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Are amphipods *Orchestia grillus* (Bosc, 1802) (Amphipoda: Talitridae) infected with the trematode *Levinseniella byrdi* (Heard, 1968) drawn to the light?

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ABSTRACT

A parasite can change its host's behavior in spectacular ways. When the saltmarsh amphipod *Orchestia grillus* (Bosc, 1802) is infected with the trematode *Levinseniella byrdi* (Heard, 1968) it is bright orange and is found in the open unlike uninfected individuals. I tested the hypothesis that infected amphipods are found in the open because *L. byrdi* reverses their innate photophobia. During daytime treatments and when placed in a dark chamber, 0% of the uninfected and 20% of the infected amphipods, on average, moved to the light chamber after 30 minutes. When placed in a light chamber, 91% of the uninfected and 53% of the infected amphipods, on average, went to the dark side after 30 minutes. These results clearly indicate that *O. grillus* is normally photophobic, but not drawn to light when infected with *L. byrdi*. Instead, *L. byrdi* appears to neutralize the amphipod's photophobia. Uninfected *O. grillus* are typically found under vegetation. I hypothesize that *O. grillus* with *L. byrdi* infections wander into open, unvegetated habitats randomly. In addition, 94% of infected amphipods could be touched by a finger in the field suggesting they can be easily caught by predators. *Levinseniella byrdi* infects at least three other amphipod hosts, *Chelorchestia forceps* (Smith & Heard, 2001), *Uhlorchestia spartinophila* (Bousfield & Heard, 1986), and *U. uhleri* (Shoemaker, 1930). The parasite-manipulation hypothesis suggests that the parasite-induced changes (conspicuous body color and neutralized light response) are adaptive for *L. byrdi* to make amphipod hosts more susceptible to bird predators, the definitive hosts. This hypothesis remains to be tested.

Key Words: behavior, intertidal zone, negative phototaxis, parasite-manipulation hypothesis, positive phototaxis, salt marshes, semi-terrestrial amphipods

INTRODUCTION

Parasites must solve a constant problem: getting from one host to the next. One solution is to alter the phenotype of the current host, such as its behavior, color or shape, to facilitate transmission to the next, i.e., parasite-manipulation hypothesis (Moore, 2002; Thomas *et al.*, 2005). This is especially true of multi-host parasites that must be trophically transmitted (transmission from prey hosts to predator hosts) (McCurdy *et al.*, 1999, Lagrue *et al.*, 2007, Johnson & Heard, 2017). A breathtaking example is the trematode parasite *Leucocloridium paradoxum* (Carus, 1835), which fills the eyestalks of the freshwater snail *Succinea putris* Linnaeus, 1758 with sporocysts (asexual broodsacs) that pulsate white, green, and black stripes (Wesenberg-Lund, 1931 as cited in Wesołowska & Wesołowska, 2013). When these vividly pulsating broodsacs burst from the eyestalk, they imitate crawling caterpillars and are eaten by birds, the definitive host of the trematode (Ataev *et al.*, 2016). In a less flashy, but still spectacular example, when the saltmarsh killifish, *Fundulus parvipinnis* Girard, 1854, is infected with larval trematodes, they shimmy and surface more than uninfected killifish, making them more susceptible to predation by definitive bird hosts (Lafferty & Morris, 1996).

