How a trematode parasite (*Microphallus* Ward, 1901) impacts the grazing behavior of an aquatic keystone species, the rusty crayfish *Faxonius rusticus* Girard, 1852 (Decapoda: Decapoda: Astacidea: Cambaridae)

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ABSTRACT

Parasites can alter a wide range of host behaviors resulting in changes in organismal interactions and ecosystem processes. One of the most important behaviors that controls food web dynamics is herbivore grazing because an alteration in grazing behavior leads to changes in trophic dynamics and ecosystem processes by changing the abundance and diversity of primary producers. To test whether parasite load can alter host grazing levels and choices, feeding trials were conducted using the keystone species, the rusty crayfish *Faxonius rusticus* (Girard, 1852), grazing on a selection of macrophyte species. We used a total of 165 wild-caught, naturally-infected crayfish individuals with a wide range of parasite loads by species of the digenetic trematode *Microphallus* Ward, 1901. Crayfish were presented with 1 g each of the macrophytes *Elodea canadensis* (Michaux), *Ceratophyllum demersum* (L), *Chara* sp., and *Potamogeton richardsonii* (A. Benn.) in a 23-hr foraging assay. Subsequently, crayfish were dissected, and parasite loads were calculated. Mixed models were then utilized to determine how parasite load affected consumption. As infection of *Microphallus* increased in the crayfish hepatopancreas, consumption of all four macrophytes significantly decreased. Melanization of *Microphallus* spp. within the hepatopancreas, the immune response to infection, did not significantly reduce crayfish macrophyte consumption. These results indicate that macrophyte consumption in the crayfish was affected by *Microphallus*.

This impact on crayfish grazing could alter macrophyte abundances in aquatic ecosystems. Because of the many ecosystem functions macrophytes play, an alteration in their abundances could lead to community-level ramifications by impacting nutrient flow and organismal abundances in aquatic ecosystems.

Key Words: community ecology, Crustacea, freshwater ecology, host-parasite relationships, parasitism, Trematoda

INTRODUCTION

Parasites have been shown to alter a variety of host behaviors, including daily and seasonal migrations (Barber et al., 2000; Johnson et al., 2018), predator avoidance (Medoc et al., 2009; Friesen et al., 2019), escape behaviors (Adamo, 2002; Medoc & Beisel, 2008), reproduction (Adamo, 2002), burrowing (Babirat et al., 2004), feeding behaviors (Wood et al., 2007), and nest building (Chapuisat et al., 2007). For example, the amphipod *Gammarus pulex* (Linnaeus, 1758) shows an increase in reactivity to light, with individuals infected by an acanthocephalan parasite being more likely to move towards light than uninfected *G. pulex* (Bauer et al., 2000). Labaude et al. (2020) found that parasite load and temperature also significantly interacted to decrease activity in *G. pulex*. Through altering a diverse range of behaviors, parasite presence supersedes the host-parasite level and change many
other organismal interactions within an ecosystem involving the host species and other species not directly infected by the parasite (Lafferty, 2017).

Most of the behavioral changes induced by parasites benefit the parasite, either through increasing the probability of the host being infected by additional parasites of the same species or of the host being consumed by the parasite’s next host in the life cycle (Buck, 2019). Parasites have either a direct life-cycle, where an individual can infect the same host species, or a complex life cycle, where there are multiple host species required for a parasite to fulfill its life cycle (Poulin, 1999). Many behavioral manipulations are seen in parasites with complex life cycles. Killifish Fundulus parvipinmis (Girard, 1854) infected with the trematode Euhaplorchis californiensis (Martin, 1950) displayed more conspicuous behaviors, such as turning over and flashing their sides, compared to uninfected individuals (Lafferty & Morris, 1996). This alteration in behavior led to birds consuming more infected killifish than uninfected fish, which benefits the trophically-transmitted trematode (Lafferty & Morris, 1996). Similarly, when G. pulex is infected with the acanthocephalan Porphophychnus tricollis (Rudolphi, 1809), the amphipod is attracted to predatory odor instead of repelled by the odor as uninfected G. pulex are (Perrot-Minot, 2007). Thus, through altering the behavior of their hosts, the trematode parasite indirectly altered food web dynamics within this aquatic community. Behavioral changes, even within a single host species, thus alter the ecosystem (Gordon, 2011).

