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Parasite Variability in the Invasive Crayfish, Faxonius rusticus, in Northern New Jersey

Nathan Martin Klunk

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Abstract

The Northeast United States has been thoroughly invaded by the well-known invasive crayfish species *Faxonius rusticus* (rusty crayfish). Similar to the exploration of man to new regions, the spread of invasive species can cause the introduction of new diseases and parasites while conversely the invader has to deal with the already existing diseases and parasites. For crayfish, the most notable of these is a fungal plague, which has a poorly understood distribution in the United States. In this study, I collected rusty crayfish in Northern New Jersey to better understand the locations where they exist in the state. I also dissected a sample of collected individuals to look for various parasites or signs of parasitism. My results suggest that rusty crayfish are more likely to experience parasitism while in a cobble substratum compared to a mud substratum. In addition, most observed signs were primary indicators of the fungal plague. Although rusty crayfish and the fungal plague are well understood separately, these findings indicate that the two organisms need to be looked at more closely together to further clarify how impactful the plague truly is to the rusty crayfish population.

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MONTCLAIR STATE UNIVERSITY

Parasite variability in the invasive crayfish, Faxonius rusticus, in Northern New Jersey

By

Nathan Martin Klunk

A Master's Thesis Submitted to the Faculty of

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A THESIS

Submitted in partial fulfillment of the requirements

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Montclair State University

Montclair, NJ

2022

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Introduction:

As various changes happen to our planet, species will continue to evolve, move, and be displaced from one area to another. One of these rising movements is the addition of exotic and invasive species to new locations. Before we continue, it is important to clarify the differences between these two terms. The term 'invasive species' describes species that are actively harming the ecosystem at hand. Invasive species can be a native or non-native to a region. Native invasive species occur when a disturbance of some sort, generally anthropogenic, provides them with ideal habitat to rapidly reproduce and grow without limitations. This expansion then becomes detrimental to the ecosystem and creates a less viable yet stable environment (Valéry *et al* 2009). The term 'exotic species' is used to describe organisms introduced to a new region outside of their home range, but which may not necessarily be causing harm. Exotic invasive species combine the two defined terms above, being introduced into an ecosystem outside of their home range and becoming harmful to the environment around them. One such exotic invasive to the state of New Jersey is *Faxonius rusticus*, commonly known as the Rusty Crayfish.

F. rusticus is a freshwater crustacean native to the Ohio River Basin. However, it has recently expanded North and Northeast from this region and begun thriving in areas of new introduction. The mechanisms of this expansion are believed to be movement through the water channels connecting these areas, but they have had a large help in the invasion process from humans, particularly anglers and aquarium owners. Northern New Jersey is no exception to this invasion with the first recorded sighting in 1968 (Durland Donahou *et al* 2019).

The organism itself has the ability to survive in a wide range of areas due to its ecology and size. For crayfish, this species is rather large reaching maximum lengths of 10 cm, with males being larger than females. It is believed they reach maturity by a length of 3.5 cm (Durland Donahou *et al* 2019). Ecologically, rusty crayfish do not dig into sediment like other species of crayfish and, therefore, prefer cobble habitat in the ecosystem for cover. Due to this, the organisms tend to live in shallow waters that receive well-oxygenated waters year round (Durland Donahu *et al* 2019). Adults tend to thrive in waters less than 1 m deep but greater than 20 cm while juveniles live near the water edge where the depth is less than 15 cm. However, the species has been reported in Lake Michigan at a depth of 14.6 m (Durland Donahou *et al* 2019). It also appears that this crayfish can live in both flowing water and still water systems. Equally important, the crayfish has a large range of temperatures it can survive in. Although they thrive between 20 and 25 °C and juveniles reach maximum growth rates between 25 and 28 °C, they are known to successfully live in a temperature range of 0 and 29 °C based on the Ohio Basin seasonal temperature fluctuations (Durland Donahou *et al* 2019).

Similar to other invasive species, rusty crayfish have been able to thrive in part by escaping stressors, such as natural competitors, predators, and parasites. However, native parasites of the invaded regions have been recorded infecting the rusty crayfish. For example, rusty crayfish have been known to succumb to parasitism from a trematode in the genus *Microphallus* (Sargent 2014). The trematode enters via the gills and moves to the hepatopancreas. This trematode has also been found to infect other crayfish species besides *F. rusticus* (Sargent 2014). In addition, crayfish have been recorded to have annelids attached to their carapace or gills. These annelids are generally considered to be ectosymbionts, but some studies have suggested a pathogenic result if it attaches to the gills. Other parasites in New Jersey that are known to infect native crayfish species include oomycetes on spiny-cheek crayfish (*Faxonius limosus*), protozoans in the common crayfish (*Cambarus bartonii*), and trematodes from Macroderoididae in White River crayfish (*Procambarus acutus*) (Longshaw 2010).

