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Euhaplorchis sp. A Effect on Social Behavior and Familiarity of Gulf Killifish (Fundulus grandis)

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Thesis of
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Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
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Nova Southeastern University
Halmos College of Arts and Sciences

April 2024

Approved:
Thesis Committee

Committee Chair: Christopher Blanar, Ph.D.

Committee Member: Lauren Nadler, Ph.D.

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

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By

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Abstract:

Trophically transmitted parasites may manipulate their hosts' phenotype (e.g., behavior, physiology, morphology) to increase the likelihood of transmission to the definitive host. In fishes, stable social groups develop familiarity over time through repeated interactions among individuals, and social preferences are often developed due to familiarity. Consequently, fishes often shoal with familiar fishes, a behavior that is likely to be protective against predation. Parasites may alter fish social dynamics in two ways: by decreasing association with familiar individuals, thereby isolating infected fish and making them more susceptible to predation by definitive hosts; and/or by incentivizing uninfected individuals to avoid infected fish in their shoal. In the present study, I tested whether Gulf killifish *Fundulus grandis* experimentally infected with *Euhaplorchis* sp. exhibited altered social preferences based on familiarity. I used a choice test methodology, allowing focal fishes to choose to associate with familiar or unfamiliar conspecific fishes held in transparent enclosures. I found that focal fish infection status had no impact on how far the fish swam. Familiarity of the stimulus groups also had no impact on who the focal fish spent more time with. Infection status and familiarity of the stimulus groups had no impact on who the focal fish spent more time with as well. Focal fish did not show a preference for familiarity or infection. Although *Euhaplorchis* sp is known to increase the probability of predation of its hosts by birds, my results suggest that it does not do so by altering host shoaling behavior.

Keywords: Complex life cycle, experimental infection, metacercariae, trematode, behavioral manipulation, Gulf killifish

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Introduction:

Parasites are a diverse group of organisms that have adapted many ways of survival and moving from host to host to complete their life cycle. Parasites are split into endoparasites (reside inside the host's body) and ectoparasites (lives on the surface of the host's body) (Bush et al., 2001). Some parasites have simple life cycles where they only have one host and some parasites have complex life cycles where they depend on multiple hosts to complete their life cycles (Auld & Tinsley, 2015; Benesh, 2016; Choisy et al., 2003; Cribb et al., 2003). In complex life cycles, a parasite may have one or more intermediate hosts before infecting the definitive (final) host where the parasite reaches sexual maturity (Auld & Tinsley, 2015). To infect a host, a parasite may penetrate the host through the host's integument or infect the next host through consumption (Auld & Tinsley, 2015; Choisy et al., 2003; Lafferty & Kuris, 2002). Consumption occurs through the host eating the parasite itself or eating an already infected intermediate host. Such parasites are described as being trophically transmitted parasites (TTP) (Kuris, 2003; Parker et al., 2009). Many TTPs have been found to manipulate their intermediate host's behavior to increase risk taking and reckless behaviors, which in return increases the likelihood of predation by the next host in its life cycle (Hughes, 2014; Kuris, 2003; Parker et al., 2009). This phenomenon was coined as parasite increased trophic transmission (PITT) by Lafferty in 1999 and has been seen in many host-parasite systems. The changes in the host caused by the parasite vary depending on the host-parasite system – what the host species are and how the parasite moves from one host to the next. In fish, transmission of endoparasites is through the environment or predation (trophic transmission) and rarely through interactions with other fish, whereas ectoparasites can move to a new host through interactions to find the next host like viruses (Belay et al., 2015).

When a parasite infects a host, the parasite can cause changes in the host's behavior through changing the host's physiology or by manipulating the host's behavior. Parasites can indirectly change the host's behavior through debilitation by targeting the endocrine and immunomodulatory systems (Adamo, 2002; Fredensborg, 2014; Lafferty & Shaw, 2013). Changes in these systems can cause the host's behavior to change mainly due to sickness and/or weakness which makes it easier for their predator to capture their prey (Hughes, 2014; Fredensborg, 2014; Lafferty & Shaw, 2013; Kuris 2003; Parker et al., 2009). These changes are part of PITT and will help the parasite continue its life cycle. An example of a PITT is *Toxoplasma gondii*. It is a single celled protist

whose definitive host is domestic cats and other species in the family Felidae. It has been found to manipulate intermediate hosts (e.g., hyenas) to reduce defensive behaviors (e.g., sheltering) and increase risky behaviors (e.g., exploration), making predation by (and parasite transmission into) cats (e.g., lions) more likely (Gering et al., 2021). Other ways hosts respond to parasites are through changes in eating, reproduction, and how the host reacts to stressful situations (Adamo, 2002; Auld & Tinsley, 2015; Lafferty & Shaw, 2013).

Another way a parasite can affect its host is through direct behavior manipulation. The behavioral manipulation of hosts has been described as an extension of the parasite's phenotype (Dawkins, 1982; Hughes, 2014; Hunter, 2018; Jolles et al., 2017) and mainly targets muscles and the nervous system to assist in the manipulation (Thomas et al., 2005). If a parasite is not in the next infectious stage of its life and able to survive, it may reduce the mortality of the host through behaviors that avoid risky and life-threatening situations (Parker et al., 2009). When a parasite is ready to move to the next host, it may manipulate its host's behavior in many ways to assist in trophic transmission by increasing conspicuous and risky behaviors along with altering social behavior (Demandt et al., 2020; Ezenwa, 2004; Helland-Riise et al., 2020; Lafferty & Morris, 1996; Loehle, 1995; Nezhybová et al., 2020; Sasal, 2003; Shaw & Øverli, 2012; Weinersmith et al., 2016). Conspicuous behaviors are behaviors that increase a prey's chance of being noticed and consumed by a predator. These behaviors include surfacing more often, taking greater risks, and reducing defensive behaviors (Nezhybová et al., 2020; Helland-Riise et al., 2020; Lafferty & Morris, 1996; Shaw & Øverli, 2012). By increasing the host's conspicuous behaviors, the host has an increased likelihood of being consumed by the next intermediate host or by the definitive host. In fish, studies have found that infected fishes exhibit a greater frequency of surfacing and other conspicuous behaviors that have the potential to attract the definitive host, piscivorous birds (Fredensborg & Longoria, 2012; Hernandez & Fredensborg, 2015). Nezhybová et al. (2020) found that a trematode, *Apatemon* sp., manipulated its intermediate host, the African killifish, to increase risk-taking behavior and enhance transmission success. By manipulating fish behavior, the fish is more likely to be consumed by its predator and therefore the parasite is able to continue its life cycle.

Manipulating and changing a host's behavior may also change the hosts' social behavior. Increasing conspicuous and risky behaviors can lead to a decrease in the shoal cohesion of a group of fish (Demandt et al., 2020; Loehle, 1995; Sasal, 2003). An advantage of fish living in groups is

to reduce the risk of predation, increase survival, and reduces the risk of PITT (Mooring & Hart, 1992; Sasal, 2003). A parasite disrupting shoal cohesion and responsiveness to predation therefore has the potential to increase predation risk along with the transmission of the parasite to its next host (Demandt et al., 2020). Demandt et al. (2020) found this type of change in the host-parasite system of the cestode *Schistocephalus solidus* and its host, the three-spined stickleback *Gasterosteus aculeatus*. In fishes, shoal cohesion is developed through familiarity with groupmates which develops over time through repeated interactions or through kin (Demandt et al., 2020; Edenbrow & Croft, 2012; Engelmann & Herrmann, 2016; Gutmann et al., 2015; Strodl & Schausberger, 2012; Versace et al., 2018; Ward & Hart, 2003). These repeated interactions help individuals better understand and predict their groupmates' behavior in different contexts, with individuals often exhibiting social preferences based on familiarity (Griffiths & Magurran, 1997; Herbert-Read, 2017; Jolles et al., 2020; Klemme & Karvonen, 2018). This social knowledge confers benefits such as improved growth rates and foraging success through cooperation, mating opportunities, reproductive success, and lower frequency of aggressive interactions (Krause & Ruxton, 2002; Ward & Webster, 2016). The many benefits of familiarity may increase the likelihood of fish preferentially interacting with individuals with whom they have previously interacted and decrease their interactions with others with whom they do not often interact (Siracusa et al., 2017; Vickruck & Richards, 2017; Ward et al., 2002).

Shoal choice has been shown to be determined by parasite infection status in many fish species with shoaling assisting in a decrease in the spread of parasites between individuals (Barber et al., 1998; Cote & Gross, 1993; Loehle, 1995; Mooring & Hart, 1992; Poulin & FitzGerald, 1989). Many parasites in aquatic environments move through the environment or predation rather than through host interactions so fish are better able to reduce parasitic infection in shoals (Bellay et al., 2015). This is known as the encounter-dilution effect, as host density increases, parasite abundance decreases (Buck & Lutterschmidt, 2017; Mooring & Hart, 1992). Shoaling behavior in the banded killifish, *Fundulus diaphanus*, has been shown to be reduced after a simulated avian predator attack when the fish are infected with the trematode *Crassiphiala bulboglossa* (Krause & Godin, 1996). Krause and Godin (1996) also observed that parasitized *F. diaphanus* spent less time in shoals and spent more time in riskier positions than their uninfected conspecifics. Parasite avoidance in shoals is important given that studies have shown parasitic infections are able to disrupt various shoaling behaviors and reduces the cohesion of the shoal through behavior

manipulation (Demandt et al., 2020; Krause & Godin, 1996; Mikheev et al., 2013). Some fish species have been found to be able to detect when another fish is infected with a parasite (Barber et al., 1998; Dugatkin et al., 1994; Mikheev et al., 2013). When studying the cestode *Schistocephalus solidus* and its intermediate host the three-spined stickleback (*Gasterosteus aculeatus*), Barber et al. (1998) discovered that when given a choice between a shoal of the same species (conspecifics) that were infected and a shoal of conspecifics that were uninfected, the focal stickleback would choose the uninfected shoal as long as the shoals were of equal size. Another study done on the three-spined sticklebacks found that individuals avoided shoals of parasitized conspecifics with the ectoparasite *Argulus canadensis* potentially due to the behavioral changes caused by the ectoparasite (Dugatkin et al., 1994).