As parasites can alter host behavior, the surrounding habitats can be impacted through direct and indirect effects of the parasite-mediated behavioral alterations (Poulin, 2010). The trematode Cartatelia australis Allison, 2021, which infects cockles Austrovenus stutchburyi (Wood, 1828), decreases the hosts’ ability to burrow into sediment, which causes an increase in species richness and density of benthic macroinvertebrates of the surrounding substrate (Mouritsen & Poulin, 2005), a change resulting from a shift in hydrodynamics between the substrate and the water. Similarly, as parasitism in the herbivorous mud snail Hydrobia ulvae (Pennant, 1777) increased, snail activity decreased, which indirectly caused surrounding Simpson’s evenness and diversity of benthic metazoans to increase, though both peaked at intermediate parasite loads (Mouritsen & Haun, 2005). Nutrient cycling within an ecosystem can also be impacted by parasite-mediated behavioral changes (Vannatta & Michella, 2011). Parasite infection of the amphipod Gammarus duebeni var. celicus (Lågeborg, 1852) increased rates of bioturbation of G. duebeni, which shifted the amphipod’s role as an ecosystem engineer (Williams et al., 2019). While parasites alter many host behaviors, parasitic-induced changes in foraging can impact the diversity and abundance of primary producers (Bernot & Lambert, 2008).

Feeding behavior has been altered by parasites in a number of different animal hosts (Hutchings et al., 2003). Gammarus pulex infected with the acanthocephalan Echinacynanchus truttae (Schrank, 1788) consumed significantly more prey than non-parasitized individuals (Fielding et al., 2003). In addition to altering the amount of prey consumed, parasites can also alter feeding preferences, such as when individuals of the three-spined stickleback Gasterosteus aculeatus (Linnaeus, 1758) are infected with both a cestode and a aculeatus Gasterosteus such as when individuals of the three-spined stickleback of prey consumed, parasites can also alter feeding preferences, 1788) consumed significantly more prey than non-parasitized in-

Echinorhynchus truttae (Schrank, 1788) infected fish, which benefits the trophically-transmitted trematode. Infection with Microphallus has been shown to impact host feeding behavior, such as when infected E. rusticus showed an increase in macrophyte consumption, especially when a predatory fish was present, but crayfish consumption of benthic macroinvertebrates was unaffected (Reisinger & Lodge, 2016). Although Reisinger & Lodge (2016) found an increase in macrophyte consumption, they did not analyze how crayfish consumption differed between different macrophytes. Cronin et al. (2002) found that another crayfish, Procambarus starkii (Girard, 1852), has feeding preferences when parasite load is not considered, so determining if feeding preferences in crayfishes are altered by parasites will help determine how parasites alter the ecological niche of crayfish. Crayfish experimentally infected with Microphallus, however, have shown reduced growth and reduced consumption of earthworms (Sargent et al., 2014). This kind of decrease in feeding behavior is commonly seen in infected hosts (Kyriazakis et al., 1998). To combat the effects of Microphallus infection, the immune responses of crayfishes include melanization, during which melanin is deposited around each of the metacercaria stage of the parasite within the hepatopancreas of the crayfish to isolate the metacercariae from any host nutrients (Cerenius & Soderhall, 2004; de Roode et al., 2013). Melanization requires high levels of phenol, which is obtained through consumption of plants high in phenolic secondary metabolites (Korkut et al., 2018). The immune response of crayfishes to parasites might, therefore, impact their feeding habits. We conducted feeding trials measuring consumption of each macrophyte to determine how infection of Microphallus and the associated immune response alters crayfish grazing of different macrophytes. We hypothesized that increasing parasite loads in a crayfish will result in decreased consumption of macrophytes. This hypothesis is counter to the finding of Reisinger & Lodge (2016) but is supported by Sargent et al. (2014), which studied the same model system, and many other examples of decreased feeding with increasing parasite load in a range of host species (Danzer et al., 2008; Hawley & Altizer, 2011; Prior et al., 2019). A common host response is anorexia, a decrease in food consumption as a common behavior across taxa, as it conserve energy used in digestion and divert it to an immune response (Dernas & Nelson, 2011). A decrease in the consumption of macrophytes should vary across macrophyte species, as different macrophytes offer different nutritional values (Barko et al., 1991).

