The effect of a pathogen on the population dynamics and reproductive method of *Asplanchna girodi*

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The effect of a pathogen on the population dynamics and reproductive method of *Asplanchna girodi*

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SUMMARY

Studies show that the abundance of *Asplanchna girodi* can be affected by many factors including food density, temperature and pH. One such aspect not yet explored is the effect that parasitism has on *Asplanchna girodi* populations. During weekly samplings of a local lake, a parasitic oomycete was discovered to frequently infect the *Asplanchna girodi* population. Based on multi-year sampling data, this study supports recent work showing that parasitism of a zooplankton has the potential to have reproductive and evolutionary consequences. I show that epidemics of this parasite, *Pythium*, occur frequently and that an increased number of males in the population can be correlated to the presence of the parasite. Sexual reproduction involving males provides for *Asplanchna girodi's* only means of genetic recombination and production of diapausing eggs that overwinter. This study shows that there is a correlation between the rate of infection by *Pythium* and the density of males in the *Asplanchna girodi* population. By increasing the number of males in a population, the rate of diapause egg production is also increased. Infection by this parasite could therefore be associated with increased sexual reproduction and genetic recombination of *A. girodi* populations.

CHAPTER 1

INTRODUCTION

Investigating and observing our environment is the key to understanding the biological processes of our surroundings. Realizing the importance of different ecosystems in a local area offers insight into how we can live more sustainably within our local environment and improve our quality of life. The purpose of this research will be to understand population dynamics of parasitism on the zooplantkter, *Asplanchna girodi*, and to determine if the effects of parasitism might be a driving factor for sexual reproduction and genetic recombination in this population.

Zooplankton in the Southeastern US

The freshwater systems of the Southeast are fairly unexplored regions in terms of limnological research. Many Northeastern and Midwestern lakes, ponds and rivers have been extensively studied and documented, but the same cannot be said for their southern counterparts (Wetzel, 2001). This lack of research is cause for concern because many Southern cities and local communities depend on local freshwater for their drinking water and commercial use water (Frick, Henderson, Moll, Furlong, & Meyer, 2001). Local freshwater bodies such as the ACF (Apalachicola Chattahoochee Flint) River Basin provide the drinking water for much of the Southeast, including large cities such as Atlanta (Vest, 1992-1993). Without further analysis of local freshwater we cannot begin to understand the impact that these complex freshwater communities have on each other, on the local flora and fauna, and on local cities and communities.

Among the best ways to fully understand a freshwater ecosystem is to start with the microscopic community. The phylum Rotifera is a group of microscopic invertebrates that can be found in nearly all freshwater systems as well as in marine systems (Wetzel, 2001). Although the phylum is relatively small, rotifers have very high reproductive rates and can maintain high densities, giving them the ability to colonize habitats rapidly (Wallace & Smith 2009). Rotifers form a critical link in many freshwater communities by connecting the microbial community of algae and cyanobacteria and the macro community that includes higher trophic levels such as arthropods and fish (Wallace & Smith 2009). Thus studying rotifers in a lake or pond could provide insight into freshwater food webs (Wallace, Snell, & Ricci, 2006). Rotifers are also responsive to many harmful elements in the water, such as toxins, metals and pesticides. Because of their responsiveness, rotifers can be used as an indicator species in screening tests for toxicity assessment in water (Snell 2005).

Asplanchna girodi: a model Rotifer

One rotifer found in many freshwater systems is *Asplanchna girodi* (hereafter called *Asplanchna*). *Asplanchna* will be studied in order to learn more about how a pathogen can affect population dynamics and sexual reproduction through infection prevalence. The dynamics of particular interest are the effects that the parasite, *Pythium*, has on the *Asplanchna* population and whether parasitism is correlated with sexual reproduction and genetic recombination in the population.

Asplanchna are predatory rotifers that swim by means of the cilia surrounding the head (Birky, 1965). The cilia not only aid in movement, but they also provide the means by which *Asplanchna* obtain food. Food particles that come into contact with the cilia are circulated toward the mouth and then ingested. This indiscriminate ingestion is believed to be how most parasites enter into the gut and eventually colonize the animal (Gilbert,

1980). An advantage of working with *Asplanchna* is that their bodies are naturally clear; foreign growth, such as a pathogen, is easily detectable when viewed under a microscope. They are also ideal organisms to use when studying infection, because they are easy to culture and maintain in a lab setting (Birky, 1965).

The reproductive cycle of *Asplanchna* is particularly important to understanding sex and diapause egg production of this species. By recognizing features of the *Asplanchna* reproductive cycle, it is easier to understand how a pathogen could profoundly influence the reproduction, development and evolution of this species. *Asplanchna* occur most often as asexual females, called amictic females. Mictic females are less commonly seen in the population and usually appear in response to environmental cues like population density (Serra, Snell, & King, 2003). Mictic females are able to reproduce sexually, providing for genetic recombination and diapause egg production. *Asplanchna* alternate between reproducing via mictic and amictic females, and this process is called cyclical parthenogenesis (Birky & Gilbert, 1971). A figure demonstrating the parthenogenetic cycle displayed by *Asplanchna* is reproduced from Birky (1971) and is shown below:

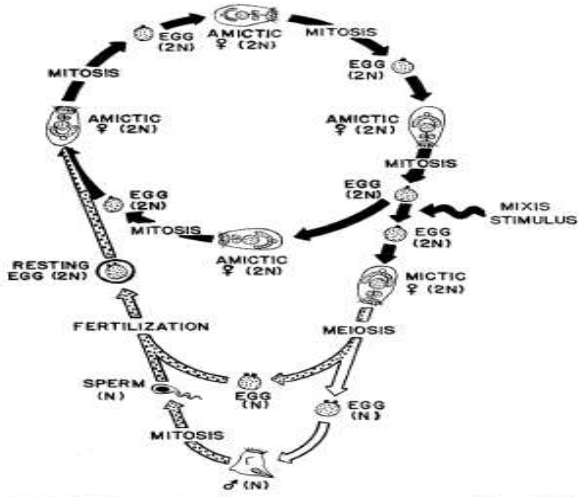


FIG. 1. Life cycle of the monogonont rotifers. "Mitosis" and "meiosis" refer to the oocyte maturation divisions in amictic and mictic females, respectively. (Reprinted from Birky, 1964).

The Advantages of Cyclical Parthenogenesis

Cyclical parthenogenesis is believed to be an evolutionary advantage for *Asplanchna* by providing a means for both genetic recombination and diapausing egg production. Typically, most *Asplanchna* are amictic females, allowing them to conserve their genetic material and simply pass on an identical copy of their genetic makeup to their daughters (Birky & Gilbert, 1971). Sexually reproducing mictic females are also consistently smaller than amictic females (Serra, Aparici, & Carmona, 2008). But by periodically reproducing sexually, *Asplanchna* reap the benefits of recombination and produce genetically diverse offspring, while still minimizing the cost of producing males (Serra, Aparici, & Carmona, 2008).

Males are rarely observed in natural populations of *Asplanchna* because they are ephemerally produced and short-lived. The appearance of male *Asplanchna* in a natural population usually coincides with higher population densities. King and Snell's lake observations found that the time when males where observed in the population usually coincided with the maximal population density (King & Snell, 1980). In addition, lab experiments showed that, unlike other species of *Asplanchna,* mictic female production in *Asplanchna girodi* is directly related to population density (Serra, Snell, & King, 2003). One theory suggests that sex is timed to coincide with high density population to increase the probability of male-female encounters (Serra, et al., 2008; Serra, et al., 2003). It has also been shown that other factors may influence the timing of sexual reproduction in rotifers, such as higher temperatures, food availability, and salinity (Serra, et al., 2003; T. W. Snell, 1986). However, little consideration has been given to the possibility that other factors like parasitism could also influence the mictic ratio in

Asplanchna populations. Population density has been identified as the single most important factor contributing to the production of mictic females in *A. girodi* (King & Snell, 1980).

Preliminary Expectations

In order to fully document the differences in population density and also the timing of sexual reproduction, multiple census counts will be done in a local, natural population of *Asplanchna*. First, the densities of *Asplanchna* observed in Lake Clara Meer in Piedmont Park will be documented, establishing a baseline by which population abundance can be accurately described. The counts on preserved animals are done so that the dynamics of the entire *Asplanchna* population, without considering parasites, can be determined. The counts on live animals are done so that the infection prevalence and percent of animals infected can be determined. Quantifying the infection rate by a major parasite of this organism will help to understand the population's dynamics, and will provide insight into whether epidemics can impact sexual reproduction of *Asplanchna*. Both preserved and live counts will be graphed and any cyclical observations and patterns will be recorded (Thomas et al. 2011).

In addition, I will study a possible adaptive mechanism to the stress of a parasitic epidemic exhibited by *Asplanchna*. Specifically, the number of males present in the population will be recorded. Males, and the resulting sexual reproduction of the population, would show whether the population increased genetic recombination and diapause egg production in response to an infection. I hypothesize that increased genetic recombination aids *Asplanchna* in recovering from a parasitic epidemic and that the presence of males and the presence of infection will be positively correlated. This could

indicate that parasitism may be one of many factors influencing the genetic recombination rate of *Asplanchna* populations.

Parasitic relationships

Natural animal population densities are influenced by a large variety of environmental factors. Symbiotic relationships play a key role in regulating population growth and development (Knight 1965). Parasitism is defined as a symbiotic relationship between a parasite and another organism (its host) that decreases the host's fitness, survival and fecundity (Kormondy 1969). Parasitism, by definition, has a negative effect on the host. These negative effects can include the shortening of the host's life cycle, or inhibition of host's reproductive cycle, both resulting in reduced host population abundance (Kormondy 1969). Parasites often have very short life spans in comparison to their hosts, which allows them to adapt and evolve more quickly. In order to survive, hosts must find a way to continually change genetic combinations in response to their quickly changing parasites (Hamilton et al. 1990). Asexual animals have only one way of possibly altering their genetic makeup, and that is through random mutations. By reproducing sexually however, parents recombine components of their own genetic makeup that have already proven to be effective in the past (Hamilton et al. 1990). Therefore, sexual reproduction seems to be a more effective means for a host to stay one step ahead of a rapidly evolving parasite (Hamilton et al. 1990).

