

Impact of Agriculture on *Echinostoma* Infection in Ramshorn Snails (*Planorbella corneus*) of Crawford County

By
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April, 26 2023



Allegheny College
Department of Biology

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**A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College**

*I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code
and to maintain the integrity of both myself and the College community as a whole.*

Pledge

Approval by the Senior Thesis Committee:

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Acknowledgements

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Abstract

The widespread use of agrochemicals around the world can lead to many unintended impacts on the environment. In aquatic systems, nutrient inputs caused by agrochemical runoff can lead to increases in primary production, causing a cascade of changes that can impact community level interactions as well as disease and transmission dynamics. Snail-trematode infections, which are present around the world, are one type of aquatic disease system with the potential to be affected by anthropogenic nutrient additions. Snail-trematode diseases have the potential to infect and harm several organisms including amphibians, rodents, birds, and humans. Two such disease systems present in Crawford County are *Echinostoma* spp. (an intestinal fluke) and *Ribeiroia ondatrae* (a trematode parasite known for causing limb malformations in amphibians). This experiment was designed to examine the impact of agriculture on *Echinostoma* infections in ramshorn snails (*Planorbella corneus*) of Crawford County, and compared snails collected from Heagy's Orchard with those from Bousson Environmental Research Reserve. Snails were collected from each site over a five week period from October to November, and screened in the laboratory for trematode parasites. Both the infection status of collected snails and the average number of trematodes found during shedding were recorded. The hypothesis for this study was that *Echinostoma* infections would be higher at Heagy's Orchard, compared to Bousson Environmental Research Reserve, due to the use of agrochemicals on the agricultural property. This prediction was confirmed, with Heagy's Orchard having higher infection prevalence compared to Bousson (51.61% compared to 13.33%), as well as Heagy's having higher infection abundance (2.65 echinostomes shed per snail from Heagy's compared to 0.56 from Bousson). These results have important implications for wildlife health in Crawford County as well as larger public health implications for snail-trematode systems around the world.

Introduction

Agrochemicals, such as fertilizers and pesticides, are used globally to help increase crop yields and fight against pests. Three of the more commonly used nutrients in agrochemical compounds are nitrogen, phosphorus, and potassium, all of which are important in facilitating the growth of the crops we harvest every day. In recent years, United States commercial fertilizer use has fluctuated between 20 and 25 million short tons of nitrogen, phosphate, and potash per year (Economic Research Service, 2019). Despite the advantages they provide to crops, agrochemicals can have drastic and long lasting negative impacts on the environment. These compounds are often over-applied and the resulting runoff can leach into soil, waterways, and even the atmosphere (National Pesticide Information Center, 2022). Due to the soluble nature of most fertilizers and some pesticides, they pose a very present threat to aquatic ecosystems, especially those in close proximity to agricultural areas.

One manifestation of high nutrient inputs caused by fertilization are algal blooms, a phenomenon that occurs due to the excess growth of algae living in both freshwater and marine environments (National Oceanic and Atmospheric Administration, 2023). Nutrient additions caused by agrochemical runoff can facilitate the growth of algae faster than it is able to be consumed by other organisms, creating a bloom, which in extreme cases can severely deplete dissolved oxygen levels in the water below. Dissolved oxygen levels are depleted by bacteria and fungi as part of the decomposition process when algal blooms begin to die (United States Environmental Protection Agency, 2023). The hypoxic and anoxic environments created by these blooms can be highly stressful, if not fatal, to other organisms in the environment (Paerl et al., 2001). In addition to depleting dissolved oxygen levels, algal blooms can also produce harmful toxins. In marine environments, genera of dinoflagellates such as *Alexandrium* are known to

produce neurotoxins; blooms of toxin-producing species such as these have been linked to excessive inputs of nitrogen and phosphorus (Paerl et al., 2001). If not managed carefully, nutrient additions from agrochemical runoff can cause events like these, disrupting whole ecosystems.

While fertilizers typically impact ecosystems through changes in nutrient availability, other agrochemicals, such as pesticides, have been observed to alter ecosystem dynamics through toxicity. Pesticides have the potential to be lethal to both target and non-target organisms; however they also have a variety of sublethal effects that can greatly disrupt community structure (Rumschlag et al., 2020). Sublethal exposure often leads to changes in the behavior of affected organisms ranging from changes in motor movements to alterations in normal feeding behaviors (Augusiak & Van den Brink, 2016). Zooplankton are one of many organisms that can be impacted by this. Zooplankton are highly sensitive to changes in water quality, and can be predictive of the overall health of ecosystems that they occupy (Serranito et al., 2016). Species of zooplankton exposed to sublethal concentrations of pesticides have shown decreased growth and feeding rates as well as altered motor behaviors, such as spinning, that could make them more susceptible to predators in the environment (Dodson et al., 1995). Behavioral changes, such as these, show that agrochemicals have the potential to indirectly alter population levels of the species they affect, thus affecting the community ecology of aquatic ecosystems. Amphibian and aquatic snail populations have also been observed to be impacted by agrochemical additions. Rohr and Crumrine (2005) observed impacts of the herbicide, atrazine, and the insecticide, endosulfan, both having impacts on aquatic ecosystem dynamics. Atrazine was found to reduce algal biomass, followed by reductions in the growth of snail and amphibian populations due to the dependence of these species on primary production. Endosulfan, however, indirectly

increased snail and tadpole growth by reducing chironomid survival and competition (Rohr & Crumrine, 2005). Agrochemicals are often used in combination with each other and can have conflicting impacts on non-target organisms, with some benefitting populations and others harming them. Because of these varied effects, the impacts of agrochemicals on ecosystem dynamics can be hard to predict, further underscoring the importance of studying the impacts of agrochemical additions on natural ecosystems.