MATERIALS AND METHODS

Experimental design

To test whether parasite load or the immune response of melanization impacts macrophyte consumption, individuals of...
**TREMATODE ALTERS CRAYFISH GRAZING BEHAVIOR**

*Faxonius rusticus* were placed in a 23-hr foraging assay and provided with the choice of four different macrophyte species to consume. After the consumption assay, the parasite (*Microphallus*) load was determined through digital photo analysis of the hepatopancreas. We quantified the percentage of the total hepatopancreas that was parasitized (non-melanized metacercariae) as well as percentage of the hepatopancreas that consisted of melanized metacercariae.

**Sample sites and crayfish collection**

Crayfish were collected from 11 sites in northern Michigan (Table 1) using modified minnow traps for all sample sites except for Burt Lake (Fig. 1). The opening on the traps was enlarged to allow crayfish access. Each trap was baited with ~5 g of canned sardines in oil that were placed in a mesh bag. Traps were left at sites for at least 24 hr and checked daily. Upon capture, crayfish were removed from the traps and placed in a 1 l plastic container, which was then placed in a cooler for transportation to the University of Michigan Stream Research Facility (Pellston, MI). Crayfish collected from the same sample location were transported in the same deli container to maintain records of location of capture. Burt Lake sampling was performed in Maple Bay State Park along the shore using hand nets. A total of 196 rusty crayfish were collected but only 165 were infected with *Microphallus* and used for analyses.

Crayfish from Burt Lake (*N = 98*) were housed in a community tank, which was a galvanized steel horse trough (237.5 cm long × 86.4 cm wide × 60.1 cm high), and crayfish from the other 10 sites (*N = 67*) were kept isolated in separate plastic containers (0.5 l) placed in a flow-through stream. Both the stream and community trough were fed with unfiltered water from the east branch of the Maple River. The crayfish were able to eat naturally occurring detritus that came into the artificial streams with the river water. There was enough detritus for the crayfish to feed *ad libitum*.

All crayfish collected were identified as *F. rusticus*. Post-orbital carapace length was measured for each crayfish using calipers. Carapace length ranged 1.3–4.0 cm, and chelae length 1.0–5.7 cm. Out of 165 crayfish individuals, 44 were female and 121 males. No female crayfish were berried during the feeding trials.

**Macrophyte collection and holding**

Four species of macrophytes were used for the feeding trials: *Elodea canadensis* (Michx.), *Ceratophyllum demersum* (L.), *Chara sp.* (L.) and *Potamogeton richardsonii* (A. Benn.). Macrophytes were collected in North Fishtail Bay, Douglas Lake (Pellston, MI) (45°34′33.04″N, 84°39′42.12″W). All macrophytes were collected using a homemade plant rake consisting of two (34 cm long), 14-tined rakes tied together with nylon rope (2.5 cm diameter), such that the prongs of each rake were facing out. Plants were always collected from the same location to decrease chemical variation in different sampling sites (see Janauer, 1981). The plants were placed in a 19 l bucket for transport to the Stream Laboratory, where they were sorted by species, and each species placed in an individual artificial stream (162 cm × 40.6 cm × 40.6 cm) lined with 0.1 mm thick polyethylene plastic sheeting. The streams were exposed to natural sunlight and weather conditions, and the water from the Maple River was filtered through nylon 0.1 cm² mesh.

**Experimental set up**

Eight artificial streams (40.6 cm × 40.6 cm × 20.3 cm) were constructed using cinder blocks (20.3 cm × 40.6 cm × 20.3 cm) and lined with 0.1 mm thick polyethylene plastic sheets. Each stream was fed with water from the Maple River, which was pumped from the river to the streams using PVC pipes. The water was first filtered through two pairs of nylon tights then entered into a 55 l

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**Table 1.** Collection sites, average parasite load (mean ± SEM), parasite load range, and average carapace length (mean ± SEM) of *Faxonius rusticus* individuals. Parasite load is defined as percent of the host hepatopancreas that is infected with metacercariae of *Microphallus* spp.