Crustaceans are common hosts for parasites and also experience remarkable transformations when infected (Moore, 1983; Maynard, *et al.* 1998; Lagrue *et al.*, 2007; Johnson & Heard, 2017; MacKay & Moore 2021). When talitrid amphipods are infected with the trematode *Levinseniella byrdi* Heard, 1968, they turn bright orange and can be found in open patches of salt marsh (e.g., unvegetated mudbanks, footpaths) during the day. Uninfected amphipods, however, are brown/gray and hide under vegetation during the day (some venture out at night) (Bousfield & Heard, 1986; Johnson *et al.*, 2009; Johnson, 2011; Overstreet & Lotz, 2016; Johnson & Heard, 2017). This suggests that *L. byrdi* affects the body color and light response of amphipods. In addition, amphipods infected with *L. byrdi* do not appear to escape potential predators as quickly as uninfected amphipods. For instance, when I collected infected *Orchestia grillus* (Bosc, 1802) by hand, the amphipods raise their antennae as I approach, some escaping only when touched. By contrast, uninfected amphipods scatter instantly once the grass is pulled back. Collecting infected amphipods can be as easy as picking orange jellybeans from the mud, whereas collecting uninfected amphipods is like chasing rabbits through the bushes. Similarly, Bousfield & Heard (1986) observed that infected *Uhlorchestia uhleri* (Shoemaker, 1930) and *U. spartinophila* (Bousfield & Heard, 1986) moved much more slowly than uninfected animals. The parasite-manipulation hypothesis predicts that these changes in amphipod traits (body color, behavior) will facilitate transmission of *L. byrdi* from the amphipod to the bird.

Like many parasites that alter their hosts' traits, *L. byrdi* has a multi-host life cycle. Briefly, the life cycle begins when infected marsh birds, the definitive hosts, excrete feces containing *L. byrdi* eggs. Eggs are consumed by the first intermediate host, hydrobiid snails, while feeding on the sediment surface. Free-swimming larvae (cercariae) penetrate the tissues of the amphipod, encyst, and asexually developed infective metacercariae within the digestive gland (see Johnson & Heard, 2017 for more details on life cycle). After three to four weeks, when the metacercariae become fully developed and infective, the host amphipod changes from their natural brown or gray to bright orange (Fig. 1) (Johnson & Heard, 2017). The life cycle is completed when a foraging bird eats an infected amphipod and the sexual adult stage of *L. byrdi* begins producing infective eggs. *Levinseniella byrdi* is found in the intertidal salt marshes of the Atlantic and Gulf coasts of North America and is known to infect the amphipods *Chelorchestia forceps* (Smith & Heard, 2001), *U. uhleri*, *U. spartinophila*, and *O. grillus* (see Bousfield & Heard, 1986; Johnson *et al.*, 2009; Overstreet & Lotz, 2016; Johnson & Heard, 2017).

<Fig. 1>

The observation that amphipods infected with *L. byrdi* can be found out in the open in the salt marsh during the day implies that *L. byrdi* reverses the photophobia of uninfected amphipods to make them photophilic and draw them to the light. Many parasites reverse the response of their hosts to light (Bakker *et al.*, 1997; Tain *et al.*, 2006). Here, I tested the hypothesis that infected amphipods are found in the open in salt marshes because they are attracted to light. I also observed the response of amphipods infected with *L. byrdi* to a potential predator in the field.

MATERIALS AND METHODS

Response-to-light experiments

To test the hypothesis that *L. byrdi* reverses the light response of amphipods, I conducted a laboratory study with *O. grillus*. With the help of field assistants, I collected infected and uninfected amphipods by hand at low tide from a salt marsh within the Great Marsh in Ipswich, Massachusetts, USA (Great Marsh, 42°43'14"N 70°51'00"W) on 1 July 2021. We placed the amphipods in plastic containers with seawater-dampened paper towels, detritus, and algae, and kept in the field in a cooler with ice packs. Salt marshes are intertidal grasslands and *O. grillus* is a semi-terrestrial amphipod that spends most of the time on the sediment surface, even when flooded. Individuals do not burrow and rarely swim. The amphipod *U. spartinophila*, a host of *L. byrdi*, is also found in this salt marsh, but was not used in the experiment. Orange amphipods were identified as *L. byrdi*-infected and collected from exposed muddy walls that line the tidal creeks and human-dug ditches at low tide. These muddy walls are free of plants and are well-lit, though even infected amphipods avoid direct sunlight and prefer the shade. Brown amphipods were identified as uninfected and collected from under live and dead plants. At the end of the experiment, amphipods were dissected to determine their status. Because the metacercariae of *L. byrdi* are relatively large (0.5 mm diameter, on average) and found in the body cavity of *O.*

grillus, the dissections were relatively simple. Amphipods were used in experiments on the same day they were collected from the field.