A well-studied species of parasite, *Euhaplorchis californiensis*, has been found to manipulate its second intermediate host, the California killifish (*Fundulus parvipinnis*), to increase the likelihood of predation by the definitive hosts, piscivorous birds (Helland-Riise et al., 2020; Lafferty & Morris, 1996; Martin, 1950; Shaw et al., 2008; Shaw & Øverli, 2012; Weinersmith et al., 2016). This host-parasite system is an example of PITT: *E. californiensis* encysts on the brain of the killifish and increases conspicuous behaviors (Lafferty & Morris, 1996; Helland-Riise et al., 2020; Shaw et al., 2008; Weinersmith et al., 2023). It was also found that *F. parvipinnis* may decrease their shoaling cohesion when exposed to *E. californiensis* likely due to an increase in scratching and darting (Hernandez, 2019). A congeneric unnamed parasite species, currently called *Euhaplorchis* sp. A, has been found in the Gulf of Mexico and southern Atlantic coasts (Fredensborg & Longoria, 2012). Studies have shown that this congenic parasite species has similar behavior manipulation effects of the second intermediate host; for example, infected fishes exhibit a greater frequency of surfacing and other conspicuous behaviors that have the potential to attract the definitive host, piscivorous birds (Fredensborg & Longoria, 2012; Hernandez & Fredensborg, 2015).

Euhaplorchis sp. A is a species of trematode with a complex life cycle (Figure 1), infecting a snail as the first intermediate host. From the snail, it produces a free-living larval stage (known as cercariae) that penetrates the skin or gill epithelium of a small-bodied fish, the second intermediate host. The parasite then travels through the body of the fish and matures into the metacercaria stage and forms a cyst on the brain of the fish host. The fish is then consumed by a piscivorous marsh bird which serves as the parasite's definitive host (McNeff, 1978).

Figure 1: Complex life cycle of *Euhaplorchis* sp. A.

Euhaplorchis sp. A is known to infect two snail species in Florida: the plicate horn snail, *Cerithideopsis pliculosa*, in the western Gulf of Mexico and the ladder horn snail, *C. scalariformis* on the Florida Atlantic coast, but there has been no molecular or morphological comparison to confirm they are the same species (McNeff, 1978; Smith, 2001). In terms of second intermediate hosts, *Euhaplorchis* sp. A has been recovered from the brains of three species of killifish (the Gulf killifish *Fundulus grandis*, the longnose killifish *F. similis*, and the marsh killifish *F. confluentus*) and the sailfin molly *Poecilia latipinna* (Hernandez & Fredensborg, 2015; McNeff, 1978), with this study focusing on the Gulf killifish. *Fundulus* spp. encompasses a group of small-bodied fishes commonly found in estuaries throughout North America and are a major source of food for aquatic and terrestrial predators, including piscivorous birds (Nelson et al., 2015). *Euhaplorchis* spp. (*E. californiensis* and *Euhaplorchis* sp. A) are commonly found in estuaries throughout North America and Mexico (Shaw et al., 2010). This widespread distribution may suggest that the host-parasite interactions between *Fundulus* spp. and *Euhaplorchis* spp. have the potential to play an important role in both population and community ecology in those estuarine ecosystems. However, very little

is known about these dynamics outside of the well-studied California system and the species of *Euhaplorchis* in the southeastern United States is still to be determined.

In both the California and Florida *Euhaplorchis-Fundulus* parasite-host system, it is noted that the *Euhaplorchis* spp. manipulate the individual behavior of the *Fundulus* spp., but little is known about how the parasite affects social behavior. Gulf killifish are a social species of fish, typically found in groups of approximately 20 individuals (Cashner et al., 2019). Shoal cohesion of many fish species is dependent upon familiarity which allows for individuals to acquire knowledge of the groupmates' individual behavior (Krause & Ruxton, 2002; Ward & Hart, 2003). This acquired knowledge in return helps each fish understand its own role in the group (Krause & Ruxton, 2002). When a fish is infected with a parasite that manipulates its behavior, the cohesion of the group decreases because the acquired knowledge and determined roles are thrown off (Krause & Godin, 1996). The trematode *Crassiphiala bulboglossa* has been found to manipulate the social behavior of the banded killifish (*Fundulus diaphanus*) (Krause & Godin, 1996). In this parasite-host system Krause and Godin (1996) found that parasitized killifish spent less time in shoals, had fewer neighbors, and typically held the peripheral (outside) shoal positions than the non-parasitized killifish. With parasites manipulating individual behavior and causing changes in the cohesion of social groups in this *Fundulus*-parasite system and *Euhaplorchis* sp. A being able to manipulate individuals, I postulate that this parasite also changes the cohesion of social groups.

I examined how *Euhaplorchis* sp. A affects the social preferences of its fish host. Individual fish may have the ability to determine if other conspecifics in their shoals are infected with parasites and avoid those infected fish even with social preferences and familiarity in play. This study reveals how infection status affects the social preference of *F. grandis* and how *Euhaplorchis* sp. A affects familiarity. I aim to increase the understanding of the interaction between *F. grandis* and *Euhaplorchis* sp. A. I hypothesize that infected fish travel longer distances than uninfected fishes and that an infected fish's shoaling behavior is affected by the infection status and familiarity of potential shoal mates. To test an infected fish's shoaling behavior, I will look at the number of visits to a shoal and the distance from a shoal. I hypothesize that an infected fish prefers to shoal adjacent to unfamiliar conspecifics and infected fish are more likely to check out or visit unfamiliar conspecifics. Understanding whether *F. grandis*, regardless of infection status, avoids conspecifics infected by Euhaplorchis sp. will shed light on how a parasitic infection alters social dynamics in fish and how parasites affect their hosts. The results will also help to better understand if fish

infected with *Euhaplorchis* sp. A avoid other conspecifics and opens further potential research on this system.

Methods:

Study species

Both intermediate host species in the *Euhaplorchis* sp. A lifecycle were maintained in the aquarium facility at the Nadler Marine Behavior and Physiology Lab at the Nova Southeastern University Guy Harvey Oceanographic Center. The first intermediate host, the ladder horn snail (*C. scalariformis*) (Figure 2a) was collected from the mangrove habitats in the Indian River Lagoon South near Fort Pierce, Florida, from which cercariae (Figure 2b) were shed to infect the parasite's second intermediate host. Although this parasite is known to infect multiple small-bodied fishes for its second intermediate host, these studies focused on the Gulf killifish, *Fundulus grandis*. The cercariae enter the fish through the skin or gill epithelium, then travel to the brain, where they develop into the parasite's next life stage, an encysted larval phase known as metacercariae (Figure 2c, d). The Gulf killifish (Figure 2e, f) were caught from the population inhabiting Spruce Creek Preserve near New Smyrna Beach, Florida. Through parasitological dissections of specimens from this population in the summer and fall 2021, this population was identified as naïve to the *Euhaplorchis* sp. A parasite.

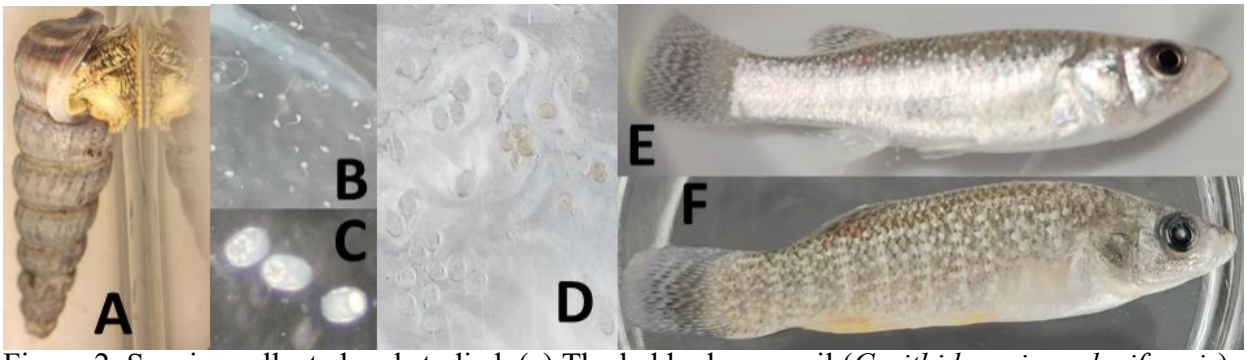

Figure 2: Species collected and studied. (a) The ladder horn snail (*Cerithideopsis scalariformis*) (b) shed *Euhaplorchis* sp. A cercariae that are infectious to a range of small-bodied fishes. (c) Once these cercariae infect a fish second intermediate host, they travel to the brain where they encyst (i.e., metacercaria), (d) and sit on the brain meninges. (e, f) One common second

intermediate host is the Gulf Killifish (*Fundulus grandis*) (e) illustrates a female and (f) illustrates a male.