<table>
<thead>
<tr>
<th>Water body</th>
<th>Latitude, Longitude</th>
<th>N</th>
<th>Average parasite load (%)</th>
<th>Parasite load range (%)</th>
<th>Average carapace length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burt Lake</td>
<td>45°29′14.28″N, 84°42′3.4″W</td>
<td>98</td>
<td>8.83 ± 0.106</td>
<td>0.00–48.74</td>
<td>3.15 ± 0.004</td>
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<tr>
<td></td>
<td>(85, 13)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Boyne River</td>
<td>45°11′47.76″N, 84°57′25.92″W</td>
<td>27</td>
<td>0.92 ± 0.028</td>
<td>0.03–2.94</td>
<td>2.60 ± 0.014</td>
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<tr>
<td></td>
<td>(16, 11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bear River</td>
<td>45°20′2.04″N, 84°55′44.04″W</td>
<td>14</td>
<td>0.84 ± 0.041</td>
<td>0.25–1.94</td>
<td>2.50 ± 0.022</td>
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<tr>
<td></td>
<td>(6, 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Black River</td>
<td>45°21′51.48″N, 84°17′53.88″W</td>
<td>7</td>
<td>0.23 ± 0.047</td>
<td>0.01–0.88</td>
<td>1.69 ± 0.040</td>
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<td></td>
<td>(4, 3)</td>
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<tr>
<td>Bear River</td>
<td>45°21′32.04″N, 84°57′24.12″W</td>
<td>7</td>
<td>0.74 ± 0.111</td>
<td>0.05–2.40</td>
<td>2.37 ± 0.102</td>
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<tr>
<td></td>
<td>(4, 3)</td>
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</tr>
<tr>
<td>Carp River</td>
<td>45°40′48″N, 84°48′49.68″W</td>
<td>3</td>
<td>0.01 ± 0.004</td>
<td>0.00–0.02</td>
<td>2.97 ± 0.004</td>
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<td></td>
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<tr>
<td>Stover Creek</td>
<td>45°18′2.88″N, 85°14′59.64″W</td>
<td>3</td>
<td>0.18 ± 0.094</td>
<td>0.00–0.50</td>
<td>2.90 ± 0.203</td>
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<tr>
<td></td>
<td>(3, 0)</td>
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</tr>
<tr>
<td>Carp River</td>
<td>45°41′3.48″N, 84°46′52.32″W</td>
<td>2</td>
<td>0.28 ± 0.195</td>
<td>0.00–0.55</td>
<td>2.75 ± 0.247</td>
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<tr>
<td>Upper Black River</td>
<td>45°27′2.16″N, 84°20′13.2″W</td>
<td>2</td>
<td>0.16 ± 0.001</td>
<td>0.16–0.16</td>
<td>1.80 ± 0.071</td>
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<td></td>
<td>(0, 2)</td>
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<tr>
<td>Upper Black River</td>
<td>45°23′33.36″N, 84°20′2.04″E</td>
<td>1</td>
<td>1.46</td>
<td>1.46</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>(0, 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Branch</td>
<td>45°32′46.68″N, 84°46′34.68″W</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.8</td>
</tr>
<tr>
<td>Maple River</td>
<td>45°32′46.68″N, 84°46′34.68″W</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>165</td>
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</tbody>
</table>
head tank. The tank had eight hoses attached to the bottom, with each hose leading into one of the eight streams. The water flowed through the streams at approximately 0.1 ± 0.05 l sec⁻¹.

Feeding trials

Each of the macrophyte samples had excess water removed by being spun in a salad spinner (Farberware® Basics, Farberware, Fairfield, CA, USA) for 20 rotations. Stems were weighed to the nearest hundredth of a gram on an Ohaus® (scout scale (model #H-5851; Ohaus, Parsippany, NJ, USA. Approximately 1 g (1.04 ± 0.03 g) (mean ± SEM) of each macrophyte was attached in each stream to a 24.8 cm × 0.6 cm glass stirring rod using 26-gauge green-painted floral wire. The rod, wire, and macrophyte was then attached to a 25 cm × 3 cm piece of hardware cloth to ensure that the plants did not float away after partial consumption by the crayfish (see Wood et al., 2018). Each macrophyte grate had only one species of macrophyte affixed to the glass rod. These macrophytes were then haphazardly placed in the streams, such that each stream had all four macrophytes, by picking up the macrophyte grates in no order and gently placing all four grates at once in the stream, so that the macrophytes were in different locations around the stream during every feeding trial.