Freshwater snails play many important roles in aquatic ecosystems from maintaining water quality to facilitating the flow of nutrients throughout the food-web (Cheng et al., 2022). Serving as a food source for organisms such as fish, turtles, ducks, and crayfish, freshwater snails are important components in aquatic food webs. Additionally, consuming both algae and detritus, snails exert top-down control on primary producers and are important in maintaining water quality (Silliman & Bertness, 2002). Snails are known for feeding via scraping, however certain species also filter feed and can help reduce water turbidity, an attribute commonly utilized by aquarium owners (Zhu et al., 2013). Due to their grazing on algae, snails are a vital component of controlling algal growth. The impact of snail grazing on algal growth is so strong that they have even proven to be effective as a biological control against algal blooms in both field and modeling experiments (Chase & Knight, 2006; Han et al., 2010; Zhu et al., 2013). Despite the great importance of snails to aquatic ecosystems, they are widely known not only for the good they do, but also for their ability to transmit a variety of diseases. Freshwater snails can serve as hosts for a variety of parasites including nematodes (roundworms) and trematodes (flatworms or flukes). Snail-borne parasites such as these have varying life cycles and can infect snails and other organisms, both aquatic and terrestrial, through various mechanisms (Lu et al., 2018). In snail-trematode systems, snails become infected by miracidia, the second, free swimming,

trematode life cycle stage. This stage then replicates and develops within the snail through several intra-molluscan stages. Finally, the snail sheds the trematode parasite as cercariae. Depending on the specific trematode species, cercariae can then move on to encyst on aquatic vegetation or infect other organisms (Lu et al., 2018). Following their development within the snail host, trematodes go on to infect several other hosts, both aquatic and terrestrial. Snail-borne trematode infections in humans are not highly prevalent within the United States; instead, they primarily impact wildlife.

The impacts of agrochemicals on snail-trematode systems are complex, in some cases exacerbating snail-borne trematode disease, and in others mitigating it (Hoover et al., 2020; Zhang et al., 2019). Snail-trematode systems have the potential to impact several organisms, both aquatic and terrestrial; therefore studying the impact of agrochemicals on systems such as these is important to understand not only host-parasite dynamics but also transmission dynamics between organisms. Molluscicides are one of the major control measures taken against snail-borne trematode infections such as schistosomiasis, a neglected tropical disease that impacts upwards of 230 million people across the globe (Colley, 2014). Molluscicide control of the disease has been successfully implemented in several countries; however, certain molluscicidal compounds have been demonstrated to be highly toxic to other organisms including fish and mammals, as well as having carcinogenic, teratogenic, and mutagenic properties (Zheng et al., 2021). Despite the success of molluscicides in snail control programs, other agrochemicals can facilitate population growth of aquatic host snails. Nutrient additions by fertilizers help facilitate the spread of disease in snail-trematode systems by way of increasing algal growth. As a result of increased primary production, snails have a more readily available food source, allowing their populations to become more robust and their individual growth to

increase. In turn, increased snail population density also increases the probability of infection by free-swimming miracidia in the water (Anderson & May, 1978). Due to increased growth and larger populations, snails are able to shed more cercariae into the water which can then infect other organisms such as mammals, waterfowl, and amphibians. A mesocosm experiment by Johnson et al., (2007) confirmed this trend, with eutrophic environments increasing the density and prevalence of infected snails, as well as doubling host snails' individual trematode cercarial production. This cascade, caused by anthropogenic nutrient additions, makes agricultural areas especially vulnerable to increased disease transmission.

Crawford County is home to both *Ribeiroia ondatrae* and *Echinostoma* spp., both of which infect ramshorn snails (*Planorbis corneus*) and aquatic amphibians. *Ribeiroia ondatrae* is known to infect snails, amphibians, and birds. In amphibians, *Ribeiroia ondatrae* encysts around developing limb buds and has been cited as a major cause of limb malformations, causing extra, missing, and misshapen limbs in a variety of species (Johnson et al., 2002). *Echinostoma* spp. is not known to cause malformations such as these, but infects a much broader range of organisms including the host snail along with birds, rodents, dogs, humans, bivalves, salamanders, tadpoles, and fish. Symptoms of echinostomiasis vary between species but commonly involve gastrointestinal inflammation (Centers for Disease Control and Prevention, 2019). In amphibians, *Echinostoma* spp. encysts in the kidneys and can be lethal to tadpoles at early developmental stages through disruption of renal function (Schotthoefer et al., 2003). With approximately 30% of Crawford County acreage being farmland, how landowners choose to manage their agricultural properties can have a great impact on surrounding ecosystems (National Agricultural Statistics Service, 2022). The influence of agriculture on ramshorn snail abundance in Crawford County has been previously observed to show that *Planorbella*

abundance was higher in agricultural associated wetlands compared to non-agricultural sites. In addition to increased *Planorbella* populations at the agricultural study site, significant differences in nitrogen concentrations between the two sites were found, suggesting a relationship between these two factors (Vigneri, 2019). Consistent with observations from the literature, this snail population abundance difference suggests that snails at agricultural sites in the county would also be able to support higher counts of trematodes.

The aim of this project was to examine differences between *Echinostoma* infections in ramshorn snails at an agricultural-associated site compared to a non-agricultural-associated, woodland site. Due to previously demonstrated differences between nutrient concentrations and snail abundance in the county, it is important to examine cercarial production within these snails as well as to consider disease risk to other aquatic and non-aquatic organisms (Vigneri, 2019). Given that both *Ribeiroia ondatrae* and *Echinostoma* spp. have the potential to infect multiple organisms, characterizing the relationship of land use differences on disease dynamics is especially important for wildlife health. Due to the observed impact of agrochemical use on *Planorbella* populations, I hypothesize that the presence of *Echinostoma* infections in ramshorn snails will be higher in those collected from an agricultural site compared to a woodland site due to increased nutrient availability from agrochemical use.

Methods

Study Sites

Study sites for this project consisted of one agricultural associated pond and one woodland pond. Both ponds are located in Crawford County, PA and are home to several organisms including fish, tadpoles, and snails. The agricultural associated pond is located on the property of Heagy's Orchard, where it sits at the foot of a hill of fruit trees (Fig. 1).