Snail collection and husbandry

Adult stage *C. scalariformis* were collected in the mangrove forests of the Indian River Lagoon South (Fort Pierce, FL). Snails were taken to the Smithsonian Marine Station (Fort Pierce, FL) where they were left to dry overnight. They were then submerged in warm seawater (28-30°C; 25 ppt) and left outside in the sun for at least two hours. The seawater was then scanned for parasites. Any parasites found were identified to species level using morphological characteristics. Snails infected with only *Euhaplorchis* sp. A were marked and later double checked to confirm infection status. In total, 290 snails were collected, of which 17 were infected with *Euhaplorchis* sp. A. Infected snails were taken to the Nadler Marine Behavior and Physiology (NMBP) Lab at the Nova Southeastern University Guy Harvey Oceanographic Center. Snails were maintained in small holding tanks (in groups of five per tank), which contained a seawater-dampened paper towel and algae wafers (Tetra PRO PlecoWafers).

Fish collection and husbandry

Fish used in this study (*F. grandis*; n = 182 fish) were collected from two different sites in the Spruce Creek Preserve (New Smyrna Beach, FL; 29°04’57.2”N 80°57’49.2”W and 29°04’41.9”N 80°57’10.0”W) using a two-pole seine net (3 mm mesh, 3 m L x2 m H). All fish identified as our focal species were placed in 10-gallon coolers (in group sizes ≤ 30 fish) filled with seawater from the collection site and continuous aeration using a battery-operated aerator for transport to the Nadler Marine Behavior and Physiology (NMBP) Lab at the Nova Southeastern University Guy Harvey Oceanographic Center. Non-target species were returned to the water immediately.

Once back at the lab, fish were split evenly across two distinct aquarium systems (in groups ≤ 25 fish each), which were allocated to the uninfected (control) and infected (parasite-exposed) treatments (100 gallons per system, 25 ppt salinity, natural 12h light:12h dark/light cycle). Fish were split evenly between each aquarium system such that experimental parasite infections (described below) were completed on paired tanks in the uninfected and infected systems. (Figure

3). All tanks were covered with a mesh to prevent fish from jumping out and contained the same number of assorted sizes of PVC pipes to act as shelters. Fish were fed Omega One freeze-dried bloodworms to satiation twice per day. If any dead fish were found during daily inspections, they were placed in a small plastic bag, labeled with the date, tank, and fish species then placed in a -20 °C freezer for dissection at a later date (using the procedure described below). Uneaten food and feces were siphoned from the tanks daily, with a deep tank clean (involved scrubbing of tank and PVC shelter surfaces) and a 25% water change in each system weekly to ensure that water quality was maintained to a high standard (tested at least twice weekly for the duration of the study using API Saltwater Master Test Kit).

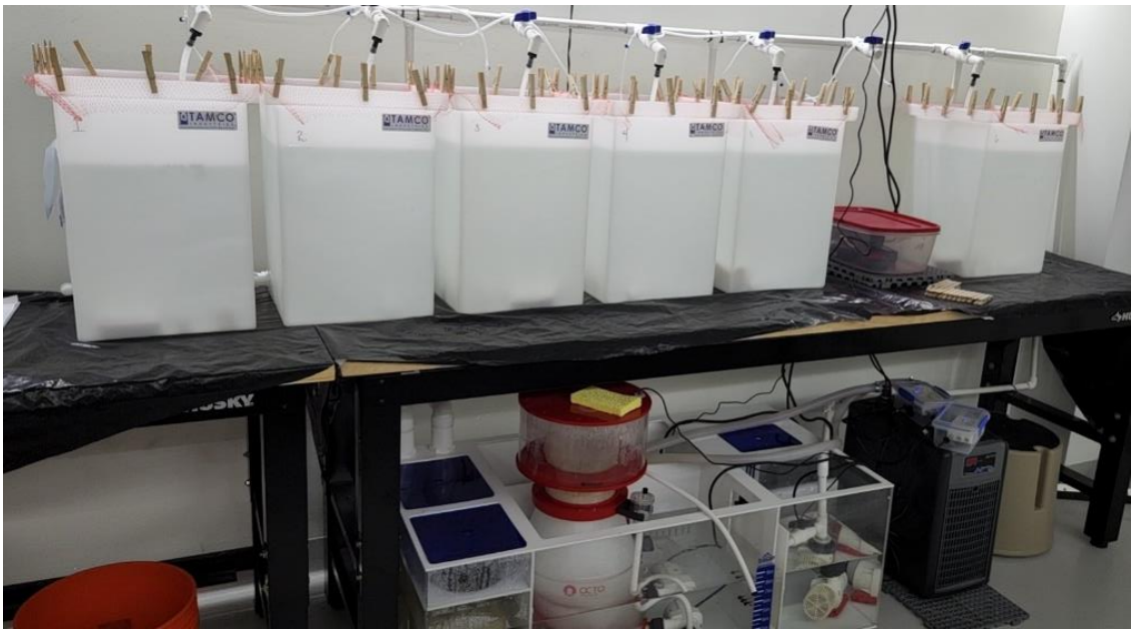

Figure 3: Set up for one of the experimental treatment aquarium systems.

Experimental parasite infection

The infected treatment aquarium system was exposed to infectious cercariae parasites using the procedure outlined below, while the tanks in the uninfected system received a sham parasite infection (simulated infection with only seawater that controlled for the disturbance of the container holding the parasites). Paired tanks from the same collection dates were exposed to

their respective treatment (sham seawater exposure or parasite exposure) on the same days. To expose fish in the infected treatment to *Euhaplorchis* sp. A cercariae, snails were shed based on a protocol previously designed for *E. californiensis* (Helland-Riise et al. 2020), and adapted for the *Euhaplorchis* sp. A parasite. Based on preliminary work, we determined that approximately 10% of the cercariae that fish were exposed to resulted in a metacercariae in the host. As previously published work suggests that *Fundulus* spp. hosts exhibit a mean infection intensity of 159.8 metacercariae in the wild (Fredensborg & Longoria 2012), each fish needed to be exposed to approximately 1,500 cercariae to achieve an ecologically relevant infection intensity.

At least 12 hours prior to each of the experimental infection events, the snails were put into mesh bags to dry overnight (Figure 4a). On the day of the experimental infection, one snail was placed in each compartment of a compartment box with seawater (25 ppt) heated to 28-30°C so that the water level fully covered each snail (Figure 4b). The compartment boxes were placed under a heat lamp for two hours, with a thermometer placed next to the boxes to make sure the temperature did not go out of range (28-30°C; Figure 4c). If snails climbed out of the water by attaching to the compartment lid, they were gently dislodged and returned to the water through gently tapping on the compartment lid. After two hours, each compartment was examined under a dissection microscope and compartments with *Euhaplorchis* sp. A were marked with an X. The snails that did not shed were returned to their tanks.

While the parasites were shedding from their snail host, water flow to the uninfected and infected tanks that were to be treated that day was stopped, and they were drained to 50%. We did not turn off the entire system so as not to disturb the tanks that were not being treated that day. At the end of the two-hour shedding period, all *Euhaplorchis* sp. A cercariae that shed were combined into a graduated cylinder. To determine the total number of parasites that shed, 2 mL of the cercariae-laden water was subsampled using a glass pipette and placed into a glass petri dish (5 cm diameter x 1.5 cm deep). The cercariae in the 2 mL of water was counted twice and the average was used to calculate the approximate total cercariae shed for that day. Each fish was exposed to a maximum of 250 cercariae per infection, and the number allocated per individual was calculated using the total number derived from sub-sampling. Fish housed in tanks together were batch exposed to cercariae. The cercariae-laden water allocation for each tank was aliquoted to scintillation vials based on how many fish were in the tank. Each tank received seven infection events and each fish was exposed to 1400 – 1600 cercariae (Table 1). Each tank

had at least three days in between sequential cercariae exposures to minimize the stress associated with the treatment.

Table 1: Summary of experimental infections. Number of fish based on the last count of fish in each tank during last infection.

Tank (n = fish)	*Estimated number of cercariae per fish*	*Average number of cercariae per fish per infection event*	*Number of infection events*	*Date of last infection event*
1 (n =16)	1568	224 +/- 28.8	7	8/14/22
2 (n = 14)	1498	214 +/- 48.1	7	8/14/22
3 (n = 14)	1518	217 +/- 35.8	7	8/12/22
4 (n = 14)	1603	229 +/- 36.5	7	8/15/22
5 (n = 10)	1567	223 +/- 32.6	7	8/12/22

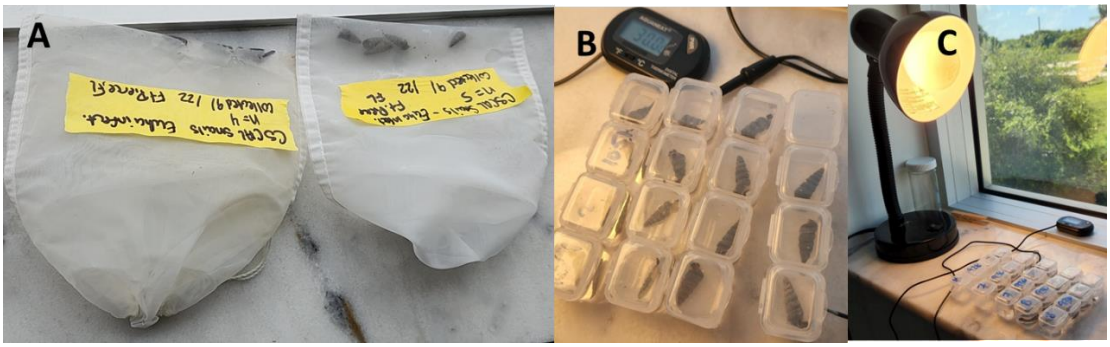

Figure 4: Shedding snails for *Euhaplorchis* sp. A cercariae. (a) Snails drying in mesh bags. (b) Snails shedding cercariae in their individual compartments. (c) Temperature maintained as snails were shedding using a heat lamp.