We attached a tether to the carapace of each crayfish to ensure that they remained in the streams for the entire duration of the feeding trials. Tethers consisted of 30 cm of fishing monofilament (0.5 mm diameter; 13.6 kg test strength) anchored with 3.5 × 5.0 cm tiles. The monofilament was attached to the tiles with a swivel to ensure the crayfish could move around the stream and reach all macrophytes without getting tangled. At one end of the monofilament, we tied a 1 cm² piece of loop-sided VELCRO® (Velcro Manchester, NH, USA). The hook-sided piece of VELCRO® was glued (using Gorilla Glue®; Gorilla Glue Co., Sharonville, OH, USA) to the carapace of the crayfish and attached to the loop-sided VELCRO®. The tile and a single crayfish were placed into the center of a stream after the plants, so the crayfish were 20.3 cm from the stream wall on all sides. Trials ran for 23 hr, starting at 1030 and ending at 0930. The streams were wiped clean between trials to prevent buildup of algae. Trials ran June–August 2019, with crayfish exposed to natural light-dark cycles and temperature fluctuations. After each trial, the stems were removed from the glass rods, spun in a salad spinner 20 times, and weighed again to give a post-trial weight.

Crayfish dissections

Crayfish were dissected and the hepatopancreas removed to determine the parasite load of *Microphallus*. Crayfish were removed from the stream and placed in the freezer at the end of the feeding trials, and frozen for 1 hr before dissection. The carapace of each crayfish was cut vertically in half with dissecting scissors and separated. The hepatopancreas was removed using forceps and placed on a 5 × 7.5 cm glass slide. Another 5 × 7.5 cm slide was placed on top of the first, compressing the hepatopancreas between the two slides, which were held together using wooden, 7.5 cm clothes pins.
**Parasite-load quantification**

Parasite load for each crayfish was quantified using a digital photo analysis, similar to the method described in MacKay & Moore (2021). Photos of the entire hepatopancreas were taken using an OT-V1 digital USB microscope (Opti-TekScope™, Chandler, AZ, USA) and stored as jpgs. Digital images were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA). Cropped photos had their brightness, saturation, and color altered such that the metacercariae of Microphallus were in colors different from the surrounding tissue of the hepatopancreas. Due to variation in hepatopancreas colors, saturation and color alterations varied slightly between samples. The images were altered so that the metacercariae were noticeably brighter than the rest of the photo. Before alteration, the hepatopancreas ranged from dark teal to bright orange, with most being a shade of mid-tone green. The metacercariae showed as white ovals. Melanized metacercariae were brown or black before processing. Once the metacercariae stood out from the rest of the hepatopancreas, the image color was changed to black and white such that the black area represented the metacercariae and the white was hepatopancreas using the binary function of Image J. The total number of black pixels (metacercariae) and total pixels (total hepatopancreas) were recorded. This process was repeated to determine the amount of melanization of the hepatopancreas, although melanization showed up as white pixels and the rest of the hepatopancreas was black pixels after photo processing.

Overstreet (2011) reported that five species of Microphallus could infect F. rusticus and that these species cannot be differentiated without genetic analyses. All five species develop metacercariae in the hepatopancreas, so the behavioral impacts of these trematodes are most probably similar. Other studies (Sargent et al., 2014; Reisinger et al., 2015; Reisinger & Lodge, 2016; MacKay & Moore, 2021) that examined potential behavioral changes of crayfish did not identify the parasite to species level. We did not genetically test metacercariae to determine what species of Microphallus infected the crayfish.

**Data analysis**

Percent consumption of each macrophyte for each feeding trial was determined by subtracting the post-trial weight from the pre-trial weight, dividing this difference by the pre-trial weight, and multiplying by 100 to determine a percentage (adapted from Frantzis & Gremare, 1992; Wood et al., 2018); (before – after)/before × 100 = consumption. Our procedure allowed for the control of subtle differences in the weights of the macrophytes in the trial. Macrophytes grow rapidly, especially when in direct sunlight (Spencer, 1986; Nielsen & Sand-Jensen, 1991; Ozimek et al., 1993). For the purposes of our study, “consumption” was defined as the net difference between crayfish consumption and macrophyte growth.