Agrochemicals used at this site include the pesticide Captan and the fertilizer 19-19-19 (P. Heagy, personal communication, October 19, 2022). Captan is a human made fungicide, with a half life ranging from 5-19 hours, with slower breakdown in settings where light is limited. Due to the quick breakdown of the fungicide in waterways, its impact on organisms is limited, however it has been demonstrated to be toxic to fish, water fleas, shrimp, and oysters (Strid et al., 2018). 19-19-19 is a water soluble fertilizer containing equal amounts (19%) of nitrogen, phosphorus, and potash (Witz, 2023). It is recommended that 19-19-19 is kept away from waterways due to the potential of nutrient additions from the fertilizer to promote excess algal growth. 19-19-19 has low toxicity for humans and wildlife (Border Chemical Company Limited, 2006).



Figure 1: A satellite image from Google Maps of the pond at Heagy's Orchard (41.56896°N, 80.16134°W). Sampling at this site occurred on the east bank of the pond due to accessibility.

Bousson Environmental Research Reserve is a wooded area that has been owned by Allegheny College since 1935 (Fig. 2, Fig. 3). Although Bousson has prior agricultural land use history dating back to the late 1800s, the property is now reserved for environmental and biological research purposes (Allegheny College ES 440, 1996). Agrochemicals are not presently used around the aquatic habitats on the property; however, adjacent landowners could use agrochemicals on their properties. Bousson Environmental Research Reserve is home to a number of aquatic habitats which host a variety of organisms including snails, tadpoles, turtles, newts, and waterfowl. South Marsh was selected due to the observed presence of *Planorbella* at the site.

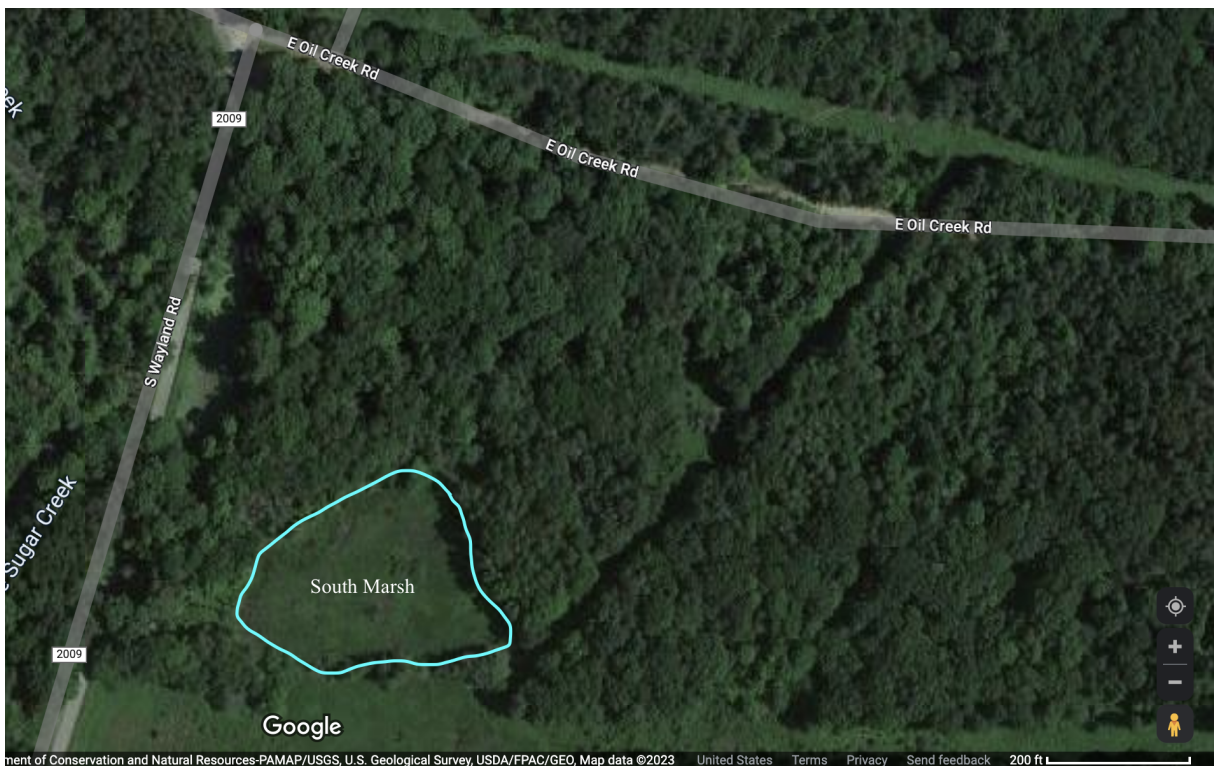


Figure 2: A satellite image from Google Maps of South Marsh and the surrounding wooded area at Bousson Environmental Research Reserve. The sample pond is labeled and outlined in blue (41.59533°N, 80.04488°W). Sampling occurred mostly on the east bank of the pond.