Paired light and dark chambers (*N* = 10 pairs) were constructed out of cardboard boxes (20 cm × 8.5 cm × 3.2 cm (l × w × h); Fig. 2). Two boxes were joined on one side with a 4-mm opening cut across the bottom of the joined wall allowing free movement between chambers while minimizing light into the dark chamber (Fig. 2). The tops of the light chambers were cut open and plastic wrap was placed on top to prevent escape and desiccation of amphipods. The bottoms of both chambers were wetted until saturated with deionized water and rewetted when necessary. The experiments were conducted on 1 July 2021 in an open garage on a warm (26 °C), overcast day away from direct light.

<*Fig. 2*>

I had three treatments for this experiment: 1) infection level (infected, uninfected), 2) ambient light (daytime, nighttime) and 3) initial placement (dark side, light side). To maintain independence, I conducted trials with infected and uninfected amphipods separately (see Table 1 for trials conducted and predictions). I used 10 amphipods per chamber pair for a total of 100 amphipods per trial. With the help of an assistant, we recorded the proportion of amphipods found in the light chamber at 0, 5, 15, and 30 min. For the daytime treatments, trials were conducted with the garage door open, and with the garage door closed for the nighttime treatments. These treatments simulated the darkness of night while eliminating the possible confounding factors that occur at night (e.g., lower temperature, higher humidity, changes in animal activity). We used headlamps with red lights turned on only during the time of observations to monitor the chambers and record results.

<*Table 1*>

Response to a potential predator

To observe how *O. grillus* infected with *L. byrdi* would respond to a potential predator, I or another researcher slowly approached infected amphipods (identified by their bright orange color) with an index finger. If an amphipod let us touch it, we tried to stroke its body and recorded the number of strokes made before it crawled or jumped away. These amphipods were clinging to the walls of the exposed mudbanks that line the tidal creeks at low tide. Creek walls are common places to find infected amphipods and it is where I have observed birds eating them (Johnson *et al*., 2009). We approached amphipods from the top (their dorsum) or their front. We made these observations on sunny or overcast days in West Creek (42°44'14.3"N 70°50'51.1"W, 28 June and 7 July 2021) in Rowley, MA, and in Sweeney Creek (15 August 2015). We were not able to approach uninfected amphipods, which are found under the grass, because they fled as soon as the grass is lifted.

RESULTS

Levinseniella byrdi *infections*

All infected amphipods had at least one *L. byrdi* metacercaria. The mean intensity (number of metacercariae per infected amphipod) was 2.6, the median was 2, and the mode was 1 ($N = 91$ amphipods). On average, infected amphipods were 15.9 mm long. Of the amphipods identified as uninfected (i.e., brown), 3% (3/93) had 1 underdeveloped *L. byrdi* metacercaria per amphipod. On average, uninfected amphipods were 17.0 mm long.

Daytime treatments

When placed in the dark chamber, none of the uninfected amphipods went to the light side at any point during the trial (Fig. 3A). On average, 20% of the infected amphipods crawled to the light chamber after 30 min (Fig. 3A). I observed one infected amphipod crawl from the dark side, investigate most of the light side, and then turn around and crawl back to the dark side. When placed in the light chamber, an average of 91% of the uninfected amphipods went to the dark side after 30 min (Fig. 3C). Most of the uninfected amphipods fled to the dark side within seconds after being placed in the light chamber. Those that remained sought the edge and corners of the chamber, which may be a thigmotactic response of the amphipods who seek the corners and edges as refuges. When placed in the light chamber, 47% of the infected amphipods, on average, remained after 30 min (Fig. 3C). When the infected amphipods were placed in the light chamber, I noted, but did not quantify, that a few ran to the dark side within seconds, while most remained in the light chamber.

<Fig. 3>

Nighttime treatments

When placed in the dark chamber, 33% of the infected and 7% of the uninfected amphipods had gone to the light side after 30 min (Fig. 3B). When placed in the light chamber, 57% of the infected and 33% of the uninfected amphipods, on average, had gone to the dark side after 30 min (Fig. 3D). Infected amphipods moved between chambers freely, exploring and eating the damp cardboard chambers. Most of the uninfected amphipods, like the infected individuals, explored the chambers, with some eating the damp cardboard. An uninfected male engaged and then carried an uninfected female when placed on the light side in one of the chambers. Mate-carrying was also observed in the field.