In order to study a parasite-host interaction, a parasite must be found that infects the host fairly regularly with epidemics occurring in the natural population. One such parasite that infects *Asplanchna* is the oomycete fungal parasite *Pythium sp.* (hereafter called *Pythium*). This parasite has been shown to reduce population growth, survivorship

and fecundity of the *Asplanchna* population (Thomas et al. 2011). The presence of the parasite and its negative effects on a local *Asplanchna* population follow a fairly common parasite-host epidemic pattern. The epidemics observed by the Duffy Lab in the natural population of *Asplanchna* result in a cyclic rise and then decline in infection prevalence. *Asplanchna* epidemics have been documented up to three times a year in Lake Clara Meer (Thomas et al. 2011). The *Pythium* infection of the *Asplanchna* is usually characterized by the hyphae growing within the body cavity, making the body opaque and spotted. The figures below contrast an uninfected *Asplanchna* with an infected *Asplanchna*.

Figure 2
Uninfected *Asplanchna girodi*

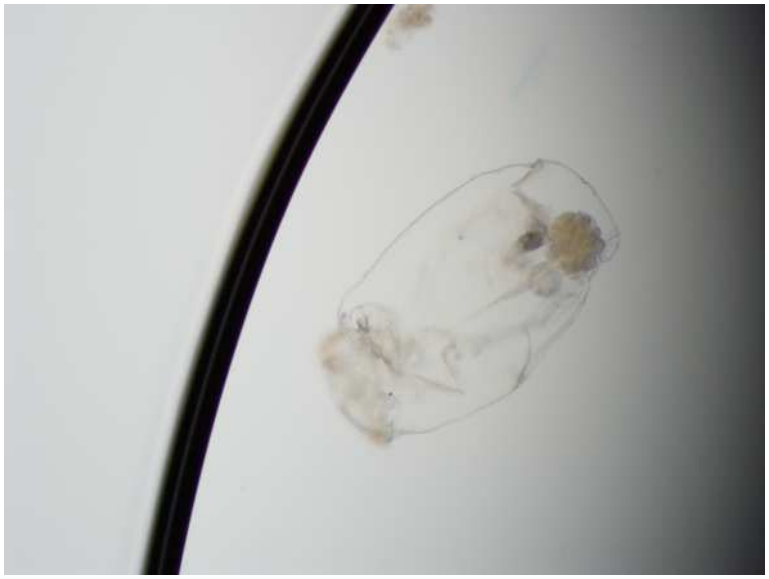
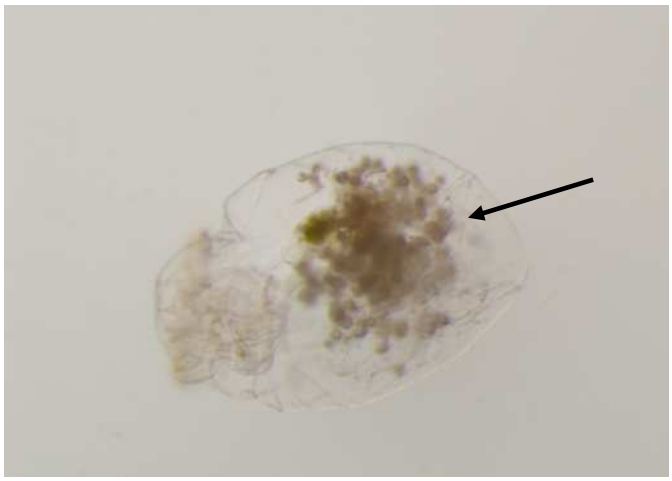


Figure 3
Asplanchna girodi infected by *Pythium*



Figures 2 & 3: The *Asplanchna* in Figure 2 is an uninfected *Asplanchna*. The *Asplanchna* in Figure 3 is infected with *Pythium*. The cilia of the mouth on both *Asplanchna* can be seen on the lower left of each body. When not infected, the *Asplanchna's* gut is transparent, and one can clearly see the gut and stomach. Once infected however, the entire body cavity is clouded with the parasite. The arrow indicates the *Pythium* hyphae growing within the body cavity. Presence of the hyphae is an indication of infection.

The parasite *Pythium* will provide an excellent model for further research because it is easy to see within an infected individual and is highly virulent on its host (Thomas et al. 2011). Studying and identifying those *Asplanchna* infected with *Pythium* would be a start to understanding the parasite-host relationship, and also reveal whether this parasite has any direct correlation to the production of mictic females, and thereby to the genetic recombination and evolution of *Asplanchna*.

The host-parasite relationship has also been shown to be important in terms of biodiversity (Hudson 2006). Parasites that are specific to one host (often called specialist parasites) have been shown to improve the overall biodiversity of an ecosystem. By only targeting a specific host, specialist parasites keep their host from attaining high density populations, allowing the growth of some other organism to instead take up those available niches (Hudson 2006). Ecosystem biodiversity, along with productivity, predictability and resilience, is among the main factors that determine overall ecosystem health (Hudson 2006). Therefore, the healthiest ecosystems would also be the ones that have the most complex relationships between host and parasite.

It has also been hypothesized that coevolution of a parasite and host can result in genetic variation, sexual reproduction and recombination (Mitchell et al. 2004). Studies with *Daphnia* parasites have demonstrated parasite mediated selection in populations of *Daphnia* in the wild (Duncan et al. 2006). But no studies have connected such findings to other zooplankton. In the case of *Asplanchna,* parasitism would be expected to have a negative effect on overall population abundance. In addition, a parasitic infection may induce the production of mictic females in *Asplanchna* populations.

