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INCREASED SURFACING BEHAVIOR IN LONGNOSE KILLIFISH INFECTED BY BRAIN-ENCYSTING TREMATODE

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ABSTRACT: Some parasites modify the behavior of intermediate hosts to increase the probability of transmission to the next host in their life cycle. In habitats where this is common, parasites play an important role in predator-prey links and food web dynamics. In this study we used laboratory observations to investigate the behavior of longnose killifish, Fundulus similis, that were naturally infected with metacercariae of the trematode, Euhaplorchis sp. A, from Laguna Madre, south Texas. In particular, we examined whether there was a relationship between the number of metacercariae lodged on the brain of the infected fish and behaviors that made the fish more conspicuous to avian final hosts. We also quantified the abundance and cercariae production of this parasite in its first intermediate snail host, Cerithidea pliculosa, and examined the seasonal variation of Euhaplorchis sp. A in F. similis. Our data demonstrated that Euhaplorchis sp. A affected the surfacing behavior of F. similis in an intensity-dependent manner. Fish with many infections spent longer time at the surface of the water than fish with few infections. Our data also show that Euhaplorchis sp. A is a common parasite in the first intermediate host and produces close to 4,000 cercariae m⁻² day⁻¹. Consequently 97% of all fish collected and necropsied were infected, with little seasonal variation in the mean abundance of the parasite. Based on our data, Euhaplorchis sp. A is a likely important to predator-prey links in Gulf of Mexico estuary food webs, similar to the closely related Euhaplorchis californiensis in southern California. We expect that other closely related species elsewhere may have similar effects on other fish hosts, emphasizing the need for incorporating trophically transmitted parasites in estuarine food web studies.

Parasites are ubiquitous and common components of aquatic ecosystems worldwide. The effects of parasites on host individuals, populations, and predator-prey interactions exert important effects on animal community structure, biomass production, and food web dynamics in these systems (Mouritsen and Poulin, 2002; Kuris et al., 2002; Lafferty et al., 2008). Many parasites are trophically transmitted, using existing predator-prey interactions to reach a final host and complete their life cycles. While most trophically transmitted parasites passively await ingestion of the infected intermediate host by a suitable final host, some have evolved the ability to modify host behavior to enhance the probability of transmission to the next host (Lafferty, 1999). One of the best examples of a parasite that modifies host behavior rests with the trematode, Euhaplorchis californiensis, that occurs in southern California estuaries. Euhaplorchis californiensis induces parasite-increased trophic transmission (PITT) by increasing the number of conspicuous behaviors in their second intermediate killifish host, Fundulus parvipinnis (Lafferty and Morris, 1996). The parasite achieves this by encysting on the brain case and altering the concentration of host neuromodulators (Shaw et al., 2009). Euhaplorchis californiensis plays an important role in southern California estuarine food webs because it is extremely abundant, and because it increases predation of infected killifish by fish-feeding birds on the order of 10–30 times relative to uninfected fish (Lafferty and Morris, 1996; Shaw et al., 2010).

Despite the huge importance of E. californiensis in southern California estuaries, no studies have examined the role of widely distributed close relatives that occur elsewhere. In the Gulf of Mexico, Euhaplorchis sp. A infects the snail first intermediate host, Cerithidea pliculosa, and has been reported throughout the geographic range of its snail host from Alabama to Central America (McNeff, 1978; Aguirre-Macedo et al., 2011; B. Fredensborg, unpubl. obs.). The genetic relationship between Euhaplorchis sp. A and E. californiensis is currently under investigation, but the 2 species appear to be very closely related (O. Miura, pers. comm.). The most important difference between these 2 parasites is that they use different species of intermediate hosts. In California, E. californiensis uses Cerithidea californica, while Euhaplorchis sp. A employs C. pliculosa as the first intermediate host on the Gulf Coast. The second intermediate host from southern California, F. parvipinnis, does not occur in the Gulf of Mexico, but infections of Euhaplorchis sp. A have been observed in the Gulf killifish, Fundulus grandis, the longnose killifish, Fundulus similis, and the saifin molly, Poecilia latipinna (McNeff, 1978; A. Longoria and B. Fredensborg, unpubl. obs.). In addition, another species of Euhaplorchis has been reported in Cerithidea scalariformis in Florida and Cerithidea costata in Florida and Puerto Rico (Cable, 1956; Holliman, 1961; Smith, 2001). It is unknown if Euhaplorchis sp. A, or any other close relative to E. californiensis in the Gulf of Mexico, is able to induce PITT in fish hosts.