To calculate parasite load, the number of pixels that contained the metacercariae was divided by the number pixels of the total hepatopancreas and multiplied by 100. These calculations were performed in Microsoft® Excel® (Microsoft, Redmond, WA, USA). The same method was used to determine the percent of the hepatopancreas that was melanized. Only crayfish that were infected, meaning they had greater than 0% parasite load and/or melanization after photo analysis, were statistically analyzed (N = 163). A linear model using the “lm” function in R was run to determine potential crayfish size effects on consumption (Bates et al., 2020). All analyses were conducted in R Core Team, 2017. There was not a significant effect of size on consumption of macrophytes (F_{1,495} = 0.96, P = 0.323) when only infected crayfish were analyzed, so crayfish carapace length, the measurement used for size, was not included in any models.

To determine if parasite load impacted crayfish consumption, a linear mixed model was run in R using the “lmer” function (Bates et al., 2015). Only parasitized crayfish (N = 163) were included in this model because the non-infected individuals might mask the impact of increasing parasite load on crayfish consumption. An ANOVA test found that crayfish parasite load was significantly impacted by site, making these two independent variables confounded (F < 0.001), and so parasite load could not be analyzed as an independent variable in this model. The dependent factor of the parasite-load model was thus consumption, with percent parasite load, species of macrophyte, and sex as independent, fixed factors. The interactions of parasite load, sex, and macrophyte were also run in the mixed model. Each crayfish used in the trial was given an identification number. Within the model, the individual crayfish identifier was a nested random factor within experimental stream number. This random factor was added to account for any differences in hunger or consumption states of individual crayfish and for potential differences in each of the eight streams. Crayfish identification number was nested within stream number because feeding trials of each crayfish took place in a specific stream. Effect sizes of each variable were determined using the command “effectsize.”

For the melanization model, consumption was the dependent factor with melanization, macrophyte species, and sex as the independent factors. The interactions of the three dependent factors were also tested in the model. Individual crayfish number nested in stream number was the random factor. Effect sizes were also determined for each variable in the melanization model using the command “effectsize.” Because two models were conducted on the same consumption data, a Bonferroni correction was performed, making the alpha-value 0.025 instead of the standard 0.05 (Morgan, 2007).

To determine if crayfish size significantly affected parasite load, a linear model was run using the command “lm” comparing crayfish parasite load with crayfish carapace length. The effect size of these size effects were also tested using the command “effectsize.”

**RESULTS**

**Parasite load**

Increasing parasite load significantly decreased consumption (F_{1,165} = 9.45, P = 2.47 x 10^{-11} ) (Fig 2, Table 2). Parasite load had a medium, negative effect on consumption (d = -0.36). Sex also significantly impacted consumption, with females consuming less macrophytes than males (F_{1,165} = 6.39, P = 0.011), and crayfish sex had a small, negative effect (d = -0.23). Consumption was also significantly impacted by macrophyte species, with crayfish consuming more Chara sp. than any other species (F_{1,165} = 28.62, P = 2.20 x 10^{-16} ). The interaction of parasite load and crayfish sex was not significant (F_{1,165} = 2.87, P = 0.092). Parasite load and macrophyte species did not interact significantly (F_{1,165} = 0.42, P = 0.737), and neither did crayfish sex and macrophyte species (F_{1,165} = 0.971, P = 0.406). The interaction effect between parasite load, sex, and macrophyte species was similarly insignificant (F_{1,165} = 1.16, P = 0.324). Centropylphium demersum, E. canadensis, and P. richardsoni all had high, negative effects on consumption, indicating these species were consumed much less than Chara sp. (d = -0.82, -0.88, -0.73, respectively).

**Melanization**

Consumption was not significantly impacted by crayfish melanization (F_{1,165} = 3.17, P = 0.077), although there was a medium negative effect (d = -0.51) (Fig 3, Table 2). Like the parasite load model, crayfish sex and macrophyte species significantly impacted consumption (F_{1,165} = 7.93, P = 0.005; F_{1,165} = 33.92, P = 2.20 x 10^{-16} , respectively). Sex had a medium, negative effect
size ($d = -0.36$). The macrophyte species had a large, negative effect on consumption ($d = -0.84$, -0.94, and -0.81 for $C.~demersum$, $E.~canadensis$, and $P.~richardsonii$, respectively). Melanization and sex did not significantly interact ($F_{1,165,0.05} = 2.03$, $P = 0.156$), nor did melanization and macrophyte species ($F_{3,495,0.05} = 0.978$, $P = 0.402$). Additionally, crayfish sex and macrophyte species did not significantly interact ($F_{3,495,0.05} = 0.48$, $P = 0.699$). The three independent factors of sex, macrophyte species, and melanization did not significantly interact ($F_{3,495,0.05} = 0.38$, $P = 0.764$).