Parasites as a mixis stimulus

The infection of *Asplanchna* by a parasite might directly increase the rate of mictic female production by an as yet unidentified mechanism. Evidence in support of this would show that *Asplanchna* populations with a high rate of parasite infection would have a high proportion of mictic females and a high density of male *Asplanchna*. From an evolutionary point of view, this relationship would suggest that parasitism may be a key factor in determining the level of genetic recombination in *Asplanchna* and its rate of evolution. Showing that parasitism in *Asplanchna* can enhance mictic reproduction and genetic recombination would be an important contribution to the understanding of the evolutionary forces shaping their adaptation. If parasitism can directly affect the frequency or timing of sexual reproduction, it would provide one more key to understanding the factors that influence a population's evolutionary path.

CHAPTER 2

METHODS AND MATERIALS

Field Sampling Methods

Sampling was done approximately once a week in Lake Clara Meer in Piedmont Park, Atlanta, Georgia from March 2008 to June 2010. Zooplankton samples were obtained by sampling at three different locations in the lake, using a kayak. At each spot a 153 nanometer Wisconsin net was lowered into the water, all the way to the bottom of the lake. The net was then drawn out of the water at approximately 1 meter per second. The zooplankton filtered into the net were washed into a sample bottle and then the process was repeated twice more, filtering into a different sample bottle each time. This process was repeated at three different locations on the lake. At each location, the contents of the Wisconsin net was added into one of three separate sample bottles, so that when sampling was done the three sample bottles had a total of three samples within them, having one water sample from each of the three locations on the lake. One of the samples was saved for live sample analysis and the other two samples were preserved in ethanol for future analysis.

Wet Lab Analysis Methods

Once water samples were obtained, cataloged and divided, the live samples were taken to the lab and scanned for infections. The scanning process was started by removing one 20ml aliquot from the live sample, pouring it into a petri dish, and scanning it under a microscope. The *Asplanchna* in the petri dish were identified, removed from the dish, and placed into an unused, dry petri dish. This method ensured that the *Asplanchna* sample will not be double counted and kept separate but alive. The

rest of the sample was discarded. The process of counting in the petri dish and transferring live *Asplanchna* to the discard beaker was repeated until the *Asplanchna* count reached at least 500. At that point the number of aliquots was recorded.

After at least 500 *Asplanchna* were identified, those *Asplanchna* were rescanned for possible *Pythium* infection. Under a dark field setting with a microscope at 25-50X magnification, the appearance of *Pythium* is quite obvious: infected *Asplanchna* have a milky white color, as opposed to their normal transparent appearance. The total number of *Asplanchna* in the sample, and the number of individuals inflected with the parasite *Pythium,* were recorded. After all *Asplanchna* were counted and the infected individuals identified, they were then be poured into a new beaker with food in it. This culture of *Asplanchna* was maintained in the lab and fed regularly. These *Asplanchna* were kept to promote the spread of infection within laboratory cultures, so that further observation of the infection could be done in the lab.

After initial live samples were counted for the infection rate, the other two water samples collected were preserved. Preservation of samples consisted of filtering each sample through a 153 nanometer filter. This process separates all of the zooplankton from the water of the samples. The zooplankton were then washed from the filter into a glass sample bottle which was filled with 50-95% ethanol and then tightly capped. The preserved samples will keep the integrity of the general population, allowing for density counts to be made later; however infection cannot be quantified accurately in preserved samples. To determine population density, the preserved samples were removed from storage and counted by filtering the sample to remove all ethanol. The zooplankton in the sample were transferred from the filter to a petri dish with water.

Once in the petri dish, densities of zooplankton were too high to reasonably look for *Asplanchna*; so a wide mouth pipette was used to transfer some of the sample into a round counter, where the male and female *Asplanchna* were counted and then transferred into a separate beaker. This process was repeated until nothing was left in the petri dish and all of the counted zooplankton were in a separate beaker. The sample was then re-preserved by filtration and washed back into the original sampling container with 50-90% ethanol and placed in storage. This procedure yielded an accurate count of the density of the sample, and also the percentage of males present in the overall population.

CHAPTER 3

RESULTS

Population Density over time

The Duffy Lab is fortunate to have multiple preserved samples spanning a period of three years, with only minor breaks in data sampling. Using this resource, the densities of the *Asplanchna* population from Lake Clara Meer were recorded, plotted and graphed over the past three years (Figure 4). Data collection started originally in March of 2008 and samples were collected approximately every seven days through June 2010. There is, however, a significant gap in sampling that occurs from October 2008 to January 2010. This gap in sampling occurs because of technical and legal issues concerning lake sampling within the Atlanta city limits. All issues were resolved within those months and sampling has remained consistent since then. By keeping track of the densities of *Asplanchna*, it is easier to see the cyclic changes that occur in the population. Previous studies have shown that temperature (and therefore the season of the year), and population density are two main factors in the *Asplanchna* life cycle (Birky 1974). By tracking their densities over a number of years we can see that there is a pattern of cycling.