We used laboratory observations in naturally infected fish to investigate if Euhaplorchis sp. A manipulates the behavior of F. similis, which is the most abundant species of killifish in Laguna Madre, south Texas. We also examined the prevalence and abundance of Euhaplorchis sp. A in the first intermediate host, C. pliculosa, and in F. similis from a site in the Lower Laguna Madre, Texas. We measured the production of cercariae by C. pliculosa and estimated the number of cercariae that killfish would be exposed to in the field.

Given the established importance of E. californiensis in southern California estuaries, it should be a high priority to determine if behavioral modification is a general trait of Euhaplorchis species. We predicted that Euhaplorchis sp. A was a common parasite in Laguna Madre. We also expected that it would elicit similar effects on second intermediate host behavior in E. californiensis, which would also indicate their importance to estuarine food webs in the Gulf of Mexico.

MATERIALS AND METHODS

Behavioral studies

In July 2008, 30 F. similis were collected with a 15-m beach seine dragged at a depth of 0.5–1 m along the shoreline adjacent to a stand of black mangrove (Avicennia germinans) at the South Padre Island Convention Center in Lower Laguna Madre, south Texas (26°8'N, 899
flakes released from sp. A cercariae, we counted and identified from [46], and in July 2009 [sp. A metacercariae were counted and identified from F. similis] for a total of 146 individuals. On all occasions, except July 2008, sp. A in second intermediate snail host, sp. A in the first intermediate snail host, Euhaplorchis similis (see Lafferty and Morris, 1996). The preliminary studies determined that surfacing behavior was the most common and easily observed behavior in F. similis, and that the frequency of surfacing varied substantially among individual fish. Surfacing presumably increases conspicuousness to an avian predator, and we, therefore, chose to quantify surfacing behavior in relation to the number of Euhaplorchis sp. A that resided on the brain of each fish. The behavioral studies took place during a 15-min period with an observer positioned 3 m from the tank. The observer’s attention was focused on the behavior of 1 fish at a time. In addition to an observer, a video camera mounted on a tripod recorded the behavior of 24 of the 30 fish used in the study. The video footage was examined later to verify the behaviors noted by the observer and to measure the time spent by each fish in the top 5 cm of the tank. Immediately following each observation period, the fish was transferred to a plastic container with 0.2 L of seawater and euthanized by adding CO₂ to the water. The standard length and wet weight were measured, and each fish was necropsied to identify and quantify metacercariae of Euhaplorchis sp. A on the brain. Thus, the infection status of each fish was unknown to the observer until the subsequent necropsy.

Infection parameters of Euhaplorchis sp. A in the first intermediate host, C. pliculosa

We examined the prevalence of infection and the abundance of Euhaplorchis sp. A in the first intermediate snail host, C. pliculosa from a mudflat adjacent to the fish collection site. In August 2008, 155 C. pliculosa were collected from 3, 15 × 15 m rectangular plots positioned along an intertidal mudflat separated by 15 m from each other and shoreward of a stand of black mangrove (A. germinans). In each plot, snails were collected by hand from 10 randomly assigned, 0.25 m² quadrats; all snails were collected for later determination of the prevalence of Euhaplorchis sp. A. Additional snails were collected to ensure a minimum of 50 snails from each plot. In the laboratory, shell length was measured to the nearest 0.1 mm using vernier calipers, and the snail gonads were necropsied and examined for the presence of cercariae and/or rediae of Euhaplorchis sp. A, which were identified according to McNeff (1978).

To estimate the exposure of F. similis to Euhaplorchis sp. A cercariae, we collected a large number of C. pliculosa from the intertidal mudflat adjacent to the black mangrove habitat described above. The snails were brought to the lab and placed individually in well plates with 20 ml of seawater. The snails were incubated at 25 C for 24 hr under a light source to stimulate cercariae shedding. All snails that shed at least 1 Euhaplorchis sp. A. A cercaria was then separated from the rest and used to calculate the mean daily production of cercariae. Each Euhaplorchis sp. A-infected snail was removed from the well plate, and the well was gently stirred with a pipette to mix the sample. Three, 1-ml sub-samples were taken from the center of the well, and the number of cercariae in each sample was counted. We estimated the total number of cercariae released by each infected snail by multiplying the average number of cercariae from each sub-sample with the total volume of water in the sample.