Once the infected tanks' cercariae-laden seawater was aliquoted, a comparable volume of seawater was aliquoted for the paired uninfected tank's sham treatment. The water from each jar was poured into the respective tank in a circle to ensure a homogenous distribution and rinsed with the tank's water to ensure that no cercariae remained in the vial. This sequence was completed for both the uninfected and infected treatments. Water flow remained off for four hours to promote fish-cercariae encounters. After four hours, water flow was returned to all tanks and the water level was refilled to the correct volume.

After two infections, one fish from the infected treatment was dissected to ensure the experimental infection protocol was resulting in host infection. This fish was found to have no *Euhaplorchis* sp. A so after a third infection event, another fish from the infected treatment was

dissected and *Euhaplorchis* sp. A metacercariae were discovered. Fish were given a minimum of six weeks before behavioral studies commenced to ensure *Euhaplorchis* sp. A metacercariae matured the stage that is infectious to the parasite's final host (K.L. Weinersmith, personal communication).

Social familiarity behavioral study

For the behavioral study, fish were rearranged from paired tanks across the two aquarium systems so that each tank was composed of 50% uninfected and 50% infected. To differentiate between uninfected and infected individuals, prior to reorganization, all fish were tagged with a unique visible implant elastomer (VIE) tag. These subcutaneous tags are small and have no adverse effects on growth or survival (Hoey & McCormick, 2006). Following this re-organization, all fish were given a period of three weeks to recover from the stress of moving home tanks and to familiarize themselves with the newly introduced fish. Past studies suggest that familiarity is accomplished in approximately two to three weeks in other small-bodied fishes (Griffiths & Magurran, 1997; Utne-Palm & Hart, 2000). The behavioral study then assessed if infection (uninfected, infected) alters killifish social preferences based on familiarity (unfamiliar, familiar) in a fully crossed design. Social preference was tested using a choice test methodology (Griffiths & Magurran, 1997; Nadler et al., 2021) in an acrylic tank (l: 76 cm x w: 30 cm x h: 30 cm; depth of water was 12 cm – figure 4b) with two porous and translucent containers that allowed the focal fish to detect both visual and olfactory cues of the social groups in each container (Figure 5).

At the start of each trial, stimulus social groups composed of 3 fish each were placed into these containers. The focal fish was then placed into a removable cylinder in the middle of the tank to acclimate for 10 minutes. The cylinder was then removed, and the focal fish was allowed to swim around the tank for a further 15 minutes of acclimation. At the conclusion of this 25-minute acclimation period, the focal fish was then video-recorded from above using a GoPro10 at 30 fps for 6 minutes. We used a white background on the bottom of the tank to ensure sufficient contrast between the fish and the bottom of the tank (Figure 5). To prevent disturbance to all fish in the experimental set up, a white tarp was draped around the tank.

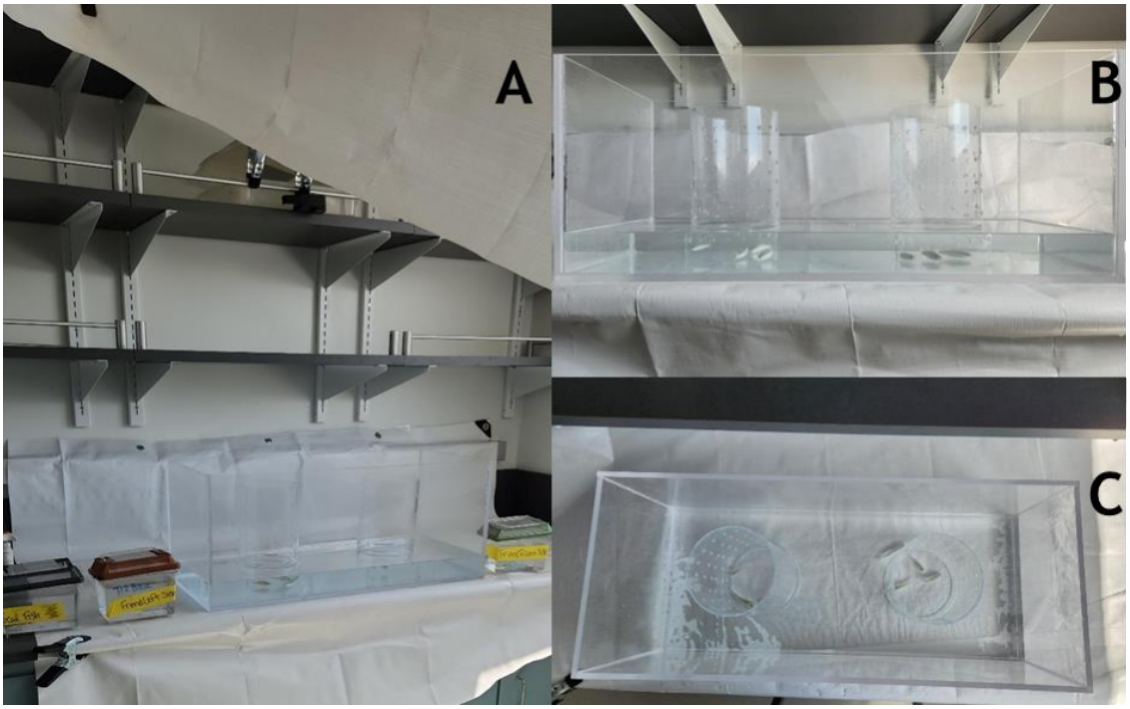

Figure 5: Tank and camera set up, including (a) the overall set up, (b) a side view, and (c) the top view.

Table 2: Combinations of stimulus groups for the experiment. All focal fish are presented with each of these combinations of stimulus groups, so each fish completes four trials.

Stimulus group combinations		
Familiar Infected	x	Familiar Uninfected
Familiar Infected	x	Unfamiliar Uninfected
Unfamiliar Infected	x	Unfamiliar Uninfected
Unfamiliar Infected	x	Familiar Uninfected

Each focal fish was tested four times, once with each combination of social groups (Table 2). The order for the social group combinations were randomized along with the order of the focal fish. The tank used for the social groups was randomized as well to avoid repetition of the social groups. With having paired tanks and the potential for familiarity amongst paired tanks,

the paired tanks were not used in the same trial. One trial a day was completed from each tank to reduce the amount of stress put on the fish.

Behavioral Trial Analysis

In total, 120 trials were completed and recorded. The videos were converted to a picture series (.jpeg format) using Prism Video File Converter and each focal fish was tracked (x and y coordinates) in 1 frame per second (every 30th frame) in ImageJ (Hoch et al., 2019). Each trial consisted of 360 frames. For each trial, the coordinates (X-Y) of the center of both social group cylinders were recorded along with the length of the tank in pixels. Focal fish location was also given in X-Y coordinates. All pixel measurements were converted to cm in Excel. The distance of the focal fish to the center of the social group container was measured in Excel using =SQRT((X$_1$-X$_A$)^2+(Y$_1$-Y$_A$)^2) and =SQRT((X$_1$-X$_B$)^2+(Y$_1$-Y$_B$)^2), with X$_1$Y$_1$ being the center coordinates, X$_A$Y$_A$ being the left focal group, and X$_B$Y$_B$ being the right focal group. The distance the fish swam from one frame to the next was calculated by =SQRT((X$_1$-X$_2$)^2+(Y$_1$-Y$_2$)^2) and added to get the total distance swam.

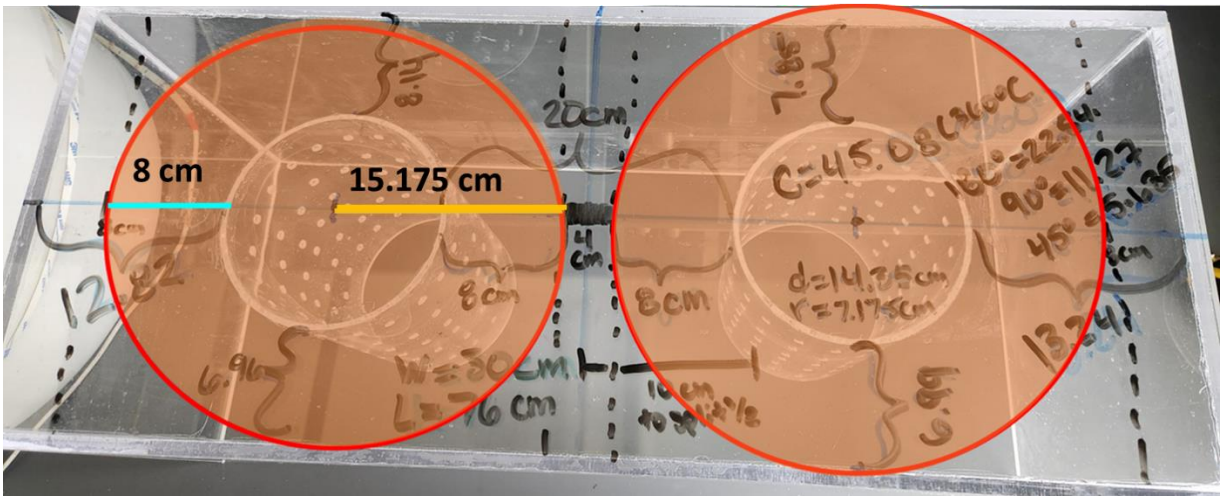

Figure 6: The interaction and neutral zones of the trial tank. The red circle represents the 8 cm or 2 body length interaction zone. Outside of the red circle is the neutral zone.