Behavior response to a potential predator

Ninety-four percent (33/35) of the infected amphipods raised their antennae when a finger approached (Table 2). Seventy-seven percent (28/35) of the infected amphipods allowed a finger to touch them at least once before trying to escape. Eight percent (3/35) of the infected amphipods jumped away before a finger could touch them, only when a researcher was within < 10 cm of the amphipod.

<Table 2>

DISCUSSION

My results demonstrate that *O. grillus* is photophobic and will flee from light. *Levinseniella byrdi* does not, however, appear to reverse this behavior. Infected *O. grillus* were not drawn to light; they appeared, instead, oblivious to the light. This was clear when infected amphipods were placed in the light chamber during the daytime treatments and they crawled to the dark side and back to the light side. Because infected amphipods are not drawn to the light, yet are found in the open in the field, my results suggest that infected amphipods move from protected, vegetated habitats to exposed, unvegetated ones randomly. Similarly, insects such as crickets and grasshoppers infected by nematomorphs were once thought to “commit suicide” by jumping in water so that adult nematomorphs could emerge (Thomas *et al.*, 2002). Instead, insects infected with nematomorphs have more erratic behavior and are more likely to encounter water than uninfected insects (Thomas *et al.*, 2002). The neutral light response of amphipods with *L. byrdi* might also explain why infected individuals are also found in protected, vegetated habitats and not exclusively in open habitats (Johnson, 2011; Johnson & Heard, 2017). Alternatively, infected amphipods may be drawn into the open by some other factor not tested such as changes in humidity, temperature, or the presence of more benthic algae (one of their foods).

It is unclear how *L. byrdi* defuses the light response in amphipods, but neurological manipulation may be responsible. Serotonin is a key neuromodulator mediating behaviors in crustaceans (McPhee & Wilkens, 1989; Weiger, 1997) and has been implicated in parasite-induced changes in crustacean behavior (Maynard *et al.*, 1998; Helluy & Thomas, 2003; Guler & Ford, 2010). For instance, when the amphipod *Gammarus pulex* (Linnaeus, 1758) is infected with the acanthocephalan parasites *Pomphorhynchus laevis* Müller, 1776 or *P. tereticollis* (Rudolphi, 1809), it switches from photophobia to photophilia and has higher serotonin levels compared to uninfected animals (Tain *et al.*, 2006). Based on transcriptional analysis *O. grillus* infected with *L. byrdi*, D.M. Rand *et al*. (unpublished data) found increased expression of genes affecting "detection of stimuli," although they did not identify the specific neurological genes expressing changes. In a transcriptional analysis of 10 genes associated with serotonin production in the amphipod *Echniogammurus marinus* (Leach, 1815) infected with a trematode, Guler *et al.* (2015) found that half were upregulated and half were downregulated.

Regardless of the mechanism, does defusing the amphipod's innate photophobia benefit *L. byrdi* or is it merely a side-effect or by-product of infection? The parasite-manipulation hypothesis predicts that it is adaptive because it will enhance transmission of *L. byrdi* to the next host, in this case a definitive bird host. For this hypothesis to be supported, infected amphipods must be more likely to be eaten by bird hosts than uninfected amphipods (Cézilly *et al*., 2010). The neutral phototaxis of infected amphipods, which may randomly bring them out into the open, suggests that amphipods would be more vulnerable to bird predation. The parasite-manipulation hypothesis, however, has not been tested for *L. byrdi*.

Researchers debate the effectiveness of host manipulation by parasites on trophic transmission (e.g., Thomas *et al*. 2005; Cézilly *et al*., 2010; Perrot-Minnot *et al*., 2012), in part because the link between a single host trait and parasite transmission is difficult to demonstrate in the field. For instance, Perrot-Minnot *et al*. (2012) found that although gammarid amphipods infected with acanthocephalan parasites were more susceptible to predation by fish, photophilia, a condition of infection, alone was insufficient to make them more vulnerable to fish predation. Their results suggest that photophilia is not an adaptive trait and that some other trait or traits associated with infection is responsible for increased vulnerability to predation.