Effects of size

Crayfish carapace length significantly affected parasite load ($F = 31.02$, $P = 8.68 \times 10^{-8}$) with a medium positive effect ($d = 0.38$).

DISCUSSION

Our results provided evidence that consumption of macrophytes by $F.~rusticus$ significantly decreased with increasing parasite load of Microphallus. Both parasite load and melanization had negative, medium effects on consumption, though the effect of melanization was not significant. Crayfish sex also significantly impacted consumption in both parasite load and melanization models, with females consuming less macrophytes than males. Species of macrophyte also significantly affected consumption because $Chara$ sp. was consumed more often than the other three species. Crayfish parasite load and sites are comingled in this study, which is to be expected as naturally-occurring infection levels of parasites are typically affected by site of capture (Sargent et al., 2014). We therefore recognize the possibility that any differences in crayfish consumption is actually due to differences in site and not in parasite load. Previous work in the same area (MacKay & Moore, 2021) found no site effects in $F.~rusticus$ behavioral traits. Although we sampled 11 sites, these 11sample sites occur within two adjacent watersheds (https://www.michigan.gov/documents/deq/wrd-mi-watersheds_559937_7.pdf). Many of the sites are connected by water flow, so site effects are even more unlikely.

Our results show that $F.~rusticus$ decrease their consumption of macrophytes with increasing parasite load, a finding that has been shown in other model systems. $Littorina~littorea$ (Linnaeus, 1758) snails infected with the trematode Cryptocotyle lingua (Creplin, 1825) consumed less macroalgae than uninfected individuals (Wood et al., 2007). Parasitized $G.~pulex$ nevertheless consumed more isopods than non-parasitized conspecifics (Dick et al., 2010), so that parasite load can either increase or decrease host feeding, depending on the context and perhaps of taxonomic group. Although we showed no significant interaction between parasite load and macrophyte type and thus no significant change in feeding preference, parasites in other studies have been shown to alter feeding preferences of infected individuals. Parasitized wooly caterpillars consume more toxic plant secondary metabolites than non-parasitized caterpillars, allowing the parasitized caterpillars to resist further infection by tachinid flies (Singer et al., 2009). Similarly, parasitized larvae of fruit flies ($Drosophila~melanogaster$ (Meigen, 1830)) preferentially consumed yeast to inhibit the ability of egg encapsulation by a wasp parasitoid (Anagnostou et al., 2010). Sticklebacks infected with the larva of the cestode Schistocephalus (Müller, 1776) also showed a change in feeding preferences, with infected fish consistently consuming lower-quality prey items than uninfected individuals (Barber et al., 2000). Since increasing parasite loads decrease crayfish consumption of

Table 2. A summary of the results of the linear mixed models conducted to determine how consumption was affected by the sex of $Faxonium~rusticus$, parasite load, melanization, and macrophyte species. Crayfish parasite load and melanization were run in two separate models. Although all interactions were run in the models, no interactions for either model were significant, so they are left off of the table.

<table>
<thead>
<tr>
<th>Model</th>
<th>Main effects</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite Load * Sex * Macrophyte</td>
<td>Parasite load, Sex</td>
<td>0.02, 0.011</td>
</tr>
<tr>
<td></td>
<td>Macrophyte</td>
<td>2.2 x 10^-16</td>
</tr>
<tr>
<td>Melanization * Sex * Macrophyte</td>
<td>Melanization, Sex</td>
<td>0.077, 0.005</td>
</tr>
<tr>
<td></td>
<td>Macrophyte</td>
<td>2.2 x 10^-16</td>
</tr>
</tbody>
</table>

Figure 2. Effect of parasite load on macrophyte consumption. Due to significant sex effects ($P = 0.011$), males and females are graphed separately. Number of females = 44 and number of males = 121. Shaded areas represent the 95% confidence interval.
TREMATODE ALTERS CRAYFISH GRAZING BEHAVIOR

Figure 3. Effect of melanization on macrophyte consumption. Due to significant sex effects (P = 0.005), males and females are graphed separately. Number of females = 44 and number of males = 121. Shaded areas represent the 95% confidence interval.