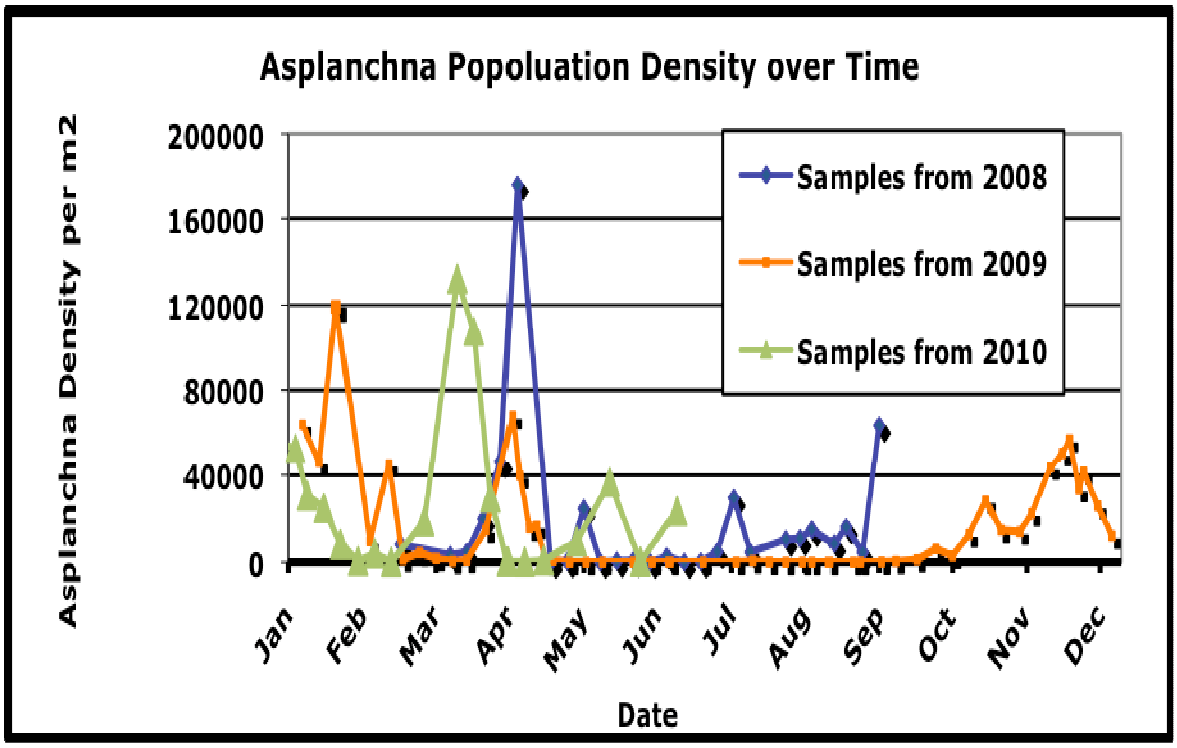


Figure 4 This graph charts the population density of the *Asplanchna* population from Lake Clara Meer for a period of three years. Data was collected starting in March of 2008 and samples were collected approximately every seven days through June 2010. The only significant gap in this timeline occurs from October 2008 to January 2010. This gap in sampling occurs because of technical and legality issues concerning lake sampling. All issues were resolved within those months and sampling has remained consistent since then.

Effects of Infection on Population Density

After observing the cycling patterns of the *Asplanchna* population, the next step in understanding the cyclic patterns of *Asplanchna* was to establish whether or not infection by known parasites was significantly correlated with *Asplanchna* population density. This was done by first comparing the population density to infection prevalence (Figure 5). Statistically, there is a significant correlation between the natural log of population density and the percent *Pythium* infection according to the Pearson's correlation coefficient ($r = 0.408$, $p = 0.017$). However, the figure indicates that though this data is significant, the relationship is also nonlinear. Overall, the data indicate that there is a significant relationship between the population density of *Asplanchna* and the *Pythium* percent infection, though this relationship appears to be nonlinear.

From these data there does appear to be a threshold of the population density below which infections are not observed. Specifically, below natural log of 10, infection is not seen in the natural population. This could be due to many factors. The most likely factor is that infection is not as easily spread in a less dense population. When *Asplanchna* are not in close contact with one another (at very low densitites), it may not be possible for *Pythium* to be contracted. There is also a point (corresponding to a natural log of 18) above which infections are rarely seen. It is currently unclear as to why these high densities do not promote as much infection, but it appears that infection of the *Asplanchna* population by *Pythium* occurs the most when the natural log of density is in between 10 and 18 (Figure 5).