If the neutral phototaxis in amphipod hosts alone is not adaptive for *L. byrdi*, perhaps it is when combined with the amphipod's potential reduction in predator escape. I found that most infected amphipods could be touched by a finger and did not try to escape immediately. Almost all amphipods raised their second antennae when my finger approached, and were thus aware a finger was there. If my fingers were bird beaks, then they would have eaten almost all infected amphipods approached. That is, infected amphipods may be highly susceptible to predators.

Here I clearly demonstrate that *O. grillus* infected with *L. byrdi* are no longer photophobic but are not photophilic either. They have a neutral response to light. I hypothesize that infected *O. grillus* randomly wander from protected, vegetated habitats into risky, open ones as a result. It remains to be seen if changes in amphipod traits (neutral light response, conspicuous color, and potential reduction in predator escape) is adaptive for *L. byrdi* by making *O. grillus* more susceptible to predation by birds. Future work should compare infected and uninfected amphipods to investigate their susceptibility to birds, the influence of other environmental factors on their movements, and experimentally test their behavior (e.g., "boldness" *versus* "shyness").

The changed behavior of amphipods with *L. byrdi* infections has implications for saltmarsh functioning. For instance, *O. grillus* is an abundant detritivore in salt marshes (Johnson, 2011; Johnson & Heard, 2017) that eats dead marsh grass (Thompson, 1984) and can accelerate grass decomposition (Lopez *et al*., 1977). *Orchestia grillus* normally lives under the dead thatch of grass and grazes detritus. In the open habitats of the marsh, where *O. grillus* with *L. byrdi* infections can be found, however, highly productive benthic microalgae grows. *Orchestia grillus* also consumes benthic microalgae in addition to detritus (Pascal & Fleeger, 2013). Infection prevalence of *L. byrdi* in *O. grillus* can be as high as 15% in the same marshes studied (Johnson & Heard, 2017). If *L. byrdi* infection reduces detrital grazing by *O. grillus*, whether by reducing the number of amphipods through bird predation or shifting its diet from detritus to algal grazing, then *L. byrdi* may indirectly control detrital stocks, much in the way that predators can indirectly control plant biomass in a trophic cascade.

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FIGURE CAPTIONS

Figure 1. *Orchestia grillus* infected with the trematode *Levinseniella byrdi* will eventually turn from their cryptic brown or gray to a conspicuous orange. Images of amphipods from the Great Marsh in northeast Massachusetts, USA.

Figure 2. Set-up of light and dark chambers.

Figure 3. Response of uninfected (grey circles) and infected (orange triangles) amphipods to light when started in the dark chamber in daytime treatment (**A**), dark chamber in the nighttime treatment (**B**), light chamber in daytime treatment (**C**), and light chamber in the nighttime treatments (**D**). Sun represents daytime treatments, moon and stars nighttime treatments. Bold symbols represent mean values; faded symbols are raw data, $N = 10$ per treatment. Raw data that overlapped and were > 0% were jittered 2%–4%.

