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ALTERED PREDATION SUSCEPTIBILITY OF MOSQUITOFISH INFECTED WITH *EUSTRONGYLIDES IGNOTUS*

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ABSTRACT: *Eustrongylides ignotus* is a parasitic nematode whose definitive hosts are often piscivorous wading birds (Ciconiiformes). Several species of small fishes are intermediate hosts, while larger predatory fish may be paratenic (transport) hosts. We examined predation susceptibility of infected mosquitofish (*Gambusia holbrooki*) to three species of predatory fishes, including juvenile largemouth bass (*Micropterus salminoides*), warmouth (*Lepomis gulosus*), and bluegill (*Lepomis macrochirus*). A 250 L aquarium with removable plexiglass divider and remote observation windows was constructed. Aquatic macrophytes were placed in the tank to provide refuge for the fishes. Predatory fish were allowed to acclimate to one half of the tank, while one infected and one uninfected mosquitofish were placed in the other. The divider was removed and an observer recorded the number of capture attempts and time required for capture. Predators were observed for behavioral alterations for 4 days after ingestion of infected mosquitofish, then examined at necropsy. Infected prey were selected preferentially in 31 of 38 (82%) trials. The number of capture attempts was 2.7 $\pm$ 0.2 ($\bar{x}$ $\pm$ SE) for infected fish and 3.9 $\pm$ 0.4 for uninfected fish. Mean time of capture was 12.4 $\pm$ 1.6 min for infected fish and 21.7 $\pm$ 2.9 for uninfected fish. Because of these differences, infected mosquitofish were more susceptible to predation ($P < 0.01$) than uninfected fish. Aberrant behavior including lethargy, convulsions, and buoyancy abnormalities was observed in eight (67%) predatory fish. At necropsy, larvae of *E. ignotus* were found in the coelomic cavity, viscera, and swim bladders of predators. Parasite-induced behavior modification of intermediate hosts may predispose them to predation by wading birds and thereby facilitate the transmission of this nematode in natural populations.

Key words: Behavior, fish, *Gambusia holbrooki, Eustrongylides ignotus,* parasitism, predation, wading birds.

INTRODUCTION

In Florida (USA) the number of reproducing wading birds (Ciconiiformes) has declined dramatically over the past 40 yr (Ogden, 1994). Spalding et al. (1993) reported that infection with the parasitic nematode *Eustrongylides ignotus* was a major mortality factor in nestling wading birds in Florida, causing losses of over 80% in some colonies. Birds become infected with this parasite after consuming fish. Although the prevalence of infected fish is low (<1% [Coyner, 1998]), we hypothesized that infected fish may be more susceptible to predation, thus increasing the probability of parasite transmission to animals in the food chain. Many parasitic helminths alter the behavior of hosts in ways that increase the probability of parasite survival (Hurd, 1990). Holmes and Bethel (1972) and Lafferty and Morris (1996) reviewed a number of the studies that have contributed to the base of evidence in support of the hypothesis that parasites modify host behavior which results in increased susceptibility to predation. For example, Brattey (1983) found that the acanthocephalan *Acanthocephalus lucii* causes changes in the pigmentation and behavior of aquatic isopods, which make them more susceptible to predation by perch, *Perca fluviatilus.* Killifish (*Fundulus parvipinnis*) infected with the trematode *Euhaplorchis californiensis* have conspicuous behaviors which lead to increased predation by piscivorous birds (Lafferty and Morris, 1996). In the present study, intermediate and paratenic hosts were studied in order to determine if infection with larval *E. ignotus* affected host behavior and susceptibility to predation.

TABLE 1. Number of times mosquitofish (*Gambusia holbrooki*) were selected first, number of capture attempts, and time until capture by predators [largemouth bass (*Micropterus salminodes*), $n = 6$; warmouth (*Lepomis gulosus*), $n = 4$; and bluegill (*Lepomis macrochirus*), $n = 2$] during 38 trials. During each trial, predators were presented with a choice between 1 infected and 1 uninfected mosquitofish.

Measurement	Infected[a]	Uninfected
Number of times (%) prey selected first	31 (82)	7 (18)
Mean number of capture attempts ± SE (range)	2.7 ± 0.2 (1–6)	3.9 ± 0.4 (1–13)
Mean number of min until capture ± SE (range)	12.4 ± 1.6 (3–48)	21.7 ± 2.9 (4–60)

[a] contained larvae of *Eustronglylides ignotus.*

METHODS

A 250 L aerated aquarium with removable plexiglass divider and remote observation windows was constructed. The tank was filled with fresh-water and maintained at 26 C. Aquatic macrophytes (*Sagittaria* spp. and *Hydrilla verticillata*) were placed in the tank to provide refuge for fish and simulate natural conditions.