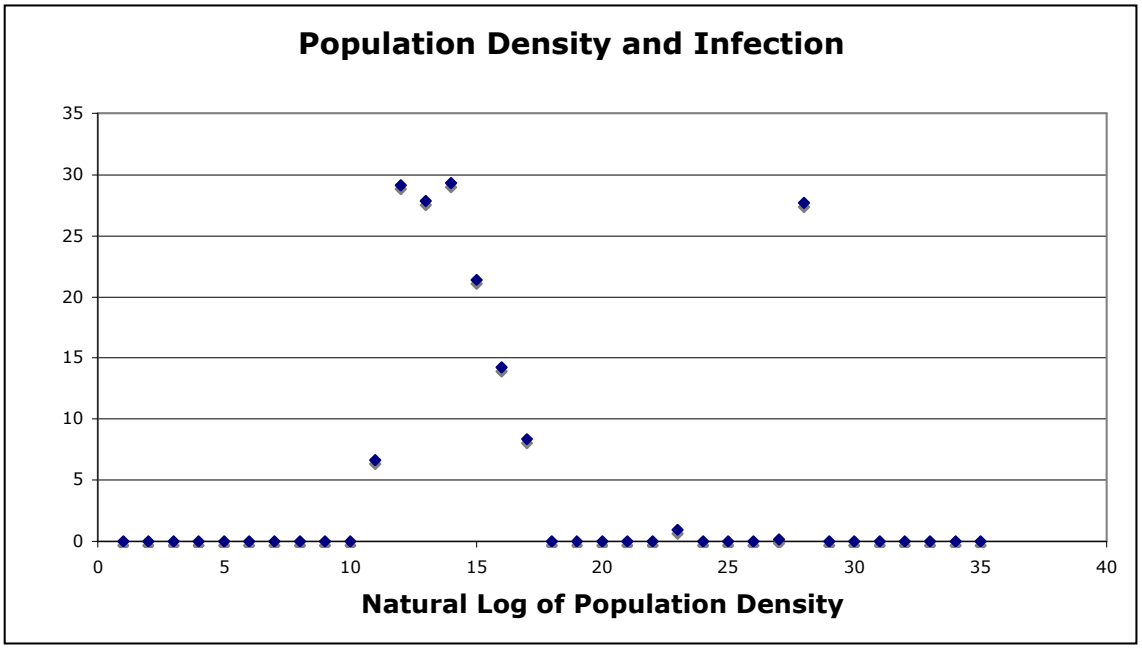


Figure 5 This figure shows the Natural Log of Population Density of *Asplanchna* versus the Infection Prevalence Percentage by *Pythium*. With a Pearson's correlation coefficient, of $r = 0.408$, and $p = 0.017$, these two variables are in a nonlinear, but statistically significant relationship.

Besides a correlation between the population density and infection prevalence it is also important to consider the instantaneous population growth rate (which is symbolized by the letter *r*). This value shows at any one time whether a population is growing ($r > 0$) or declining ($r < 0$) with respect to its population from the time of previous sampling. To determine the instantaneous population growth rate of the *Asplanchna* population the following equation was used:

$$r = \frac{\ln(N_{t+1}) - \ln(N_t)}{t}$$

where t is time and N is the population size

After finding the instantaneous population growth rate, for the *Asplanchna* population, *r* was graphed in relationship to the infection prevalence documented in the live samples. When graphed, it appears that the relationship between *Pythium* infection prevalence and the instantaneous population growth rate is a negative relationship (Figure 6). This could possibly indicate that when the *Pythium* parasite is present, the *Asplanchna* growth rate is negatively affected. However, when tested with a Pearson's correlation the results show that r = -0.012, and p = 0.974. A negative correlation coefficient indicates a negative linear correlation but a high p-value indicates that there is no statistically significant relationship between the two variables.

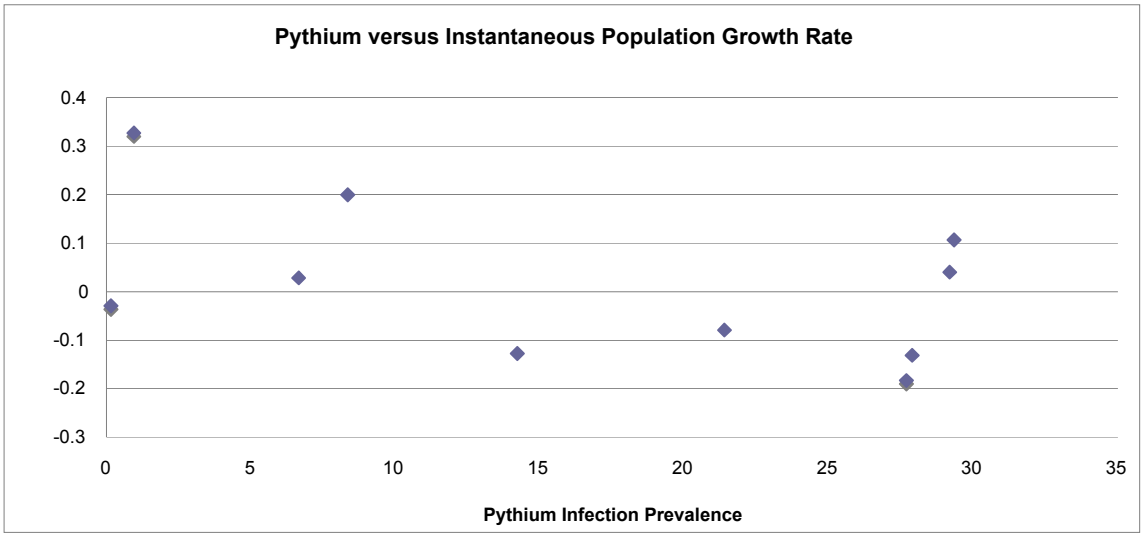


Figure 6 This scatter plot shows how the rate of infection affects the instantaneous population growth rate (r). It can be seen that infection rate does have a mildly negative impact on the Asplanchna population which is supported by a negative correlation coefficient of r=-0.012. However a high p-value of 0.974 indicates that this relationship is not significant.