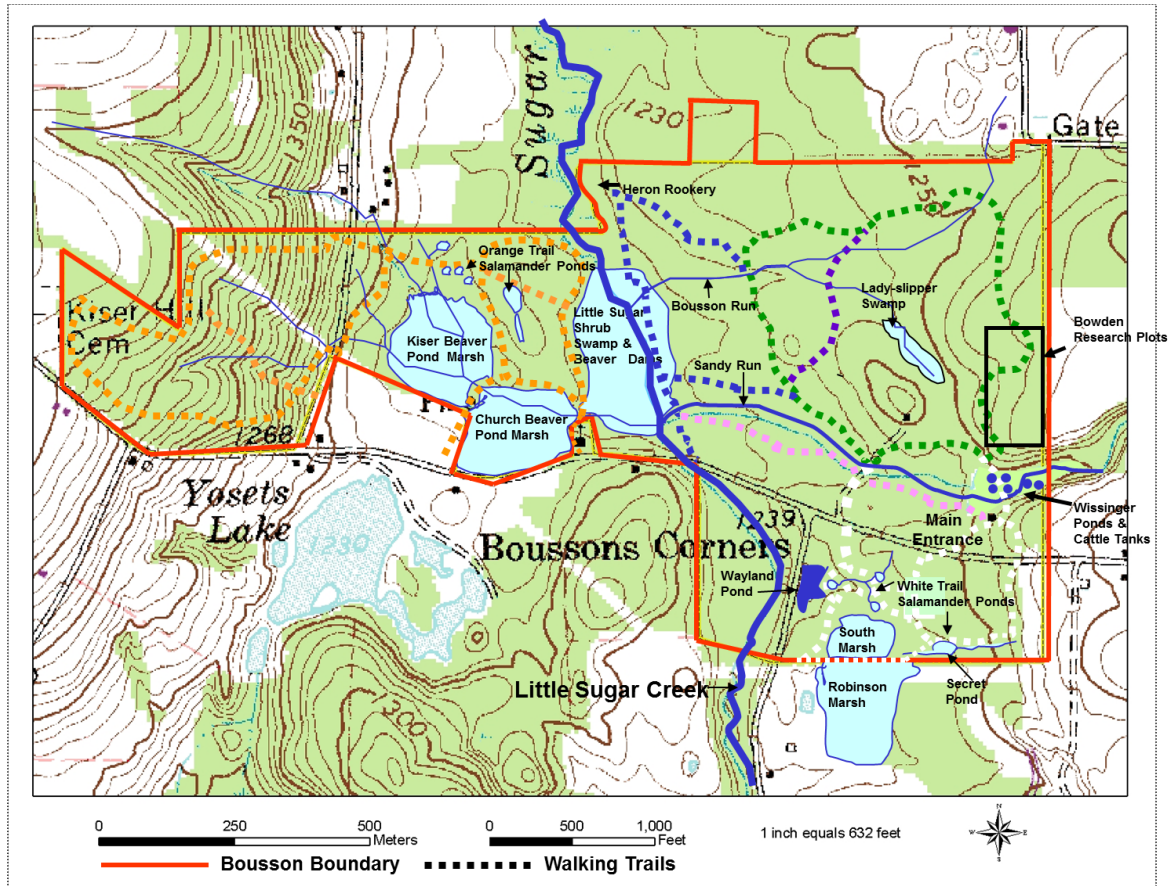


Figure 3: A map of Bousson trails and ecological features provides a clear view of the location of South Marsh on the property of the research reserve (Goeller, 2009).

Snail Collection

Snail collection from the two sites largely depended on weather conditions. Both sites were sampled from October to November. Bousson Environmental Research Reserve was sampled on October 5th, 14th, 21st, 28th, and November 4th. Heagy's Orchard was sampled on October 14th, 21st, and 28th, as well as November 4th and 8th. Snails collected on November 8th had total mortality during screening and were not counted in the results. Collection occurred along the perimeter of each pond and was targeted along areas nearby vegetation. After sampling, the shore was marked with flagging tape so that no section of pond was sampled twice. Collection followed a dip net measured sweep method in accordance with methods from the

literature (Difranco, 2014). Following these methods, the sampler swept through a one meter section of water, marked off with a meter stick by a research assistant. The sampler bumped the substrate at the bottom three times throughout the sweep, once at the beginning of the sweep, once at the middle, and once at the end (Difranco, 2014). The collected sample was then dumped into a sorting tray and searched for *Planorbella*; empty shells were cast aside and not counted in the sample. *Planorbella* were identified based on their morphology which includes a flat, coiled shell shaped similarly to that of a ram's horn from where they get their common name (Ramshorn snails field guide, n.d.). The diameter of adult shells typically range from $\frac{1}{8}$ to $\frac{3}{4}$ of an inch (Ramshorn snails field guide, n.d.). Snail counts were recorded in a field notebook and then placed in a bucket of pond water for transportation back to the laboratory. Water and snails from the two sites were kept separate for the duration of the study. Collection periods at each site were limited to one hour. When possible both sites were sampled on the same day in the early morning.

Lab Husbandry

Following collection, snails were brought to the laboratory to acclimate to laboratory temperatures for 24-48 hours before screening. While waiting to be screened, snails were kept in buckets based on the site they were originally collected from. Snails were not returned to the field during the duration of this experiment to prevent recapture. During their time in the lab, snails were fed frozen spinach and Sera Micron growth food one to two times a week. Every 7-14 days, $\frac{1}{4}$ of bucket water was changed out with dechlorinated water. Dechlorinated water was prepared as needed using forced aeration.

Assessment for Parasites

Following acclimation to the laboratory, snails were assessed for *Echinostoma* cercariae. During screening, snails were kept separately in vortex tubes that were labeled with the site they originated from. Each tube was cleaned prior to screening and contained 30-35 mL of dechlorinated water. Shedding followed procedures similar to that of other parasitic flukes (Al-Jubury et al., 2020). Snails were kept in vortex tubes under heat lamps for 12-24 hours to promote the release of cercariae. After exposure to heat and light, the tubes were then observed under a microscope for the presence of released cercariae (Al-Jubury et al., 2020). For microscope observation, a dissecting microscope was used with magnification kept at 40x. Cercariae were identified by moving up the water column from the shell of the snail to the top of the water column. Cercariae were classified based on movement and were only counted if they were moving in a circular, zigzag, or random motion; those that were floating in the water column but not moving were not counted towards the infection abundance.

Identification of *Chaetogaster*

The presence of *Chaetogaster*, a hypothesized trematode predator, was noted throughout the study and its appearance was recorded. *Chaetogaster* was identified through the use of microscope images and videos sent to a professional at North Carolina State University (S. Hopkins, personal communication, November 29, 2022). For identification of *Chaetogaster*, one snail specimen was sacrificed. *Chaetogaster* was removed from the sacrificed snail specimen under a dissecting microscope; images of which were taken at 10x magnification on a compound microscope (Fig. 4).