Infection parameters Euhaplorchis sp. A in second intermediate host, F. similis

We collected F. similis on 4 different occasions to examine the prevalence and abundance of Euhaplorchis sp. A infections (May 2008

RESULTS

Behavioral studies

All 30 fish used in the behavioral study harbored Euhaplorchis sp. A metacercariae on the brain case (Fig. 1). However, the number varied greatly from 55 to 549 metacercariae per fish (Fig. 1). There was no significant relationship between fish standard length and the number of parasites per fish (Fig. 1). The collected fish were preserved in 5% formalin and transported to the University of Texas–Pan American. Fish collected in July 2008 were used for the behavioral study and were, therefore, transported live to the lab (see above), where the standard and total lengths were measured to the nearest mm and the biomass was determined to the nearest 0.01 g for all fish. The brain of the fish was removed and transferred to a microscope slide and covered with a coverslip. Slight pressure was added to the coverslip before inspecting the slide using a compound microscope at 100×. All Euhaplorchis sp. A metacercariae were counted and identified according to McNeff (1978).

Infection parameters of Euhaplorchis sp. A in the first intermediate host, C. pliculosa

The mean density of C. pliculosa on the mudflat was 41.1 m⁻² (SE = 8.05), and the mean snail size was 19.8 mm (SE = 0.4, range = 9.5–28.6 mm). The mean prevalence of Euhaplorchis sp. A infections in the snails was 7.2% (SE = 2.9) from the 3, 15 × 15 m plots. A simple estimate of the density of snails infected with
Euhaplorchis sp. A is, therefore, 41.1 snails m$^{-2}$ × 0.072 = 2.96 infected snails m$^{-2}$.

The mean number of Euhaplorchis sp. A cercariae produced in 24 hr was 1,338.8 (SE = 142.1, range: 27–2,413, n = 28). The estimated mean cercariae density on the mudflats is then 2.96 infected snails m$^{-2}$ × 1,338.8 cercariae snail$^{-1}$ day$^{-1}$ = 3,962.8 cercariae m$^{-2}$ day$^{-1}$.

**Infection parameters Euhaplorchis sp. A in second intermediate host, F. similis**

The total mean prevalence of Euhaplorchis sp. A across all 4 sampling events was 97.8% (SE = 2.18), with a mean abundance of 159.8 metacercariae per fish (SE = 9.67, range: 0–549).

**Euhaplorchis** sp. A was common in *F. similis* on all sampling occasions (Fig. 2). However, the mean abundance differed significantly among sampling events (1-way ANOVA, *F* = 3.51, df = 3, *P* = 0.017) with fish from the July 2008 sample harboring a significantly higher mean abundance of metacercariae compared to fish collected in November 2008 (Tukey test, *P* = 0.009). No significant relationship was detected between fish standard length and the number of metacercariae harbored on the brain across the 4 sampling events (linear regression: $R^2 = 0.05$, *F* = 1.19, *P* = 0.287, n = 146; Table I, Fig. 3). The variance-to-mean ratio increased from small to mid-sized fish, after which it seemed to reach a plateau (Table I).

**Table I. Distribution of Euhaplorchis sp. A in longnose killifish from Lower Laguna Madre, Texas, by size category.**

<table>
<thead>
<tr>
<th>Size category (mm)</th>
<th>n</th>
<th>Mean intensity ±SD</th>
<th>Range</th>
<th>Variance-to-mean ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–29</td>
<td>58</td>
<td>168.4 ± 103.7</td>
<td>0–413</td>
<td>63.9</td>
</tr>
<tr>
<td>30–50</td>
<td>77</td>
<td>160.0 ± 124.2</td>
<td>0–549</td>
<td>96.5</td>
</tr>
<tr>
<td>51–74</td>
<td>11</td>
<td>158.5 ± 122.8</td>
<td>13–437</td>
<td>95.2</td>
</tr>
</tbody>
</table>

**Discussion**

Trophically transmitted parasites are essential elements in most aquatic food webs (Lafferty et al., 2006, 2008). In particular, parasites that increase the predation rate of intermediate hosts to vertebrate definitive hosts may be of disproportionate importance to food web structure (Thomas and Poulin, 1998; Lefevre et al., 2009). In the present study, we determined if Euhaplorchis sp. A, a trematode that infects longnose killifish in the Gulf of Mexico, can change the behavior of its host to make it more conspicuous to a bird final host, similar to *E. californiensis* in California.