Correlations between Infection Rates and Male prevalence

A negative correlation between infection prevalence and the population density is a classical sign of parasitism (Wetzel 2001). Even though in this particular study there was not a statistically significant relationship between the two, *Pythium* has already been shown in a previous study by Thomas et al. to reduce host fecundity, fitness and life span (Thomas et al. 2011). However, no correlation has yet been made to establish how much impact parasitism has on the sexual life cycle of *Asplanchna*. The percentage of males in the population is a significant indicator of sexual reproduction occurring in the population because *Asplanchna* normally reproduce asexually, and the presence of males in the population indicates that overall the population of *Asplanchna* has switched from reproducing asexually, to sexually. Therefore, the rate of *Pythium* infection was compared by date to percentage of males in the *Asplanchna* population (Figure 7). As seen in the graph, once the infection prevalence increased, so generally did the presence of males.

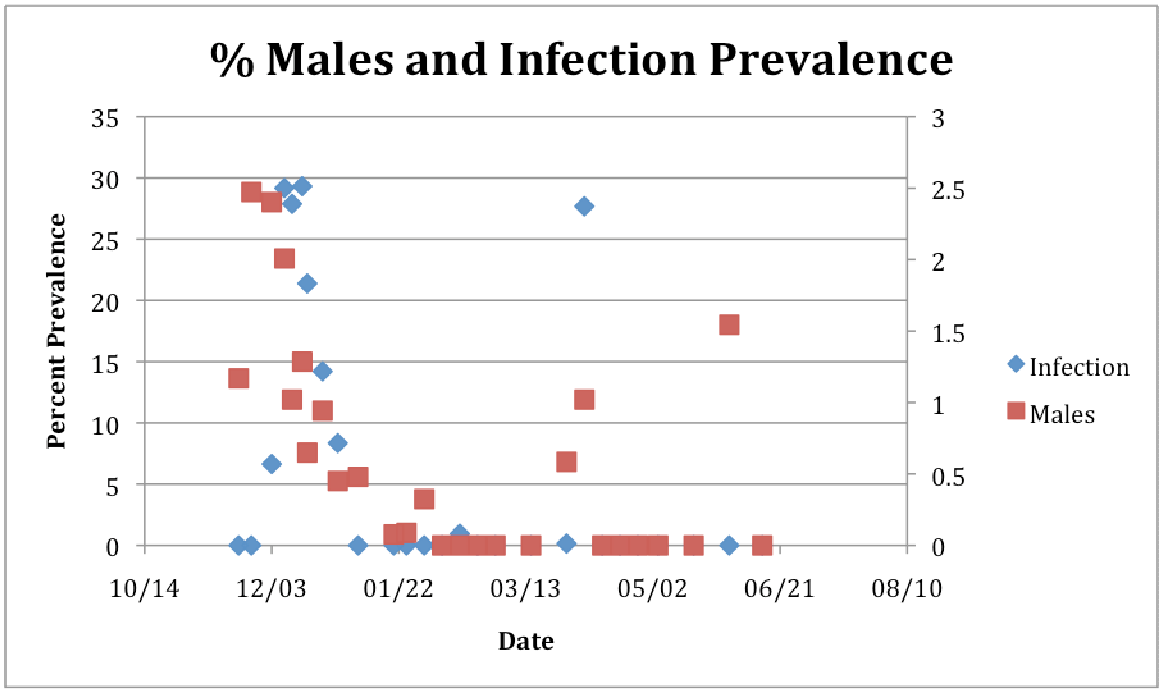


Figure 7 This figure plots the rate of infection along with the percentage of males present in the Population of Asplanchna. In general, the rate of infection parallels the same rises and falls as the percentage of males in the population.

Besides comparing the rates of infection and male presence by date, the two sets of data were also compared directly to one another (Figure 8). As infection prevalence increased within the population, so did the presence of males within the population. The Pearson's correlation coefficient was 0.447 ($p = 0.022$). This indicates that there is a statistically significant relationship between the two variables.

