
digitatum	Pred/prey community	tree holes	(Finke et al. 1997[‡])
Tripteroides			
bambusa	Competition, Community structure	bamboo stumps, artificial containers, tree holes	(Sota et al. 1994[¥], Sunahara and Mogi 1997[†], 2002a[£],b[†])
sp.	Community structure		(Sunahara and Mogi 2004[¥])
tenax	Pred/prey community	Pitcher plant	(Mogi and Chan 1996[¥])
Uranotaenia			
novobscura	Community structure	Tree holes / bamboo stumps	(Sota et al. 1994[¥])
Wyeomyia			
smithii	Competition	Pitcher plant	(Miller et al. 1994[‡])

Table 2. Parasites and pathogens of mosquitoes and their known effects on survival, reproduction, growth, behavior, and ecology. *infection of larval stage. ¥ develop in phytotelmata, artificial containers, or temporary ground pools † Reference mentions implications of parasites on population or community ecology of mosquito hosts. § Reference specifically addresses role of parasites on population or community ecology of mosquito hosts.

Parasite	Mosquito	Effects	References
Apicomplexa			
Ascogregarina taiwanensis	*Aedes albopictus**¥	-Reduced oviposition, hatch rate	(Yeh et al. 1994)
		-Sex-specific reduction in size	(Tseng 2004†)
	*Aedes aegypti**¥	-Differential negative effect on *A. aegypti* / *A. albopictus*	(Juliano 1998§, Aliabadi and Juliano 2002§)
		-Rendered oviposition sites more attractive	(Reeves 2004)
	*Ochlerotatus triseriatus**¥	-Increased larval mortality; reduced fecundity; delayed development	(Comisky et al. 1999)
A. barrette	*Ochlerotatus triseriatus**¥	-Reduced fecundity and size	(Van Rhein 1999 in Aliabadi and Juliano 2002)
Plasmodium falciparum	*Anopheles gambiae*¥	-Reduced fecundity (oocysts);	(Hogg and Hurd 1997)
		-Increased feeding-associated mortality	(Anderson et al. 2000)
		-Size-based, intensity-based mortality	(Lyimo and Koella 1992)
		-Precocious mortality not reduced	(Robert et al. 1990)
		-Increased feeding	(Koella et al. 1998b)
	Anopheles punctulatus	-Increased feeding attempts	(Koella and Packer 1996)
P. gallinaceum	*Aedes aegypti*¥	-Reduced fecundity; reduced blood feeding success	(Freier and Friedman 1976, Rossignol et al. 1986)
		-Stage-specific changes in host-seeking	(Anderson et al. 1999, Koella et al. 2002)
P. yoelii nigeriensis	*Anopheles stephensi*¥	-Reduced fecundity	(Hogg and Hurd 1995a†, Ahmed et al. 1999)
		-Infected vertebrates hosts results in decreased bloodmeal size	(Hogg and Hurd 1995b, Taylor and Hurd 2001)
		-Reduced fertility	(Jahan and Hurd 1997, Ahmed et al. 1999)
P. chabaudi	*Anopheles stephensi*¥	-Increased blood feeding	(Ferguson and Read 2004)
		-Increased sugar feeding	(Rivero and Ferguson 2003)
		-Reduced fecundity	(Ferguson et al. 2003)
P. berghei	*Anopheles stephensi*¥	-Reduced feeding success	(Li et al. 1992)

Ciliates			
Lambornella clarki	*Aedes sierrensis*[*¥]	-Mortality compensatory or depensatory in food-limited populations	(Washburn et al. 1991[§])
		-Reduced survival, flight activity	(Yee and Anderson 1995a,b)
		-Inhibit host-seeking	(Edgerter et al. 1986, Edgerter and Anderson 1989)
Microsporida			
Edhazardia aedis	*Aedes aegypti*[¥]	-Reduced fecundity, increased mortality	(Becnel et al. 1995)
		-Differential effects on reproduction and mortality based on mosquito development.	(Koella et al. 1998a)
		-Decrease blood feeding success, stage specific	(Koella and Agnew 1997)
		-reduced size of adult	(Nasci et al. 1992)
	Aedes aegypti[*¥]	-Differential sex-specific mortality based on resource availability	(Agnew and Koella 1999)
Trematoda			
Plagiorchis elegans	*Aedes aegypti*[*¥]	-Reduced adult emergence	(Schwab et al. 2003[§])
		-Larval mortality increased, instar-specific	(Nguyen et al. 2002[§])
		-Delayed development	(Dempster et al. 1986)
		-Infected larvae rendered oviposition sites repellent to females.	(Zahiri et al. 1997b[†], Zahiri and Rau 1998[†])
		-Induced anorexia	(Zahiri et al. 1998)
		-Sites that held infected larvae received fewer eggs	(Lowenberger and Rau 1994[†])
	Aedes atropalpus[*¥]	-Infected larvae rendered oviposition sites repellent to female conspecifics and *A. aegypti*	(Zahiri et al. 1997a[†])
Plagiorchis noblei	*Aedes aegypti*[*¥]	-Decreased activity	(Webber et al. 1987a,b)
Hematolechus sp.	*Aedes albopictus*[*¥]	-Delayed development, increased mortality, malformed adults	(Aparecida de Carvalho et al. 2002)
Nematoda			
Brugia pahangi	*Aedes aegypti*[¥]	Reduced fecundity	(Javadian and Macdonald 1974)
		Reduced flight	(Molyneux and Jefferies 1986 and reference therein)

B. malayi	*Armigeres subalbatus*	Reduced fecundity	(Ferdig et al. 1993)
Dirofilaria repens	*Aedes aegypti*[¥]	Ingested less blood; reduced fecundity	(Javadian and Macdonald 1974)
D. immitis	*Aedes aegypti*[¥]	Reduced fecundity Altered flight activity	(Courtney et al. 1985) (Berry et al. 1987)
	Aedes trivittatus[¥]	Fecundity sometimes reduced Altered flight activity	(Christensen 1981) (Berry et al. 1988)
Romanomermis culicivorax	*Aedes vexans*[*¥]	Delayed development	(Galloway and Brust 1985)
	Aedes aegypti[*¥]	Decreased activity	(Welch 1960, Wise de Valdez in preparation[†])
	Aedes sp. [*¥]	Reduced survival	(Petersen 1984)
Strelkovimermis spiculatus	*Aedes aegypti*[*¥]	Decreased activity	(Wise de Valdez in preparation)
Viruses			
Western Equine Encephalomyelitis Virus	*Culex tarsalis*[¥]	-Reduced life expectancy, reproductive effort, generation time. -Reduces survivorship	(Maiimood et al. 2004) (Lee et al. 2000)
Eastern Equine Encephalomyelitis Virus	*Anopheles quadrimaculatus*	-Reduced survival dependent upon inoculation	(Moncayo et al. 2000)
La Crosse Virus	*Coquillettidia perturbans*	-Reduced survival and fitness	(Scott and Lorenz 1998)
	Culiseta melanura[¥]	-Adverse effect on overwintering eggs	(McGaw et al. 1998)
	Ocherotatus triseriatus[¥]	-Increased feeding attempts, reduced success	(Grimstad et al. 1980)
Dengue Virus	*Aedes aegypti*[¥]	Increased probing time	(Platt et al. 1997)