The focal fish is considered to be interacting with a social group if it is within 8 cm of the social group (i.e., approximately 2 body lengths) (Figure 6). The total number of times the focal fish was within the 8 cm was calculated for each stimulus group. With the tank having a smaller width, there was not always 8 cm between the edge of the cylinder and the edge of the tank. This caused visits to be counted when the fish was swimming at the edge of the tank and not intentionally interacting with the stimulus group due to a lack of a neutral zone along the length of parts of the tank (Figure 6). Therefore, the number of visits within 4 cm (approximately 1 body length) was also calculated. The total number of times or visits the focal fish made to each social group was calculated and the proportion of visits was found by dividing the visits to the unfamiliar stimulus group by the number of visits to the familiar stimulus group. When the proportion is above 1, the focal fish visited the unfamiliar stimulus group more and below 1, the focal fish visited the familiar stimulus group more. When the focal fish visits each stimulus group evenly, the proportion is at 1.0. The infected social group average distance was subtracted from the uninfected social group average distance to get the difference in centimeters. A negative difference indicates the focal fish was closer on average to the uninfected social group and a positive difference indicates the focal fish was closer on average to the infected social group.

Statistical Analysis

General Linear Modelling (GLM) was used in JMP v.17.01 to test for the effect of focal fish infection status (infected vs uninfected controls) on focal fish total swimming distance, focal fish distance differential (mean distance from unfamiliar vs familiar potential shoalmates), and the proportion of visits to unfamiliar vs familiar potential shoalmates. Lastly, GLM was also used to assess whether the distance differential (mean distance from potential shoalmates) was affected by focal fish infection status, the infection status of potential shoalmates, and the familiarity of those shoalmates.

Results

Total Distance the Focal Fish Swam

Infection of the focal fish did not have a significant effect on the total distance swam (R^2 = 0.02; p = 0.1896). The infected fish swam on average 1648.2 cm and the uninfected fish swam 1815.7 cm (Figure 7), but this difference was not significant.

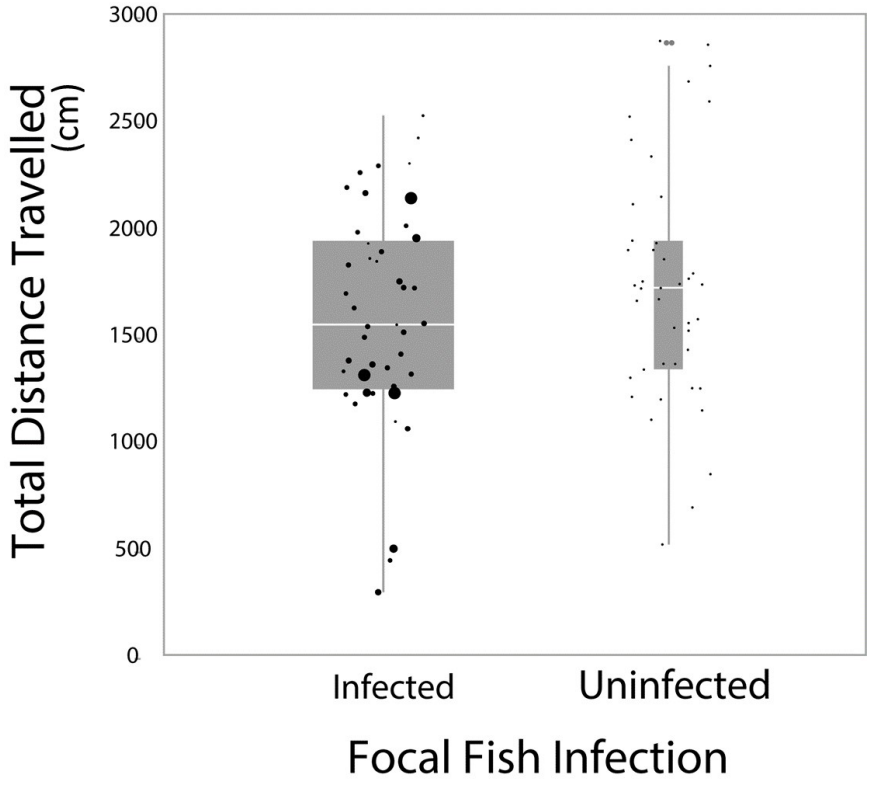

Figure 7: The total distance infected and uninfected focal fish swam. The uninfected fish swam a little farther on average than the infected fish, but it was not found to be significant. The data points are randomly jittered, and the size of each data point is scaled to the infection intensity of the individual fish. There does not appear to be a pattern in the infection intensity of the infected focal fish, no significance was found.

Difference in Average Distance from the Stimulus Groups – Stimulus Group Infection

The infection of the stimulus group did not have a significant effect on the difference in average distance from the stimulus group ($R^2 = 0.03$; $p = 0.1081$) (Figure 8).

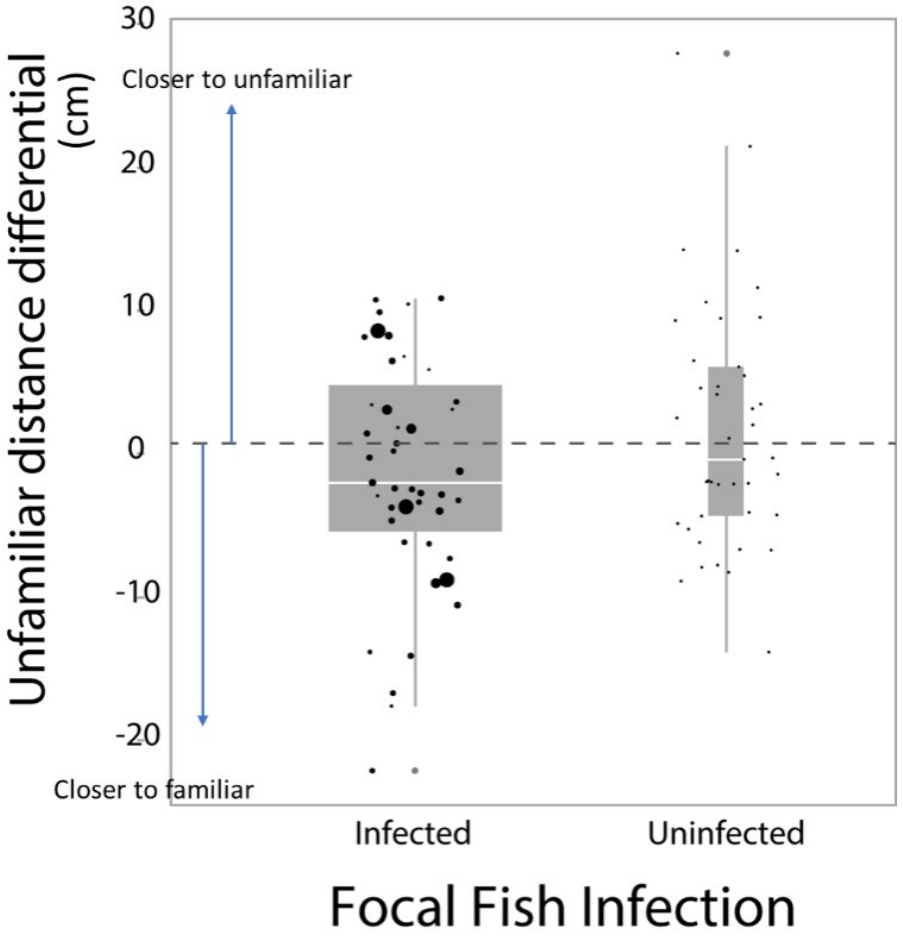

Figure 8: The difference in average distance from each stimulus group based on familiarity. The line at zero represents a focal fish having no preference and being the same distance from each stimulus group. A difference less than zero means the focal fish was closer to the familiar group and a difference greater than zero means the focal fish was closer to the unfamiliar group. No significance was found. The data points are randomly jittered, and the size is scaled to the infection intensity. There does not appear to be a pattern in the infection intensity of the infected focal fish.

Proportion of Visits to the Stimulus Groups – Stimulus Group Infection

The infection of the stimulus group did not have a significant effect on the proportion of visits to the stimulus group ($R^2 = 0.0121$; $p = 0.3069$) (Figure 9).

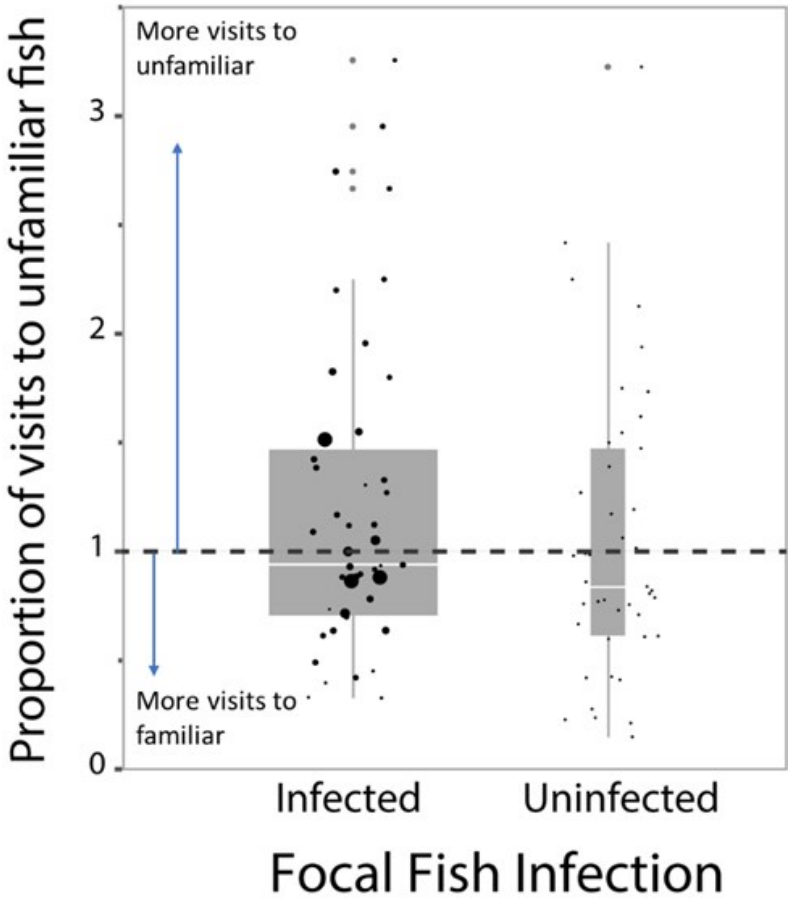

Figure 9: The proportion of visits to the stimulus groups based on familiarity. The line at one represents a focal fish having no preference and visiting the stimulus group the same number of times. A proportion less than one means the focal fish visited the familiar group most often and a proportion greater than one means the focal fish visited the unfamiliar group most often. There was no significance found. The data points are randomly jittered, and the size is scaled to the infection intensity. There does not appear to be a pattern in the infection intensity of the infected focal fish.