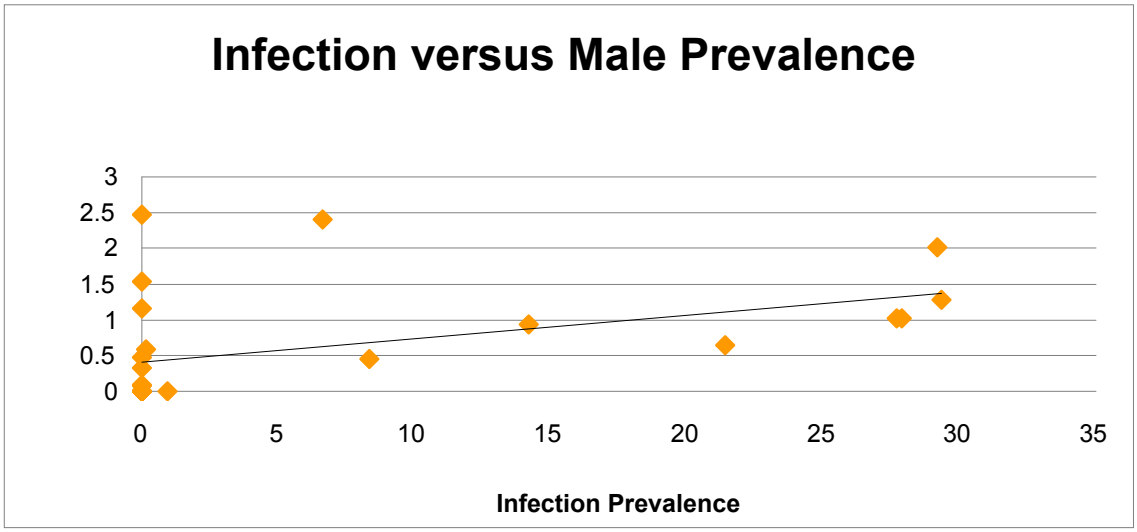


Figure 8 This scatter plot shows the infection prevalence in the Asplanchna population along with the percent of males found in the population. Overall there is a general trend upwards as shown by the linear trend line. The Pearson's correlation coefficient was 0.447 ($p = 0.022$). This indicates that there is a statistically significant relationship between the two variables. This data shows overall that as the Infection prevalence increases, the percentage of males in the population does as well.

The correlation between *Pythium* infection rate and the percentage of males in the population is certainly significant; however the correlation could also be due to another factor. As Figure 2 already indicates, there is an established significant correlation between the population density of *Asplanchna* and *Pythium* infection rate. Upon further investigation, there is also a correlation between the population density of *Asplanchna* and percentage of males in the population ($r = 0.482$, $p = 0.013$). A significant relationship such as this could indicate that the previous relationship between infection prevalence and males is only due to their respective relationships with *Asplanchna* population density.

CHAPTER 3

DISCUSSION

Finding any sort of correlation between infection prevalence and sexual reproduction in *Asplanchna* is of interest, because it could indicate that parasitism in this case can influence adaptation in its host. Since *Pythium* infections of *Asplanchna* are significantly correlated with male production, this provides preliminary evidence that infection by a parasite may actually increase genetic recombination in hosts. Because *Asplanchna* usually reproduce asexually, the increased presence of males in the population indicates a switch from the amictic reproduction to the mictic reproduction. This reproductive switch previously has been attributed to environmental factors such as population density, food abundance and temperature (Serra, Snell, & King, 2003). The results of this study suggest that mictic reproduction in *Asplanchna* may also be modulated by parasitic infection.

Study Limitations

The data collected by the Duffy Lab over the past three years clearly shows a seasonal pattern of *Asplanchna* abundance in Lake Clara Meer. This variation in population density can be attributed to various factors like temperature and food abundance. Dramatic increases in population density are observed regularly in the spring when temperature and food are both favorable for growth. Population density then drops off as temperatures plummet in the late fall and winter. Abiotic and biotic factors such as temperature, population density and food abundance, certainly play important roles in the cycling of the *Asplanchna* population. Unfortunately, it is not possible to isolate and separate these causes using observational field data. In addition, though there is a

correlation between *Pythium* infection and male abundance, both of those factors are also significantly correlated with population density. Again, isolating these factors from natural population data is not possible and, in reality, these three variables are probably all interacting in a natural setting.

By only looking at field data, this study provides a valuable insight into the natural occurrences of rotifer density trends, parasitic involvement and male abundance. However, the lack of lab experimentation provides opportunity for continued study. The results of these observations argue for various lab experiments to replicate the observations that we made in nature. Besides culturing the parasite and attempting to replicate these findings in lab, it would also be important to find out why there is a significant relationship between infection prevalence and male abundance. Because significant infection rates are only reached when population density was also high, it remains unclear if infection by can serve as an amplifier of the mixis signal in the *Asplanchna*. If a reliable way of infecting individuals in lab can be developed, an experiment could be designed in which the *Asplanchna* population had a high rate of infected individuals, but was not allowed to attain a high population density. If sexual reproduction was still initiated at similar rates as observed in nature, it could then be concluded that parasitism alone might serve as a mixis stimulus. Also, if in lab an *Asplanchna* population was allowed to grow and was observed regularly, the average density threshold at which *Asplanchna* switch to sexual reproduction could be found. Experiments could be conducted to determine whether infection with the *Pythium* parasite alters the density threshold for mictic reproduction in *Asplanchna* populations. If

sexual reproduction was then initiated this would support the idea that parasitism is a significant stimulus for mictic reproduction.

The main hindrance to conducting these in lab experiments is the fact that the parasite, *Pythium*, has not been able to be grown, cultured or even transmitted with great consistency in lab. The best known laboratory method of infection by this parasite is to take an infected *Asplanchna* individual from a field sample and place it in a beaker with other non-infected *Asplanchna*. This method is effective only in spreading and keeping the disease present in lab cultures. No effective or quantitative way of transmitting the disease without a starting host has yet been discovered.

Conclusion

Although lacking laboratory evidence, the findings of this study still suggest a hypothesis that *Pythium* infection modifies the mixis response of *Asplanchna* populations. Sexual reproduction involving males provides for *Asplanchna girodi's* only means of genetic recombination. This study shows that there is a correlation between the rate of infection by *Pythium* and the density of males in the *Asplanchna girodi* population. Infection by this parasite could therefore be associated with increased sexual reproduction and genetic recombination of *A. girodi* populations.

Observation of the relationship between infection prevalence and male abundance in nature gives rise to questions about the relationship between parasite and host evolution. If further studies in lab can be done, it is quite possible that these would also support the idea that parasitic infection can alter the rate of genetic recombination of its rotifer host and therefore its evolutionary trajectory.

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