The most notable of crayfish parasites are the fungal plagues found originally in North America. Caused by the oomycete *Aphanomyces astaci*, the plague currently ranks in the top 100 deadliest invasive species of the world (Bouallegui 2021); this can be attributed to its now large distribution and mortality rate. For species like *Astacus astacus* (noble crayfish) and *Pacifastacus leniusculus* (signal crayfish), the plague has been found to have a mortality rate as high as 100% (Makkonen *et al* 2014; Jussila *et al* 2014) in invaded locations such as Finland (Makkonen *et al* 2014; Jussila *et al* 2014), Taiwan (Hseih, Huang, and pan 2016), and Brazil (Peiró *et al* 2016) just to name a few; some first detections are still being found today in South America and Latin America in locations like Costa Rica (Martín-Torrijos *et al* 2021).

In addition, *A. astaci* can be split into five different strains based on genotype. These being *Astacus* strain (As), *Pacifastacus* strain I (PsI), *Pacifastacus* strain II (PsII), *Procambarus* strain (Pc), and *Orconectes* strain (Or) (Bouallegui 2021) with each strain named after the crayfish species it was first found on. These being the noble crayfish (As), signal crayfish (PsI and PsII), *Procambarus clarkii* (Louisiana Crayfish, Pc) and *Orconectes limosus*, which is now *F. limosus*, (spinycheek crayfish, Or). Due to this large pool of variations, the fungal plague has greatly aided invasive species in their takeover of invaded environments (Bouallegui 2021). Luckily, large documentation exists of this plague in invaded environments as mentioned above. However, information is lacking about the disease in its native continent, which is North America.

Despite the mass studies on the topic, *A. astaci* currently does not have a lot of data in the United States nor is its distribution well delineated at this moment in time (Martín-Torrijos *et al* 2021). Most studies primarily focus on invaded areas with a few exceptions. Specifically in terms of *F. rusticus*, there are a few studies showing low levels of the parasite, including in the lower Susquehanna Watershed in Pennsylvania (Butler *et al* 2020). Therefore, it is extremely important at multiple levels to look for signs of this fungus in Northern New Jersey as well.

This invasive population of F. rusticus mixed with the ability of crayfish parasites to invade multiple species makes Northern New Jersey a desirable region to survey crayfish parasite populations. Such a survey could give us a better understanding of how the community at hand may be adapting to the invasive species. As mentioned above, native *Microphallus* in Wisconsin are known to parasitize the invasive crayfish (Sargent 2014). Therefore, similar interactions may occur with native parasites in Northern New Jersey as well. This research can serve as an insight for further research on evolving parasite-host interactions occurring with invasive populations of F. rusticus. In addition, it will ideally recognize potential sightings of fungal plague infection on rusty crayfish that many studies within the United States have not done yet.

Methodology:

Using USGS and local fisherman knowledge, I selected three locations to collect specimens in the Passaic Watershed (Figure 1). These locations were designated Ringwood Creek, Rockaway River, and Ringwood 2. Ringwood Creek is a cobblestone substrate stream with large amounts of shrubbery along its banks and a few trees. Rockaway River is a mud substrate creek with a mass of trees along its banks and a few shrubs. In addition, there is a large proportion of aquatic vegetation toward the center of the river. Ringwood 2 is a tributary creek for the Ringwood River and has a mud substrate as well where observations were taken. The vegetation was similar to rockaway and, instead of aquatic vegetation, had sparse chunks of cobble spread throughout.

At each location, I hand netted for roughly an hour followed by crayfish traps, which I baited with raw chicken (usually drumsticks). Traps were placed every other week starting in June. I collected *F. rusticus* as long as they had a large dark red spot on the carapace, which is a primary

and unique indicator for identification of the species. If the spot was present, but difficult to see, I also confirmed identification by the purple coloring on the claws and blue coloring on the legs. I did not keep individuals if they did not meet a 3.5 cm minimum in length, excluding claws, to prevent/limit juvenile collection. At each location, I measured pH, general hardness (GH), flow rate, and temperature for abiotic factors. I used a handheld pH meter (Oakton AO-35423-01 EcoTestr pH 2+) to measure pH and temperature. I measured flow rate with a stopwatch, tennis ball, and measuring tape. Specifically, I used large visual markers (i.e. trees/large rocks/etc.) where the traps were set, which were the same markers during each recording at each location, and placed a tennis ball at one and let it float to the other. The stopwatch started once I took my hand off the tennis ball and stopped once it reached the other marker. Then, I would measure the distance from the start and end point to. The time and distance were then used to calculate flow rate. I recorded GH with LaMotte Insta-Test strips. I performed each abiotic test at the location of trap placement and every day of collection/trap checking.