Difference in Average Distance from the Stimulus Group – Familiarity and Infection

The infection and familiarity of the stimulus group did not have a significant effect on the difference in average distance from the stimulus group ($R^2 = 0.0108$; $p = 0.756$) (Figure 10).

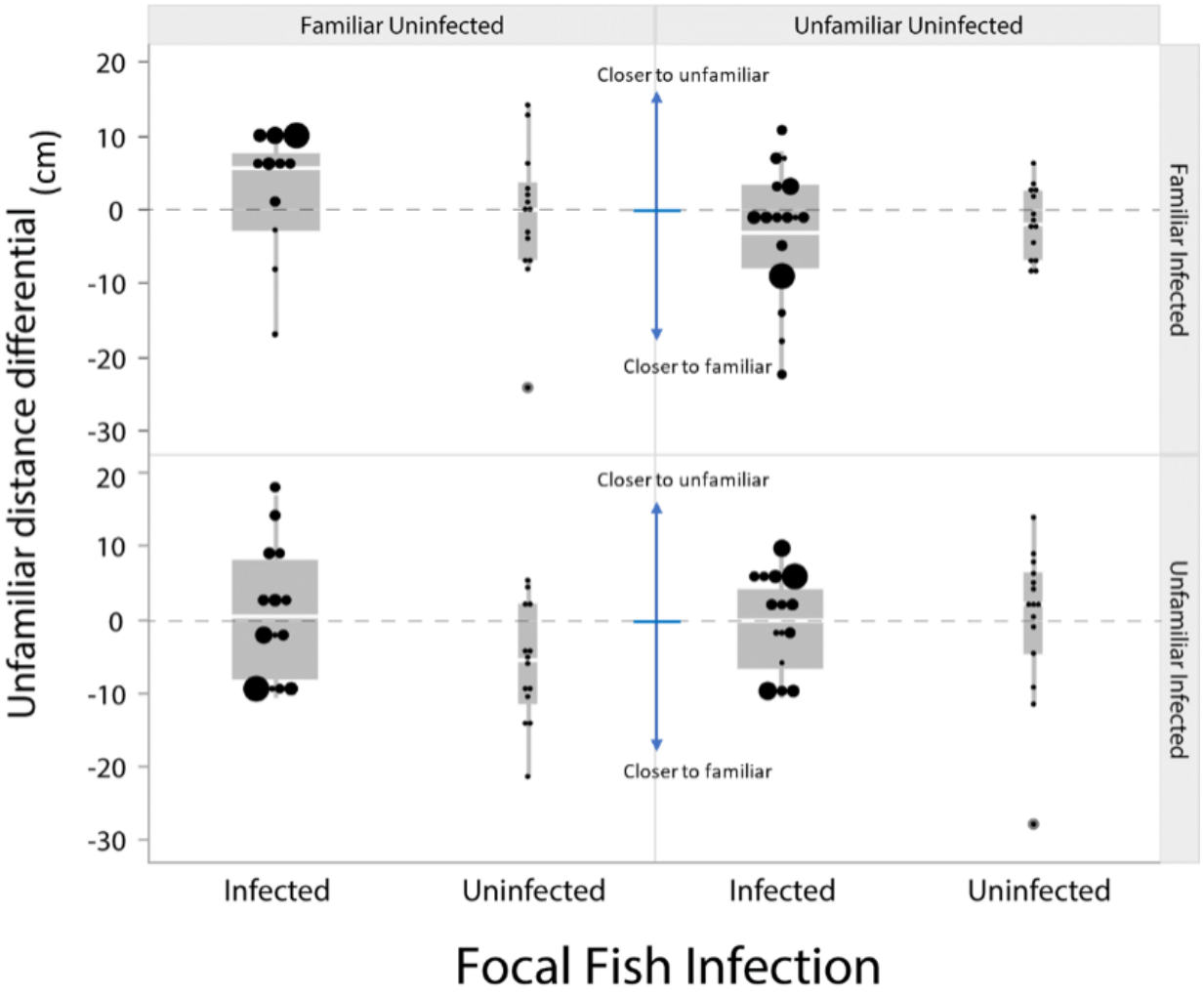

Figure 10: The difference in average distance from each stimulus group based on familiarity and infection of the stimulus groups. The line at zero represents a focal fish having no preference. A difference less than zero means the focal fish was closer to the familiar group and a difference greater than zero means the focal fish was closer to the unfamiliar group. There is a lot of variation around zero, although no significance was found. The data points are randomly jittered, and the size is scaled to the infection intensity. There does not appear to be a pattern in the infection intensity of the infected focal fish.

Discussion

Focal fish infection did not have a significant impact on how far the fish swam during a trial and infected fish did not swim farther than uninfected fish. Therefore, I reject my hypothesis that stated infected fish travel longer distances than uninfected fishes. *Euhaplorchis* sp. A does not appear to have an impact on Gulf killifish's swimming ability or the fish's inclination to explore an arena. This suggests *Euhaplorchis* sp. A directly manipulates its host behavior and does not cause changes in systems that may cause debilitation and weakness (Adamo, 2002; Fredensborg, 2014; Hughes, 2014; Hunter, 2018). The direct manipulation of a parasite is typically done through an extension of the parasite's phenotype (Dawkins, 1982; Hughes, 2014; Hunter, 2018). Further studying the physical and phenotypic changes *Euhaplorchis* sp. A has on Gulf killifish can help to better understand the full impact this parasite has on its fish hosts.

Stimulus group familiarity did not significantly impact the focal fish's distance from the groups or the number of visits. Therefore, I reject my hypothesis that infected fish prefer to shoal adjacent to unfamiliar conspecifics and infected fish are more likely to visit or check out unfamiliar fish. Focal fish did not spend more time with familiar shoal mates although these fish generally are curious and will investigate unfamiliar conspecifics. The fish in this study did not have a significant preference for familiar or unfamiliar fish and therefore familiarity may not be a large driving force in shoaling of Gulf killifish. There may be instances where familiarity does not benefit a fish's shoaling (Godin et al., 2003; Gómez-Laplaza & Fuente, 2007). An absence of predators or other stressors may reduce a fish's preference to shoal with familiar fish over unfamiliar fish (Godin et al., 2003; Gómez-Laplaza & Fuente, 2007). During the trials, there was no risk of predation and other stressors were reduced so a need to shoal and obtain the benefits of shoaling is not as needed. Further research on the motivations underlying shoaling behaviors in Gulf killifish is needed to fully understand why this species shoals and whether familiarity plays any part of shoaling.

In a study on *F. diaphanus*, it was found that test fish preferred to shoal with a larger shoal of conspecifics of similar body size under a predator threat (Krause & Godin, 1994). In this study, the focal fish and stimulus groups had a larger variety of body sizes with some fish being as small as 3 cm and some as large as 7.5 cm. This difference in fish body size may have impacted the likelihood of a focal fish shoaling with a particular stimulus group. Body size and shoaling in this

fish species may have similar impacts as in *F. diaphanus* and may be why there was no significance in shoaling. This study also used three fish for each stimulus group which is a small shoal. Increasing the number of fish in each shoal and further exploring the size of the shoal may give insight into a shoal size preference like what was found in *F. diaphanus*.

Infection status and familiarity of the stimulus groups did not significantly impact the focal fish's distance from the groups. Therefore, I reject my hypothesis that infected fish shoaling behavior, specifically the difference in distance, is affected by infection status and familiarity of potential shoal mates. The infection status of potential shoal mates does not affect their shoaling decisions. Gulf killifish may not be able to detect the infection status of other fishes. Fishes use visual and chemical cues to detect infection in another fish (Krause & Godin, 1994; Krause et al., 1999). Gulf killifish infected with *Euhaplorchis* sp. A may not give off chemical cues that clue in individuals on infection. Visual cues like an increase in conspicuous behavior may not be enough for the killifish to change an uninfected fish's behavior and cause an avoidance to infected fish. Conversely, if Gulf killifish are able to detect infection status of other fishes, the infection status may not cause enough of a change in behavior or other factors to cause killifish to avoid infected fish. Further studying the visual and chemical cues of Gulf killifish and *Euhaplorchis* sp. A would help to better understand the interaction between infected and uninfected individuals.