Mosquitofish (*Gambusia holbrooki*) were selected as a prey model because they were found to be infected with *Eustrongylides ignotus* under natural conditions, they were abundant where wading birds foraged, infections could be detected in live fish, and they were easy to capture and maintain in captivity (Spalding et al., 1993). Mosquitofish were collected with dip nets from an urban retention pond in Alachua County (29°39.4′N, 82°24.5′W) that was enzootic for *E. ignotus.* Fish were transported to the laboratory in aerated coolers. Live fish were examined by trans-illumination to confirm infection with larvae of *E. ignotus.* One infected and one non-infected fish (of the same gender and approximate size) were introduced to one side of the tank and allowed to acclimate for 24 hr.

Centrarchids including bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salminoides*), and warmouth (*Lepomis gulosus*) were selected as predator models because they prey on mosquitofish and were often part of the diet of wading birds (Loftus and Kushlan, 1987). Experimental and control predators were collected by seine net from a natural pond (29°33.3′N, 82°36.4′W) where *E. ignotus* had never been reported from fishes (Coyner, 1998). All predators were placed in aerated holding tanks and allowed to acclimate to captivity for 1 wk. One predator was then introduced to the opposite side of the test aquarium from mosquitofish and allowed to acclimate for 24 hr. The divider was removed and an observer recorded which mosquitofish was selected first, the number of capture attempts, and the time required for capture. Trials were repeated one to six times for each predator. The same predators were used in multiple trials for the experiment, but were not reused if they exhibited any aberrant behaviors after a trial. Each predator was observed for behavioral alterations for 4 days after ingesting mosquitofish, then killed by cervical dislocation and examined at necropsy.

Selection preference, number of capture attempts, and mean length of capture time for infected and non-infected fish were compared separately using 2-sample *t*-tests (SAS Institute, 1988). As predators were presented with new prey items for each trial, all observations were treated as independent. Though the effects of prior infection on the number of capture attempts and capture time are unknown, we feel strongly that prior infection did not influence predator choice of infected versus uninfected prey fish. Fisher's Exact Test (Siegel, 1956) was used to compare the number of larvae in predators with abnormal behavior.

RESULTS

Over a 4-mo-period, 38 trials were performed (Table 1). Infected mosquitofish were selected before uninfected fish in 31 of 38 (82%) trials. To avoid predation, mosquitofish attempted to remain motionless and conceal themselves in vegetation. Infected mosquitofish appeared to move erratically and often left the cover of vegetation.

The mean number of capture attempts was greater for uninfected mosquitofish than infected fish ($t = 2.91$, $P = 0.024$, df $= 74$). In addition, mean time of capture was greater for uninfected than infected prey ($t = 2.82$, $P = 0.0031$, df $= 74$). When a capture attempt was made, the

TABLE 2. Number and location of parasites recovered 4 days post ingestion (PI), and observations of aberrant behavior of predatory fishes after ingestion of mosquitofish (*Gambusia holbrooki*) infected with larvae of *Eustrongylides ignotus.*

Species of predatory fish	Number mosquitofish ingested	Number larvae collected 4 days PI	Location of larvae in predator[a]	Aberrant behavior observed[b]
Lepomis gulosus	1	1	CC	A
Lepomis gulosus	3	3	CC, CC, DM	None
Lepomis gulosus	4	3	CC, IM, K	A, B, C
Lepomis gulosus	5	3	CC, CC, SB	B, L
Lepomis macrochirus	2	2	CC, CC	None
Lepomis macrochirus	3	2	CC, IM	B, L
Micropterus salmoides	1	1	CC	A, C, L
Micropterus salmoides	2	2	CC, IM	None
Micropterus salmoides	3	2	IM, SB	B, L
Micropterus salmoides	3	3	CC, CC, IM	None
Micropterus salmoides	5	4	CC, CC, DM, K	B, C, L
Micropterus salmoides	6	3	CC, G, IM	L
Totals	38	29		

[a] Location: CC = coelomic cavity, DM = dorsal musculature, G = gonads, IM = intestinal mesentery, K = kidney, SB = swim bladder.
[b] Aberrant behavior: A = loss of appetite (refused food), B = buoyancy problems, C = convulsions, L = lethargy.

mosquitofish attempted to outmaneuver the predator and seek refuge in other patches of vegetation. Infected fish were often captured on the initial attempt and appeared unable to maneuver or swim as fast as uninfected fish.

During the 4 days after ingestion of infected mosquitofish, aberrant behavior was observed in 8 of 12 predatory fish (Table 2). These behaviors were not observed in control fishes and included lethargy (n = 6), buoyancy abnormalities (5), loss of appetite (3), and convulsions (3). Three of the lethargic fish remained motionless near the surface or suspended vertically in the water column and were caught easily by hand. Predators with loss of appetite refused to eat when offered uninfected immobilized prey. Convulsions included erratic head shaking and full body tremors, most of which occurred within 3 hr of ingestion of infected fish.