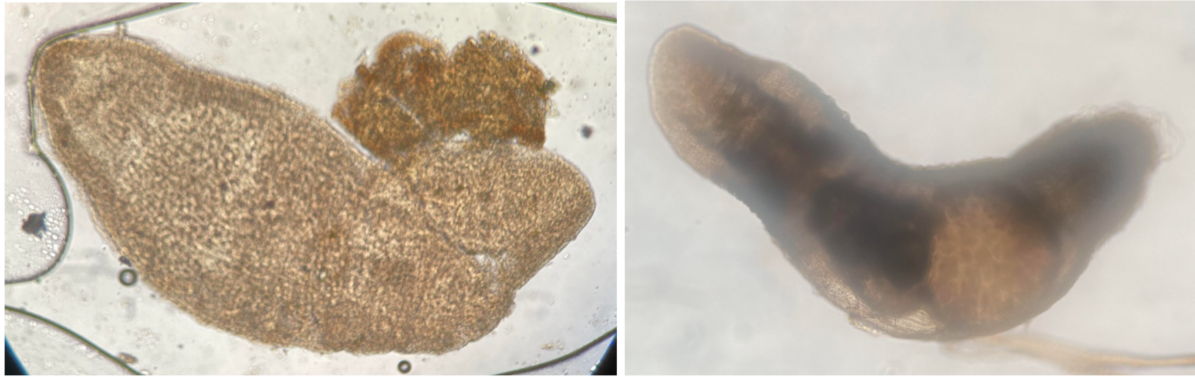


Figure 4: Microscope images of *Chaetogaster* taken at 10x magnification under a compound microscope. The image to the left used a flat microscope slide, while the image to the right used a well slide.

Statistical Analyses

I ran two different statistical models to assess two different metrics of snail infection: the prevalence of infected snails and the infection abundance (i.e., the number of echinostomes observed per snail, including "0" from the snails that were not infected). My first statistical model on infection prevalence was a generalized linear mixed model (using the "glmer" function in R) and it tested whether the binomial response variable "infected" (yes or no) differed between location (categorical variable Bousson or Heagy's). I included "Sample Date" as a random factor in this model because I was not interested in testing how infection changed through time, but I needed to account for the repeated sampling of the same location. My second statistical model on infection abundance was a linear mixed effects model (using "lmer" in R) and it tested whether the continuous response variable "infection abundance" differed between location (categorical variable Bousson or Heagy's). As with model 1, I included "Sample Date" as a random factor in this model because I was not interested in testing how infection changed through time, but I needed to account for the repeated sampling of the same location.

Results

Across the duration of the study a total of 121 ramshorn snails were collected, 72 were from Heagy's Orchard while the remaining 49 were from Bousson Environmental Research Reserve. Due to mortality either before or during screening, only 62.81% of the specimens collected were included in the results. A total of 45 snails were screened from Bousson Environmental Research Reserve, while 31 were screened from Heagy's Orchard. Infection prevalence was calculated using the total number of snails screened from each site as opposed to the total number collected from each site to account for mortality during the screening process. Out of all specimens screened, snails from Heagy's Orchard showed a higher prevalence of 51.61% compared to Bousson's 13.33% ($F_{1,5} = 10.160$, $P = 0.001$) (Fig. 5). Infection prevalence was calculated as the percentage of snails from each site that was infected with *Echinostoma* spp., regardless of the number of trematodes occupying each host snail.

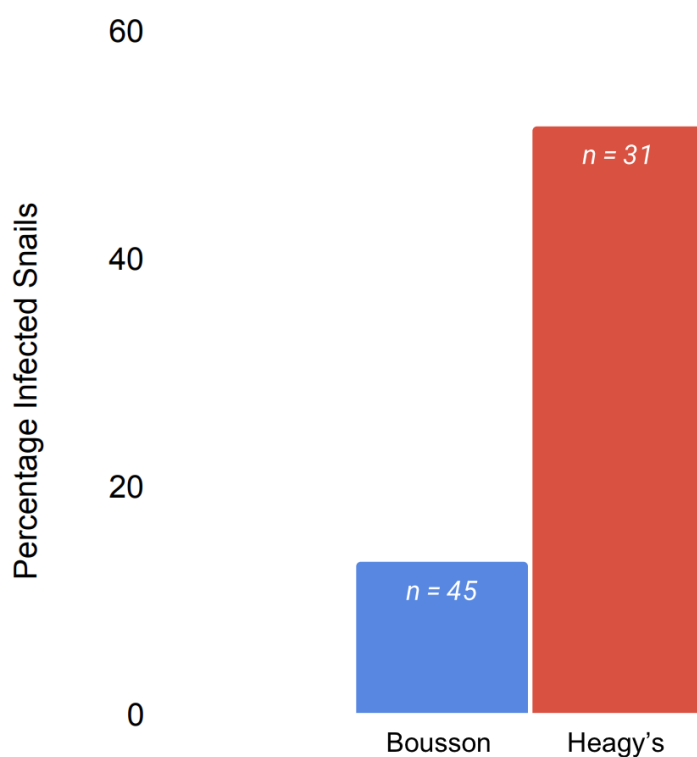


Figure 5: *Echinostoma* spp. Infection Prevalence by Location. Infection prevalence at Heagy's Orchard is represented in red, while prevalence at Bousson Environmental Research Reserve is represented in blue. *Planorbella* from Heagy's Orchard showed a higher infection prevalence than those screened from Bousson Environmental Research Reserve with 51.61% of snails at Heagy's Orchard being infected compared to Bousson's 13.33% ($F_{1,5} = 10.160$, $P = 0.001$).