We demonstrated that Euhaplorchis sp. A significantly increases the time that the fish host spends at the surface of the water column, and increases the number of surfacing events in an intensity-dependent way. These behaviors are consistent with those previously reported by Lafferty and Morris (1996), who found that heavily infected *F. parvipinnis* were much more likely to fall prey to piscivorous birds than uninfected controls. Our data suggest that Euhaplorchis sp. A probably makes infected fish more conspicuous and vulnerable to predation by avian definitive hosts, although we did not confirm predation by birds in this study. The location of metacercariae on the brain case of killifish makes them well positioned to influence host behavior, which is presumably linked to altered concentrations of dopamine and serotonin in the brain of infected killifish (Shaw et al., 2009).

Euhaplorchis sp. A was relatively common in the first intermediate host, *C. pliculosa*, and high production of cercariae by the snail intermediate host provides an estimated density of cercariae of almost 4,000 m$^{-2}$ day$^{-1}$. Accordingly, Euhaplorchis sp. A was very common in *F. similis* with very few uninfected individuals. The mean abundance of Euhaplorchis sp. A in the longnose killifish was lower than that observed in *F. parvipinnis* (Shaw et al., 2010), which is probably a reflection of a lower density of infected first intermediate hosts in Laguna Madre, and also could be partly because *F. similis* is a smaller species. However, the mean number of metacercariae found on the brain of fish collected in the field was well within the expected range necessary to induce the behavioral changes observed in our laboratory experiments (Fig. 1).

There was no significant relationship between fish standard length and mean abundance of Euhaplorchis sp. A, which was
surprising (Table I, Fig. 3). Typically, a positive relationship between mean abundance host size/age would be expected due to accumulation of parasites over time (Shaw et al., 1995). In our study, larger fish clearly did not harbor more metacercariae than intermediate-sized fish. It is possible that larger and, hence, heavily infected fish are removed from the population by predation, which was indicated in the *E. californiensis*-*F. parvipinnis* system in California (Shaw et al., 2010). Our data show that the variance-to-mean ratio plateaus in mid-sized fish (Table I), a characteristic that has been used to infer that heavily infected individuals are disproportionately removed from the population (Rousset et al., 1996). Larger fish may also be less susceptible to new infections. Experimental infections are needed to verify if host size influences the success of cercariae infection.

There was very little seasonal variation in the mean abundance of *Euhaplorchis* sp. A. A metacercaria in longnose killifish, with the highest mean abundance recorded in July 2008 (mean = 218.4).

The abundance of *Euhaplorchis* sp. A in both snail and fish hosts suggests a high rate of transmission to avian definitive hosts in Laguna Madre. Previous studies showed that the prevalence of *Euhaplorchis* sp. A in *C. pliculosa* from 2 other locations in the Lower Laguna Madre was 8.3% and 14.8%, respectively (B. Fredensborg, unpubl. obs.). Prevalence in the present study (7.2%) is, therefore, a conservative estimate of the prevalence of *Euhaplorchis* sp. A in *C. pliculosa* in the area. Laguna Madre is a shallow lagoon that harbors a rich and abundant community of fish-eating birds that often are observed foraging in the relatively shallow water close to the mangrove trees (Farmer, 1991; Withers, 1996), and successful captures of longnose killifish by birds have been observed (B. Fredensborg, pers. obs.). There is, therefore, little doubt that the abundant *F. similis* serves as important prey for wading birds in the area. Based on the number of *Euhaplorchis* metacercariae in the killifish, we expect that the majority of fish-eating birds are infected with *Euhaplorchis* sp. A.

Previous studies indicate that *Euhaplorchis* sp. A is a widely distributed parasite in the Gulf of Mexico. It has been reported in *C. pliculosa* from Dauphin Island, Alabama (prevalence 4.9%), and from the Yucatan peninsula (prevalence 3.0%), suggesting a wide geographic range of this species (McNeill, 1978; Aguirre-Macedo, 2011). Similar species, i.e., *Cercaria coruscantis* and *Euhaplorchis* sp., have been reported in *C. scalariformis* in Florida, and *Cercariae caribea* X in *C. costata* in Puerto Rico (Cable, 1956; Smith, 2001). No behavioral studies have been published for any of those species. Based on our data, we expect that several related species in the *Euhaplorchis* species complex may serve a similar function in different regions and on different fish species. The very well documented effects of *E. californiensis* and other parasites on salt marsh ecosystems in southern California could be generally applicable to estuarine systems that harbor a similar parasite fauna. In addition, the high productivity of cercariae in the snail first intermediate hosts significantly contributes to estuarine biomass production (Kuris et al., 2008; Thielges et al., 2008). The abundance of *Euhaplorchis* sp. A and its effect on the behavior of *F. similis* is, therefore, further evidence that parasites should be factored into future food web studies in estuarine ecosystems.

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LITERATURE CITED