At necropsy, larvae of *E. ignotus* were collected from all predators, although some larvae (n = 9) were not recovered from those predators receiving multiple infections (Table 2). All larvae were fourth-stage, motile, and not encapsulated. Larvae were identified in the coelomic cavity, dorsal musculature, and viscera. Sixteen of 29 larvae (55%), were collected from the coelomic cavity in 11 of 12 (92%) predators. The locations of remaining larvae (n = 29) were the intestinal mesentery, (6; 21%); dorsal musculature, (2; 7%); kidney, (2; 7%); swim bladder, (2; 7%); and ovary, (1; 4%). All control fishes were negative for larvae of *E. ignotus.*

Abnormal behavior was not observed in four predators, although larvae were collected from them (Table 2). The mean number of larvae collected from predators with observed aberrant and normal behavior did not differ significantly (Fisher's Exact Test, P = 0.081).

DISCUSSION

Although infected mosquitofish had a well defined abdominal mass (larval parasite encysted in coelomic cavity), it was unclear if physical appearance was a factor in prey selection. Movement of infected mosquitofish is the most plausible explanation since centrarchids are sight predators and cue on movement (Williams, 1994). Although both infected and non-infected mosquitofish sought refuge in vegetation, infected fish appeared to exhibit frequent erratic movement. Coiled larvae often moved inside the cysts and may have caused discomfort and erratic movement in infected mosquitofish.

Whatever the mechanism, larvae of *E. ignotus* produced an effect on mosquitofish, which made infected fish more susceptible to predation. Holmes and Bethel (1972) examined predator-prey relationships and suggested two strategies by which parasites increased the likelihood of intermediate host predation including (1) reduction in host stamina and locomotor efficiency and (2) host disorientation. Larvae of *E. ignotus* are large relative to mosquitofish body size, and probably produce a physical and metabolic burden that may reduce and inhibit the fish's ability to avoid capture.

Infected mosquitofish are probably more susceptible to predation by foraging wading birds also. Herons and egrets use many of the same visual cues to select prey as centrarchids (e.g., movement and prey exposure) (Kushlan, 1978). Dence (1958) reported that common shiners (*Luxilus cornutus*) infected with larval stages of the cestode *Ligula intestinalis* were sluggish, less gregarious, and frequented shallow warm waters near shore more often than uninfected fish, even when avian predators were present. When fish did evade capture by retreating to deeper water, they returned to shore quickly. In another study of the same parasite, van Dobben (1952) reported that 30% of fish in the stomach contents of great cormorants (*Phalacrocorax carbo*) were infected, whereas only 7% of fish in the population at large were infected. He concluded that parasitized fish were more susceptible to predation by piscivorous birds than non-parasitized fish. Lafferty and Morris (1996) reported similar findings with parasitized killifish.

Nestling wading birds fed large numbers of mosquitofish may be at greater risk of infection with *E. ignotus* than nestlings fed other prey items such as crustaceans and medium size fishes. Frederick and Collopy (1988) reported that small fishes (including mosquitofish and small centrarchids) made up a significant portion of the diet of nestling snowy egrets (*Egretta thula*) and little blue herons (*Egretta caerulea*) in Florida, while crustaceans and medium-sized fishes were observed in nestling great blue herons (*Ardea herodias*) (Hoffman et al., 1994). Although large fish may be paratenic hosts, some species of medium sized fishes, selected by great blue herons for their nestlings, may be too small to consume infected mosquitofish. These differences in prey selection may help explain the observations by Spalding et al. (1993), who reported higher prevalences of *E. ignotus* in snowy egret and little blue heron nestlings than in great blue heron nestlings from Florida. Most nestling great blue herons collected by Spalding et al. (1993) were from estuarine sites in Florida Bay, which may explain the low prevalence of infection also, because the parasite has not been reported from salt water fishes.

Adult wading birds that select large fish with multiple infections of *E. ignotus* are probably at greater risk than birds that forage on small fish. Solitary adult great blue herons forage in deeper water and take significantly larger prey than do smaller herons and egrets, which often forage in groups (Hoffman et al., 1994). This supports Spalding et al. (1993) who observed higher prevalences of *E. ignotus* in adult great blue herons than in adults of smaller wading bird species.

MANAGEMENT IMPLICATIONS

Although the prevalence of infected fish at certain wading bird foraging sites is low (<1%), these sites may pose a potential health risk to foraging birds because infected fish may be selected preferentially over non-infected fish. Agencies responsible for monitoring and managing surface waters should be informed of this potential wildlife disease problem and personnel taught to recognize the parasite in fish intermediate hosts. Periodic monitoring of fish from certain sites may be recommended, especially during wading bird breeding seasons.

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