When screened for trematode parasites, snails from Heagy's Orchard showed higher infection abundance of *Echinostoma* spp. compared to those from Bousson Environmental Research Reserve ($F_{1,5} = 8.159$, $P = 0.004$) (Fig. 6). Infection abundance was calculated as the number of trematodes observed during screening of each snail regardless of infection status. On average, snails from Bousson Environmental Research Reserve had 0.56 echinostomes per snail ($SE = 0.289$) while snails from Heagy's Orchard had an average of 2.65 echinostomes per snail ($SE = 0.475$). The lowest count of trematode parasites observed at both sites was zero, while the highest count observed at each site were ten at Bousson Environmental Research Reserve and fifteen at Heagy's Orchard.

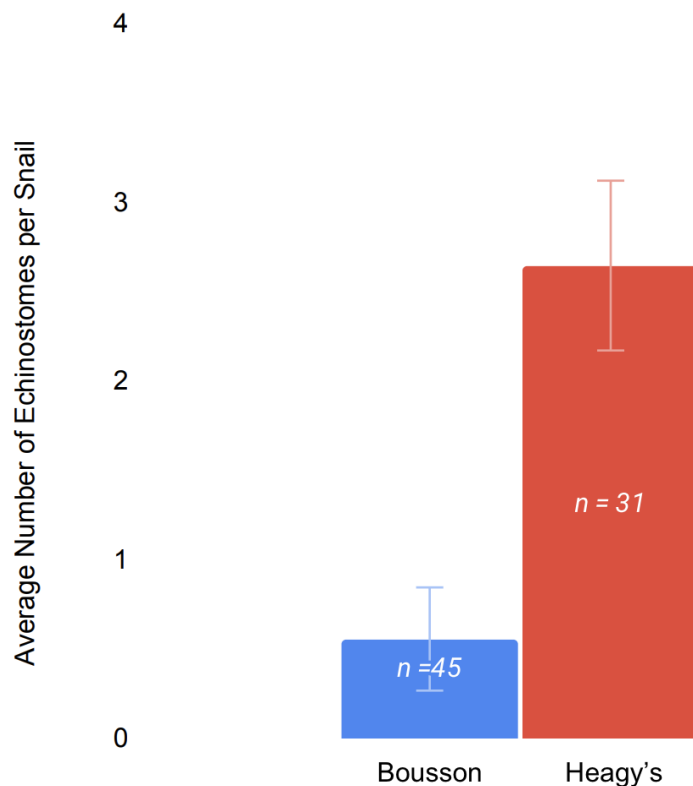


Figure 6: Infection Abundance by Location. Infection abundance at Bousson Environmental Research Reserve, represented in blue, compared to Heagy's Orchard, represented in red.

Planorbella at Heagy's Orchard had higher infection abundance, with more echinostomes per snail observed from the site. On average, snails screened from Bousson Environmental Research Reserve had 0.56 echinostomes per snail ($SE = 0.289$) while snails screened from Heagy's Orchard had an average of 2.65 echinostomes per snail ($SE = 0.475$) ($F_{1,5} = 8.159$, $P = 0.004$). Error bars represent ± 1 standard error.

Chaetogaster presence was only observed at Heagy's Orchard, with none being observed colonizing snails from Bousson Environmental Research Reserve. Out of the 31 snails screened from Heagy's Orchard, 51.61% had echinostomes and were classified as infected (Fig 5.). Infected snails from Heagy's Orchard were colonized by an average of six *Chaetogaster* per snail

(SE = 2.0), compared to uninfected snails which were colonized by an average of 10.9 *Chaetogaster* per snail (SE = 4.17) (Fig. 7). Infected snails were colonized by a lower range of *Chaetogaster* per snail (0-30) than uninfected snails (0-50).

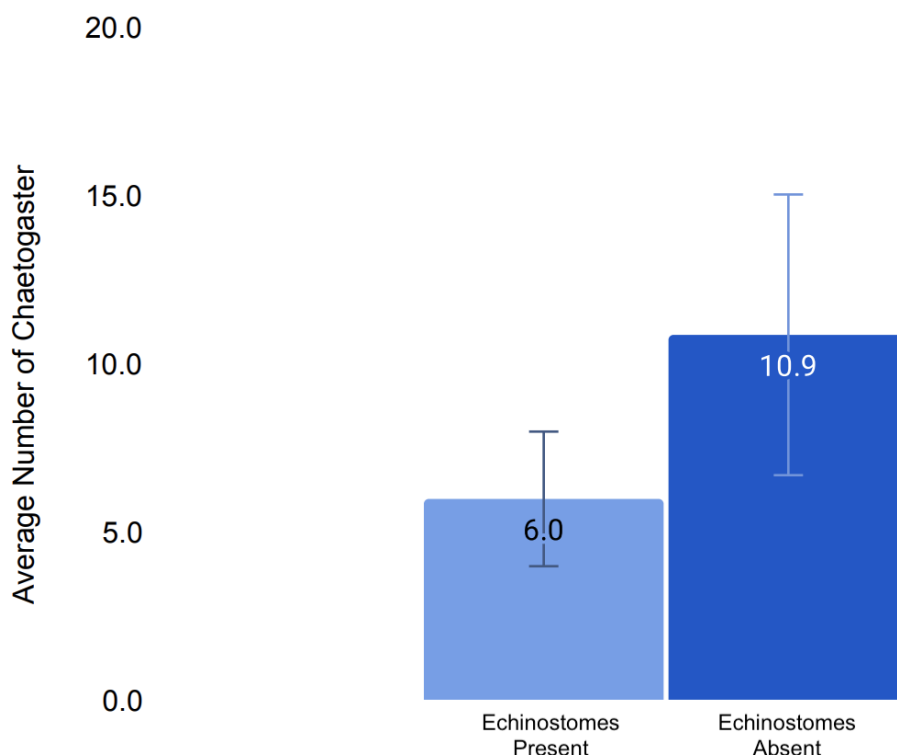


Figure 7: Average Number of *Chaetogaster* compared to Echinostome Presence. Snails with echinostomes, represented in blue, had a lower average number of *Chaetogaster* per snail (6.0) (SE = 2.0) than snails where echinostomes were absent, represented in red, which had a higher average number of *Chaetogaster* (10.9) (SE = 4.17). Error bars represent +/- 1 standard error.