The Gulf killifish in this study were from two naïve populations and experimentally infected. Doing a similar experiment on a naturally infected population would help to determine if the experimentally and naturally infected fish have similar reactions to infection. *Euhaplorchis* sp. A also infects three other species of fish (*F. similis*, *F. confluentus*, and *P. latipinna*) and doing similar studies on these fish would increase our understanding of how *Euhaplorchis* sp. A affects its intermediate fish hosts and their behavior. Furthermore, a parasite may also be able to impact its host's personality which in return can potentially drive selection of some personality traits that increase a parasite's survival (Barber & Dingemanse, 2010). These differences in personality traits of fish may alter their shoaling behaviors and therefore studying personality traits before and after experimental infection may help to better understand the full impact of *Euhaplorchis* sp. A. Examining personality traits of naturally infected fish and comparing those traits to the experimentally infected fish may show potential personality traits that may have been selected for by the parasite as well. An increase in intraspecific aggression in uninfected fish compared to infected fish was observed in this study, which led to an increase in fish loss. This may be caused

by *Euhaplorchis* sp. A decreasing aggression in infected fish. Fully exploring the personality traits of fish including aggression and boldness both pre- and post- parasite exposure and in naturally infected fish could be used to further study this observation.

Conclusion

This study found that infection of the focal fish and stimulus groups along with familiarity did not impact swimming distance, visits, or distance from each stimulus group. *Euhaplorchis* sp. A does not appear to change *F. grandis* swimming ability or inclination to explore an arena. Focal fish, regardless of infection status, did not spend time closer to or visit unfamiliar shoalmates more often, although globally these fish are curious and will investigate unfamiliar fish. Shoaling in Gulf killifish may not be based on familiarity. The infection status of potential shoal mates does not affect a focal fish's shoaling decisions. If Gulf killifish are able to detect infection, it does not appear that it is a big factor when interacting with conspecifics, but they also may not be able to detect infection. Overall, behavior manipulation by *Euhaplorchis* sp. A does not seem to involve the types of social interactions tested in this study. This study is one of few done on *Euhaplorchis* sp. A and the first to examine familiarity and infection on social behavior. These results broaden our understanding of the social behavior in host-parasite system and opens future studies to further investigate the behavior manipulation of *Euhaplorchis* sp. A.

References

Adamo, S. A. (2002). Modulating the modulators: Parasites, neuromodulators and host behavioral change. *Brain, Behavior and Evolution*, *60*, 370-377. https://doi.org/10.1159/000067790

Auld, S. K., & Tinsley, M. C. (2015). The evolutionary ecology of complex lifecycle parasites: Linking phenomena with mechanisms. *Heredity*, *114*, 125-132. https://doi.org/10.1038/hdy.2014.84

Barber, I., & Dingemanse, N. J. (2010). Parasitism and the evolutionary ecology of animal personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4077-4088. https://doi.org/10.1098/rstb.2010.0182

Barber, I., Downey, L. C., & Baithwaite, V. A. (1998). Parasitism, oddity and the mechanism of shoal choice. *Journal of Fish Biology*, *53*, 1365-1368. https://doi.org/10.1111/j.1095-8649.1998.tb00256.x

Bellay, S., De Oliveira, E. F., Almeida-Neto, M., Mello, M. A. R., Takemoto, R. M., & Luque, J. L. (2015). Ectoparasites and endoparasites of fish form networks with different structures. *Parasitology*, *142*(7), 901-909. https://doi.org/10.1017/S0031182015000128

Benesh, D. P. (2016). Autonomy and integration in complex parasite life cycles. *Parasitology*, *143*(14), 1824-1846. https://doi.org/10.1017/s0031182016001311

Buck, J. C., & Lutterschmidt, W. I. (2017). Parasite abundance decreases with host density: Evidence of the encounter-dilution effect for a parasite with a complex life cycle. *Hydrobiologia*, *784*, 201-210. https://doi.org/10.1007/s10750-016-2874-8

Bush, A. O., Fernández, J. C., Esch, G. W., & Seed, J. R. (2001). *Parasitism: The Diversity and Ecology of Animal Parasites*, Cambridge, Cambridge University Press.

Cashner, R. C, Schaefer, J., Warren Jr., M. L., Echelle, A. A., Galvez, F., & Ghedotti, M. J. (2019). Fundulidae: Topminnows. In Warren, M. L. & Burr, B. M. (Eds), *Freshwater fishes of North America: Characidae to Poeciliidae* (551-608). The John Hopkins University Press.

Choisy, M., Brown, S. P., Lafferty, K. D., & Thomas, F. (2003). Evolution of trophic transmission in parasites: Why add intermediate hosts? *The American Naturalist*, *162*(2), 172-181. https://doi.org/10.1086/375681

Cribb, T. H., Bray, R. A., Olson, P. D., Timothy, D., & Littlewood, J. (2003). Life cycle evolution in the Digenea: A new perspective from phylogeny. *Advances in Parasitology*, *54*, 197-254. https://doi.org/10.1016/s0065-308x(03)54004-0

Dawkins, R. (1982). *The Extended Phenotype: The Gene As the Unit of Selection*. Oxford University Press: W H Freeman & Co.

Demandt, N., Praetz, M., Kurvers, R. J. J. M., Krause, J., Kurtz, J., & Scharsack, J. P. (2020). Parasite infection disrupts escape behaviours in fish shoals. *Proceedings of the Royal Society B*, *287*(1938). https://doi.org/10.1098/rspb.2020.1158

Dugatkin, L. A., FitzGerald, G. J., & Lavoie, J. (1994). Juvenile three-spined sticklebacks avoid parasitized conspecifics. *Environmental Biology of Fishes*, *39*, 215-218. https://doi.org/10.1007/BF00004940

Edenbrow, M., & Croft, D. P. (2012). Kin and familiarity influence association preferences and aggression in the mangrove killifish *Kryptolebias marmoratus*. *Journal of Fish Biology*, *80*(3), 503-518. https://doi.org/10.1111/j.1095-8649.2011.03181.x

Engelmann, J. M., & Herrmann, E. (2016). Chimpanzees trust their friends. *Current Biology*, *26*(2), 252-256. https://doi.org/10.1016/j.cub.2015.11.037

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology*, *15*(3), 446-454. https://doi.org/10.1093/beheco/arh028

Farrell, D. (2023). Parasites versus predation: The role of chronic and acute parasite exposure in infection risk and anti-predator behavior [Master's thesis, *Nova Southeastern University*]. NSU Works.

Fredensborg, B. L. (2014). Predictors of host specificity among behavior-manipulating parasites. *Integrative and Comparative Biology*, *54*(2), 149-158. https://doi.org/10.1093/icb/icu051

Fredensborg, B. L., & Longoria, A. N. (2012). Increased surfacing behavior in longnose killifish infected by brain-encysting trematode. *The Journal of Parasitology*, *98*(5), 899-903. https://doi.org/10.1645/GE-3170.1

Gering, E., Laubahc, Z. M., Weber, P. S. D., Hussey, G. S., Lehmann, K. D. S., Montgomery, T. M., Turner, J. W., Perng, W., Pioon, M. O., Holekamp, K. E., & Getty, T. (2021). *Toxoplasma gondii* infections are associated with costly boldness toward felids in a wild host. *Nature Communications*, *12*. https://doi.org/10.1038/s41467-021-24092-x

Griffiths, S. W., & Magurran, A. E. (1997). Familiarity in schooling fish: How long does it take to acquire? *Animal Behaviour*, *53*(5), 945-949. https://doi.org/10.1006/anbe.1996.0315

Gutmann, A. K., Špinka, M., & Winckler, C. (2015). Long-term familiarity creates preferred social partners in dairy cows. *Applied Animal Behaviour Science*, *169*, 1-8. https://doi.org/10.1016/j.applanim.2015.05.007

Helland-Riise, S. H., Nadler, L. E., Vindas, M. A., Bengston, E., Turner, A. V., Johansen, I. B., Weinersmith, K.L., Hechinger, R. F., & Øverli, Ø. (2020). Regional distribution of a brain-encysting parasite provides insight on parasite-induced host behavioral manipulation. *The Journal of Parasitology*, *106*(1), 188-197. https://doi.org/10.1645/19-86

Herbert-Read, J. E. (2017). Social behavior: The personalities of groups. *Current Biology*, *27*(18), R1015-R1017. https://doi.org/10.1016/j.cub.2017.07.042

Hernandez, R. N. (2019). Behavioral defense against parasites: California killifish move, dart, and scratch more during trematode cercaria exposure and attack [Master's thesis, University of California San Diego]. eScholarship.

Hernandez, R. N., & Fredensborg, B. L. (2015). Experimental test of host specificity in a behaviour-modifying trematode. *Parasitology*, *142*(13), 1631-1639. https://doi.org/10.1017/S0031182015001171

Hoch, J. M., Bermudez, A. C., Coury, O. S., Donahou, A. S., Jeffers, C. N., Ramsaran, D., LaMartina, M., & Spadafore, S. (2019). The influence of personality on small fish migration and dispersal in the Everglades. *Wetlands*, *39*(5), 991-1002. https://doi.org/10.1007/s13157-019-01147-w

Hoey, A. S., & McCormick, M. I. (2006). Effects of the subcutaneous fluorescent tags on the grown and survival of a newly settled coral reef fish, *Pomacentrus amboinensis* (Pomacentridae). *Proceedings of the 10th International Coral Reefs Symposium*, 420-424.