Figure 1

Figure 2

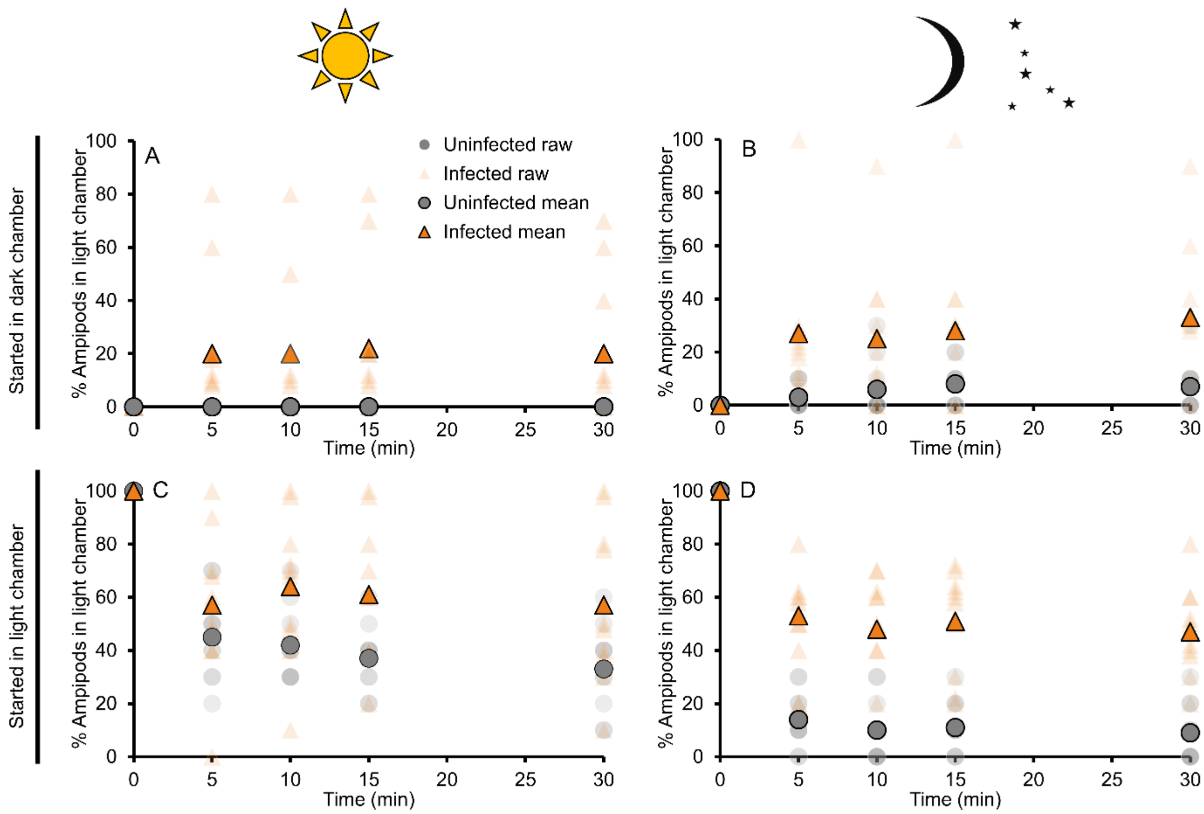
Started in dark chamber
Started in light chamber
% Ampipods in light chamber
A
B
C
D
Uninfected raw
Infected raw
Uninfected mean
Infected mean
Time (min)
0
5
10
15
20
25
30
0
20
40
60
80
100

Figure 3

Table 1. Trials and predictions.

	Sunlight	**Dark**
Infected on light side	Remain on light side	Randomly distributed
Uninfected on light side	Flee to dark side	Randomly distributed
Infected on dark side	Go to light side	Randomly distributed
Uninfected on dark side	Remain on dark side	Randomly distributed

Table 2. Response of *Orchestia grillus* infected with *Levinseniella byrdi* to the approach of an index finger. Numbers in parentheses are the number of times behaviors were observed.

Approached from the top
Raised antennae, allowed finger to stroke back 1–5 times before crawling away (5)
Raised antennae, allowed finger to stroke back once or twice before jumping off the mudbank into the water and swimming back to the mudbank (4)
Raised antennae, jumped into water before finger could get within 2 cm (2)
Did not raise antennae, jumped off wall as soon as finger touched back (1)
Did not raise antennae, jumped off creek wall when finger was < 10 cm away (2)
Approached from the front
Raised antennae, palpated or touched finger with secondary antennae, allowed finger to stroke head and body 1–4 times before crawling away (16)
Raised antennae, palpated or touched finger with secondary antennae, crawled or jumped away (3)
Raised antennae, allowed finger to stroke its back, rolled over on its side allowing finger to stroke side before crawling away (1)
Raised antennae, allowing finger to stroke head and body, crawled onto finger (1)