When average echinostomes shed from each snail were compared against *Chaetogaster* presence or absence, colonized snails showed lower average echinostomes per snail. Colonized snails had an average of 2.10 echinostomes per snail (SE = 0.556), whereas uncolonized snails had an average of 3.80 echinostomes per snail (SE = 1.971). A higher number of snails from

Heagy's Orchard were colonized (21) than uncolonized by *Chaetogaster* (10). Colonized snails shed a lower range of echinostomes on average (0-8) than uncolonized snails (0-15).

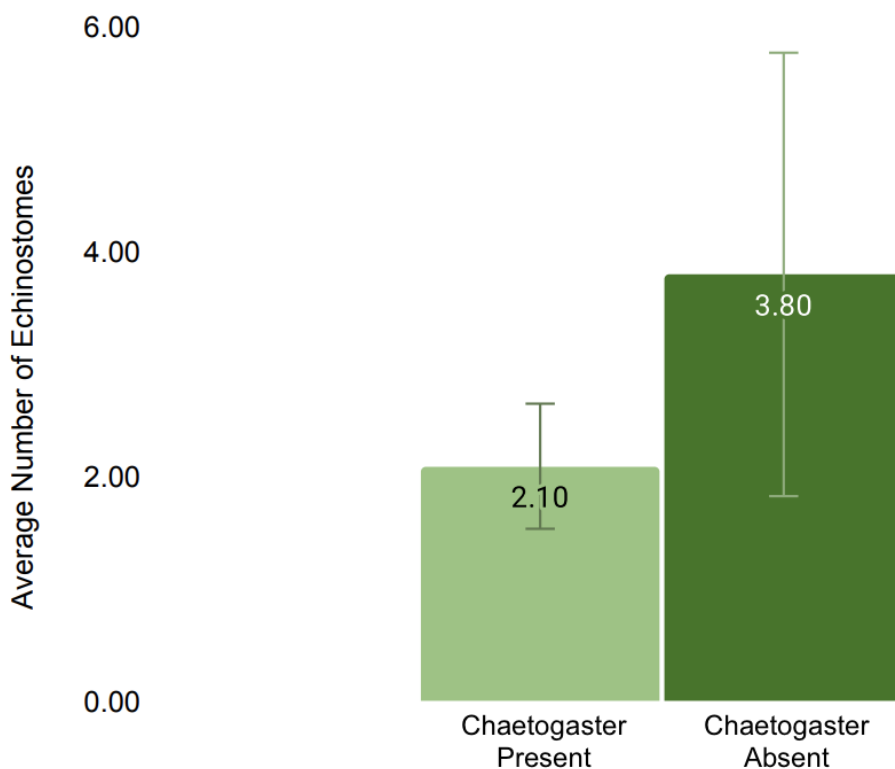


Figure 8: Average Number of Echinostomes compared to *Chaetogaster* Presence. Colonized snails, represented in blue, had a lower average number of echinostomes per snail (2.10) (SE = 0.556) compared to uncolonized snails, represented in red, which had a higher number of echinostomes per snail (3.80) (SE = 1.971). Error bars represent +/- 1 standard error.

Discussion

The impact of agrochemical use on snail abundance in Crawford County has been previously demonstrated, with agricultural sites having higher populations of aquatic snails compared to non-agricultural sites (Vigneri, 2019). The present study sought to further explore the disease-related implications of this finding, characterizing differences in pathogen abundance and prevalence at agricultural and non-agricultural sites within the county. Because more robust

snail populations should coincide with increased parasite prevalence, the hypothesis for this study was that *Echinostoma* pathogen presence would be higher in snails collected from agricultural-associated sites compared to woodland sites due to increased nutrient availability from agrochemical use. A pond located on the property of Heagy's Orchard was used for the agricultural site, while South Marsh, on the property of Bousson Environmental Research Reserve, was used for the non-agricultural site. Snails screened from Heagy's Orchard had an overall higher infection prevalence compared to Bousson Environmental Research Reserve, with about half of the snails from the site being infected. Additionally, pathogen abundance was also higher in snails from Heagy's Orchard compared to snails from Bousson Environmental Research Reserve, with snails from the agricultural site having higher counts of echinostomes regardless of infection status.

Two of the measures taken in this study were infection prevalence ($P = 0.001$) and infection abundance ($P = 0.004$), both of which were found to be significantly impacted by study site location. Both infection prevalence and abundance were found to be higher at Heagy's Orchard compared to Bousson Environmental Research Reserve, suggesting that not only are there more infected host-snails at the agricultural site, but that they are able to support higher cercarial production as well. While nutrient level differences were not tested between sites, Vigneri (2019) described differences, specifically between nitrogen levels, between ponds on the property of Davenport Fruit Farms compared to Bousson Environmental Research Reserve, with nitrogen levels being higher at agricultural-associated ponds. Considering the land use similarities between Heagy's Orchard and Davenport Fruit Farms, as well as Heagy's use of 19-19-19, it is likely that nutrient availability in the pond at Heagy's Orchard is higher than the levels present at Bousson Environmental Research Reserve. Additionally, the observations of

this study support Vigneri's findings, as more snails were collected from Heagy's Orchard than were collected at Bousson Environmental Research Reserve. While snail abundance was not specifically being measured in this study, the method of snail collection used in this study, the dip net measured sweep, is similar to those used to measure *Planorbella* abundance (Difranco, 2014). These findings and observations are consistent with those in the literature, which state that higher nutrient availability promotes parasitic infections through a cascade of effects where larger populations of host snails correspond with higher infection prevalence (Johnson et al., 2007).