Hughes, D. P. (2014). On the origins of parasite-extended phenotypes. *Integrative and Comparative Biology*, *54*(2), 210-217. https://doi.org/10.1093/icb/icu079

Hunter, P. (2018). The revival of the extended phenotype: After more than 30 years, Dawkins' Extended Phenotype hypothesis is enriching evolutionary biology and inspiring potential applications. *EMBO Reports*, *19*. https://doi.org/10.15252/embr.201846477

Jolles, J. W., Boogert, N. J., Sridhar, V. H., Couzin, I. D. & Manica, A. (2017). Consistent individual differences drive collective behavior and group functioning of schooling fish. *Current Biology*, *27*(18), 2862-2868. https://doi.org/10.1016/j.cub.2017.08.004

Jolles, J. W., Mazue, G. P. F., Davidson, J., Behrmann-Godel, J., & Couzin, I. D. (2020). *Schistocephalus* parasite infection alters sticklebacks' movement ability and thereby shapes social interaction. *Scientific Reports*, *10*. https://doi.org/10.1038/s41598-020-69057-0

Klemme, I., & Karvonen, A. (2018). Experience and dominance in fish pairs jointly shape parasite avoidance behaviour. *Animal Behavior*, *146*, 165-172. https://doi.org/10.1016/j.anbehav.2018.10.2022

Krause, J., & Ruxton, G. D. (2002). Living in Groups. Oxford University Press.

Krause, J., & Godin, J. -G. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Ciprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*, 128-136. https://doi.org/10.1111/j.1439-0310.1994.tb01063.x

Krause, J., & Godin, J. -G. J. (1996). Influence of parasitism on shoal choice in the banded killifish (*Fundulus diaphanous*, Teleostei, Cyprinodontidae). *Ethology*, *102*, 40-49. https://doi.org/10.1111/j.1439-0310.1996.tb01102.x

Krause, J., Ruxton, G. D., & Godin, J. -G. J. (1999). Distribution of *Crassiphiala bulboglossa*, a parasitic worm, in shoaling fish. *Journal of Animal Ecology*, *68*(1), 27-33. https://doi.org/10.1046/J.1365-2656.1999.00262.X

Kuris, A. M., (2003). Evolutionary ecology of trophically transmitted parasites. *Journal of Parasitology*, *89*, S96-S100.

Lafferty, K. D., (1999). The evolution of trophic transmission. *Parasitology Today*, *15*(3), 111-115. https://doi.org/10.1016/S0169-4758(99)01397-6

Lafferty, K. D., & Kuris, A. M. (2002). Trophic strategies, animal diversity and body size. *Trends in Ecology & Evolution*, *17*, 507-513. https://doi.org/10.1016/S0169-5347(02)02615-0

Lafferty, K. D., & Morris, A. K. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, *77*(5), 1390-1397. https://doi.org/10.2307/2265536

Lafferty, K. D., & Shaw, J. C. (2013). Comparing mechanisms of host manipulation across host and parasite taxa. *The Journal of Experimental Biology*, *216*(1), 56-66. https://doi.org/10.1242/jeb.073668

Lee-Jenkins, S. S., & Godin, J. -G. J. (2010). Social familiarity and shoal formation in juvenile fishes. *Journal of Fish Biology*, *76*(3), 580-590. https://doi.org/10.1111/j.1095-8649.2009.02512.x

Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecological Society of America*, *76*(2), 326-335. https://doi.org/10.2307/1941192

Martin, W. E. (1950). *Euhaplorchis californiensis* n.g., n. sp., Heterophyidae, Trematoda, with notes on its life cycle. *Transactions of the American Microscopical Society*, *69*(2),194-209. https://doi.org/10.2307/3223410

McNeff, L. L. (1978). Marine cercariae from *Cerithidea pliculosa* Menke from Dauphin Island, Alabama; Life cycles of heterophyid and opisthorchiid digenea from Cerithidea Swainson from the eastern Gulf of Mexico. [Master's thesis, University of Alabama]. University of Alabama Libraries.

Mikheev, V. N., Pasternak, A. F., Taskinen, J., & Valtonen, T. E. (2013). Grouping facilitates avoidance of parasites by fish. *Parasites & Vectors*, *6*. https://doi.org/10.1186/1756-3305-6-301

Mooring, M. S., & Hart, B. L. (1992). Animal grouping for protection from parasites: Selfish herd and encounter-dilution effects. *Behaviour*, *123*(3/4), 173-193. https://doi.org/10.1111/1365-2435.14309

Nadler, L. E., McCormick, M. I., Johansen, J. L., & Domenici, P. (2021). Social familiarity improves fast-start escape performance in schooling fish. *Communications Biology*, *4*. https://doi.org/10.1038/s42003-021-02407-4

Nadler, L. E., Adamo, S. A., Hawley, D. M., & Binning, S. A. (2023). Mechanisms and consequences of infection-induced phenotypes. *Functional Ecology*, *37*(4), 796-800. https://doi.org/10.1111/1365-2435.14309

Nelson, J. A., Deegan, L., & Garritt, R. (2015). Drivers of spatial and temporal variability in estuarine food webs. *Marine Ecology Progress Series*, *533*, 67-77. https://doi.org/10.3354/meps11389

Nezhybová, V., Janac, M., Reichard, M., & Ondrackova, M. (2020). Risk-taking behaviour in African killifish- a case of parasitic manipulation? *Journal of Vertebrate Biology*, *69*(1), 1-14. https://doi.org/10.25225/jvb.20022

Parker, G. A., Ball, M. A., Chubb, J. C., Hammerschmidt, K., & Milinski, M. (2009). When should a trophically transmitted parasite manipulate its host? *The Society for the Study of Evolution*, *63*(2), 448-458. https://doi.org/10.1111/j.1558-5646.2008.00565.x

Poulin, R., & FitzGerald, G. J. (1989). Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). *Behavioral Ecology and Sociobiology*, *24*(4), 251-255. https://doi.org/10.1007/BF00295205

Sasal, P. (2003). Experimental test of the influence of the size of shoals and density of fish on parasite infections. *Coral Reefs*, *22*, 241-246. https://doi.org/10.1007/s00338-003-0313-6

Shaw, J. C., Hechinger, R. F., Lafferty, K. D., & Kuris, A. M. (2010). Ecology of the brain trematode Euhaplorchis californiensis and its host, the California killifish (*Fundulus parvipinnis*). *The Journal of Parasitology*, *96*(3), 482-490. https://doi.org/10.1645/GE-2188.1

Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H., & Øverli, Ø. (2008). Parasite manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the trematode *Euhaplorchis californiensis*. *Proceedings of the Royal Society B*, *276*(1659), 1137-1146. https://doi.org/10.1098/rspb.2008.1597

Shaw, J. C., & Øverli, O. (2012). Brain-encysting trematodes and altered monoamine activity in naturally infected killifish *Fundulus parvipinnis*. *Journal of Fish Biology*, *81*(7), 2213-2222. https://doi.org/10.1111/j.1095-8649.2012.03439.x

Siracusa, E., Boutin, S., Humphries, M. M., Gorrell, J. C., Coltman, D. W., Dantzer, B., Lane, J. E., & McAdam, A. G. (2017). Familiarity with neighbours affects intrusion risk in territorial red squirrels. *Animal Behaviour*, *31*(2), 11-20. https://doi.org/10.1016/j.cub.2020.10.072

Smith, N. F. (2001). Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia*, *127*(1), 115-122. https://doi.org/10.1007/s004420000560

Strodl, M. A. & Schausberger, P. (2012). Social familiarity modulates group living and foraging behaviour of juvenile predatory mites. *Naturwissenschaften*, *99*(4), 303-311. https://doi.org/10.1007/s00114-012-0903-7

Thomas, F., Adamo, S., & Moore, J. (2005). Parasitic manipulation: Where are we and where should we go? *Behavioural Processes*, *68*(3), 185-199. https://doi.org/10.1016/j.beproc.2004.06.010

Utne-Palm, A. C., & Hart, P. J. B. (2000). The effects of familiarity on competitive interactions between three-spined sticklebacks. *Oikos*, *91*(2), 225-232.

Versace, E., Damini, S., Caffini, M., & Stancher, G. (2018). Born to be asocial: Newly hatched tortoises avoid unfamiliar individuals. *Animal Behaviour*, *138*, 187-192. https://doi.org/10.1016/j.anbehav.2018.02.012

Vickruck, J. L., & Richards, M. H. (2017). Nestmate discrimination based on familiarity but not relatedness in eastern carpenter bees. *Behavioural Processes*, *145*, 73-80. https://doi.org/10.1016/j.beproc.2017.10.005

Ward, A., & Webster, M. (2016). *Sociality: The behaviour of group-living animals*. Springer Cham. https://doi.org/10.1007/978-3-319-28585-6

Ward, A. J. W., Axford, S., & Krause, J. (2002). Mixed-species shoaling in fish: The sensory mechanisms and costs of shoal choice. *Behavioral Ecology and Sociobiology*, *52*, 182-187. https://doi.org/10.1007/s00265-002-0505-z

Ward, A. J. W., & Hart, P. J. B. (2003). The effects of kin and familiarity on interactions between fish. *Fish and Fisheries*, *4*(4), 348-358. https://doi.org/10.1046/j.1467-2979.2003.00135.x

Weinersmith, K. L., Hanninen, A. F., Sih, A., McElreath, R., & Earley, R. L. (2016). The relationship between handling time and cortisol release rates changes as a function of brain parasite densities in California killifish *Fundulus parvipinnis*. *Journal of Fish Biology*, *88*(3), 1125-1142. https://doi.org/10.1111/jfb.12894

Weinersmith, K. L., Nadler, L. E., Bengston, E., Turner, A. V., Birda, A., Cobain, K., Dusto, J. A., Helland-Riise, S. H., Terhall, J. M., Øverli, Ø., & Hechinger, R. F. (2023). Experimental infections with *Euhaplorchis californiensis* and a small cyathocotylid increase conspicuous behaviors in California killifish (*Fundulus parvipinnis*). *Journal of Parasitology*, *109*(4), 362-376. https://doi.org/10.1645/23-35