Adult crayfishes are voracious grazers, and their grazing pressure is a key factor in structuring aquatic macrophyte communities (Chambers et al., 1990; Parkyn et al. 1997; Carreira et al., 2013). Crayfishes have been defined as both keystone species and ecological engineers in aquatic habitats because of the intensity of their grazing pressure (Reynolds et al., 2013). A crayfish population of Faxonius virilis (Hagen, 1870) was inversely related to submerged macrophyte biomass and turbidity and directly related to conductivity, such that macrophyte biomass and turbidity decreased with increasing crayfish population and conductivity (Roessink et al., 2017). As rusty crayfish became established in Wisconsin, USA, submerged macrophyte richness declined (Wilson et al., 2004). Because of their disproportionately large impact on macrophyte abundance, crayfishes are keystones species in freshwater ecosystems. By decreasing the amount of macrophytes consumed by F. rusticus, species of Microphyllus could be indirectly minimizing the ecological role of this crayfish as a keystone species. The impact of F. rusticus on macrophyte biomass could be altered as consumption of macrophytes significantly decreased with increasing parasite load.

Our results indicated that in addition to a decrease in macrophyte consumption with increased crayfish parasite load, macrophyte consumption is also impacted by crayfish sex and the macrophyte species (Fig 2). In addition, female crayfish consumption of macrophytes was less than male consumption (Fig 3). These results disagree with the Hamilton-Zuk hypothesis, which predicts that parasites have a higher impact on males than females (Zuk, 2009). Faxonius rusticus typically does not reproduce during the summer months (Martin & Moore, 2007, 2010), so the additional energetic load females have for reproduction would not come into account, which could explain our results of these study are counter to the Hamilton-Zuk hypothesis. Since crayfish sex and levels of parasitism both independently influence crayfish grazing, habitats that have differing parasite loads and/or sex ratios would have differential grazing pressure on macrophyte beds, even though the interaction between sex and parasite load was not significant. Many more male crayfish were collected than female crayfish at the various sites, which could artificially inflate the sex differences in our results. Due to the high sample size of both males and females (N = 121 and 45, respectively), however, the sex effects should still be representative of the populations. At one end of the spectrum, habitats with a male-skewed population and reduced parasite loads would have higher grazing pressure on Chara sp. beds, female-skewed populations that are heavily parasitized would exact less grazing pressure on the macrophyte. At both ends of the spectrum, grazing pressures for the other macrophytes would be less severe than those seen in Chara sp. As a result of these differential grazing pressures, the relative abundances of the four macrophytes would be significantly impacted, albeit indirectly, by parasite load and crayfish sex ratio. Because both the parasite load and the sex of F. rusticus populations can independently alter the grazing of the crayfish, these changes in crayfish grazing can have community-level ramifications due to the many ecological roles of macrophytes in freshwater communities.

Altering the relative abundances of macrophytes could impact the community (Madsen et al., 2001; Douglas & O’Connor, 2003; Thomaz & da Cunha, 2010). Chara sp. plants grow in dense mats and, thus, aid in sediment stability (Kufel & Kufel, 2002). Conversely, P. richardsonii stems grow vertically and provide essential nursery habitats for larval fish (Bogut et al., 2007). Faxonius rusticus individuals infected with Microphyllus consumed more Chara sp. than P. richardsonii, so that the relative abundance of these macrophytes can be affected by crayfish grazing. This alteration in macrophyte relative abundance could, in turn, affect turbidity and water flow in the aquatic environment, impacting other biota (Champion & Tanner, 2000). Macrophytes, and their associated biofilms, are able to absorb nitrogen, making it bioavailable for other fauna in the community (Levi et al., 2015). Macrophytes also provide homes to benthic micro and macroinvertebrates, which are a key part of aquatic food webs (Humphries, 1996; van den Berg et al., 1997). Consumption of ecologically important macrophytes decreases with increasing parasites, which can alter nutrient cycling and survivorship of many aquatic organisms.

Our study illustrates the potential for community-level ecological impacts of parasite-manipulated host behavior as has...
been seen in other studies (Buck, 2019). As parasites manipulate host behavior, the ecological niche of the host can be altered in turn, leading to larger-scale ecological ramifications.

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