These findings have important implications for snail-borne trematode disease risk in Crawford County, especially for amphibian populations which can be harmed by both *Echinostoma* spp. and *Riberiroidia ondatrae*. Previous mesocosm experiments have demonstrated the potential of eutrophication to promote trematode parasitic infections and increase amphibian disease risk (Johnson et al., 2007). While the mechanism behind this finding lies primarily in increased nutrient availability, other agrochemicals, such as pesticides, may also increase disease risk to other organisms. Pesticides have demonstrated immunosuppressive effects on amphibians, which may increase their susceptibility to parasitic infections (Brodin et al., 2009; Johnson et al., 2007; Rohr et al., 2008). The combined effects of both fertilizer and pesticide use puts amphibians at significant risk of contracting snail-borne trematode infections. In addition to amphibian disease risk, other organisms, such as waterfowl, are also at risk of contracting parasitic infections due to increased prevalence and abundance of infections in host snails. It is important to note that many of these organisms travel between multiple sites; waterfowl, for instance, have the potential to transport trematode eggs to other sites through fecal deposits (Roy & St-Louis, 2017). In Crawford County, disease risk surrounding these systems mainly pertains

to wildlife; however, in other parts of the world where snail-trematode infections impact people, this project brings up several public health concerns. This study, as well as those from the published literature, demonstrate the potential of agrochemical use to exacerbate snail-trematode infections including those that affect humans such as Schistosomiasis and Paragonimiasis (Johnson et al., 2007). In this way, the data collected during this experiment may serve as a case study to illustrate the effects of land use differences on snail-borne trematode abundance and prevalence. Symptoms of trematode infections in humans are wide ranging, including anemia, gastrointestinal discomfort, and impaired development in children (Colley et al., 2014). The risks that these infections pose to both human and wildlife populations underscore the importance of sustainable land management practices including those associated with limiting agricultural runoff. The exacerbation of snail-trematode diseases by agrochemical use may also help inform officials on areas to target treatment and mitigation efforts.

Also observed in this study was the presence of *Chaetogaster*, a commensal oligochaete known to colonize aquatic snails (Fernandez et al., 1991). *Chaetogaster* is hypothesized to be a trematode predator, as its presence has been observed to limit trematode infections in snails; however the mechanism by which it achieves this is understudied (Fernandez et al., 1991; Hobart et al., 2021). During this study *Chaetogaster* was only observed on snails collected from Heagy's Orchard, therefore its potential influence on infection differences between the two sites cannot be commented on. The prevalence of *Chaetogaster* increased throughout sampling dates and was observed in snails collected from Heagy's Orchard on October 21st, 28th, and November 4th. 14.28% of snails screened from October 21st were colonized by *Chaetogaster*, this proportion increased for the following two dates with 100% of screened snails from October 28th being colonized, and 92.31% of snails collected on November 4th being colonized. It is important to

note; however, that due to the unsuspected presence of and unfamiliarity with the oligochaete, this rise in prevalence could also be due to growing familiarity with the organism. Both infected and uninfected snails from Heagy's Orchard were colonized by *Chaetogaster*. The data collected in this study indicate that *Chaetogaster* and echinostomes share an inverse relationship; however the mechanism by which this occurs is unclear. Lower average counts of *Chaetogaster* were observed in infected snails compared to uninfected snails. In addition to this, snails colonized by *Chaetogaster* shed lower average numbers of echinostomes compared to uncolonized snails. No statistics were run on this data due to the absence of a priori hypotheses concerning *Chaetogaster*. Although no causal mechanism for these observations can be discussed based on the data collected during this experiment, there may be some type of competitive relationship between the two organisms. The data also does not rule out the possibility of trematode predation by *Chaetogaster*; however, no predatory behavior was observed in this study. The impact of *Chaetogaster* on trematode infections in snails remains an interesting topic of future study both in the county and beyond.

Limitations of the study focus largely on the time frame the study was conducted in. Sampling of both sites depended largely on weather conditions. Originally, the sampling for this project was intended to last eight weeks; however, weather limitations reduced the sampling period to five weeks. The first week of sampling only focused on Bousson Environmental Research Reserve, and was done to make sure that there were planorbid snails at the site before beginning the project. The final week of sampling was only done at Heagy's Orchard and not Bousson Environmental Research Reserve due to unfavorable weather; however, the whole sample had total mortality during screening and was removed from the data. There may have also been discrepancies between what was identified as infected versus not infected due to

growing familiarity with the appearance of trematodes as the study progressed. Additionally, mortality could have also impacted the data. Snails from Heagy's Orchard had a higher mortality rate before and during screening, with only 43.05% of the original sample that was collected being screened compared to 91.84% of Bousson's original sample being screened. It is unclear what accounted for these differences though it could have been due to differences from the snails' habitats compared to the laboratory conditions, which were much warmer compared to the outside environment.

While providing important data on the presence of snail-trematode infections in Crawford County, many areas of the present experiment could be improved upon in future research. Increasing sample size, either by way of a longer or more frequent sampling period, would further increase the reliability of the study. Laboratory husbandry procedures should also be updated in the future to limit mortality. Due to the high mortality seen in this study, it is recommended that snails be kept at more favorable and highly monitored laboratory conditions such as keeping them in small, aerated aquarium tanks with plantlife. In future studies, these tanks should be monitored for changes in water quality, with dead snails being removed from the tanks as needed. To further limit mortality, screening time should also be reduced to 1-2 hours, with shedding procedures being repeated multiple times throughout the study using the same snails. Snails also should have been incubated in dark conditions before exposure to light. To improve the accuracy of the results from this study, shedding should also be followed with a molecular analysis, likely qPCR, which would improve the accuracy of trematode identification. While water samples were collected during this study, nutrient test kits gave inconclusive results. The accuracy of this experiment could also be improved by using a more precise method of nutrient analysis. While this experiment provides important insights into the prevalence of

snail-trematode infections in Crawford County, future research should focus on the presence of infection among other organisms such as amphibians and birds to get a better understanding of transmission between organisms within the county. Additionally, the role of *Chaetogaster* in snail-trematode systems of Crawford County could also be further studied now that its presence has been observed.

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