Upon collection, I initially examined individual crayfish for signs of parasitism on the abdomen and carapace as long as they met the previously stated length minimum. I looked for worm trails on the Carapace and red spots/opaqueness on the ventral side of the abdomen. Post-examination, I put the crayfish in a -20 °C freezer until a later date for dissection.

At a later date, I unthawed individual F. rusticus prior to handling by setting them out at room temperature for between 30 to 45 minutes (size dependent). I gave another external examination for potential parasitism using similar signs noted above under a dissecting microscope. I then cut open the crayfish starting with the cephalothorax. I made initial incisions on the cephalothorax by cutting caudally to cranially starting where it meets the abdomen and laying with its dorsal side up. Then, I thoroughly examined the gills to look for any abnormalities from possible parasitism. If I found abnormalities, I removed and preserved them in ethanol for later examination. I proceeded to move cranially to check the antennal glands for abnormalities and removed abnormalities if found. I finished a dissection by looking abdominally for any signs of fungal infection internally, especially if I noticed an opaque abdomen externally. I made incisions cranially to caudally where the carapace and abdomen met and, again, with the dorsal side up. I proceeded to slowly remove the muscles within the abdomen peeling it back from caudal to cranial. I examined both the muscle and inside of the exoskeleton for abnormalities. Specifically, I looked for potential calcifications (shown in Figure 2) on non-calcified locations of the body for this is a primary sign of the fungal plague. If anything was preserved, I looked at it under a microscope to identify if it was a parasite or not.

To analyze the data, I performed ANCOVAs on the Date and Site data and performed ANOVAs on the abiotic data. All of this was performed in RStudio. Site and Date were first tested for independence. Then, I ran them through ANCOVAs for their percent of parasitized crayfish data. I ran this under the null hypothesis that neither site nor date significantly contributed to the amount of percent of caught crayfish parasitized and using an alpha of 0.05. I then ran post-hoc analysis on any significant results. This again was tested with an alpha of 0.05 and under the null hypothesis that no two sites/dates had significantly different means when compared to each other one on one. For the ANOVAs, I performed 1 ANOVA per abiotic factor and set an alpha for 0.05. This was tested under the null hypothesis that no factor showed a significant difference among site means.

Results:

A total of 24 crayfish all representing the target species were collected from the three sampled locations (shown in Figure 1). No bycatch species were caught throughout the experiment. Ringwood Creek had 15 individuals collected from it while the other two sites amounted to nine individuals combined (four from Rockaway River and five from Ringwood 2). Over the course of the experiment, Ringwood Creek had a mean of 3 crayfish collected per visit (standard deviation (SD)= 2.24), Rockaway River had a mean of 0.67 (SD=0.84), and Ringwood 2 had a mean of 1 (SD=1) crayfish collected (Table 1; Figure 2).

In total, 14 individuals expressed external signs of infection; 11 from Ringwood Creek, two from Rockaway River, and one from Ringwood 2. Ten crayfish lacked obvious signs of parasitism. The percent of crayfish parasitized can be seen in Figure 4. The 14 infected individuals also expressed unusual calcifications on their soft-bodies, specifically their abdomen, shown in Figure 4. Looking by dates, July 6 had a mean of 1.67 crayfish collected per site (SD=2.08), July 7 had a mean of 1.67 individuals (SD=1.15), July 20 had a mean of 0.67 individuals (SD=1.15), July 22 had a mean of 2 individuals (SD=3.46), and August 5 had a mean of 2 individuals (SD=0), which is shown in Table 2 and represented in Figure 5. In addition, the percent of crayfish parasitized can be seen in Figure 5. A test of independence was performed on both sites and dates under the null hypothesis that they are independent of percent of caught crayfish parasitized. It resulted in p-values of 0.839 (site) and 0.911 (date) and therefore the null hypothesis could not be rejected (shown in Table 3). In addition, an ANCOVA was performed on sites and dates under the null hypothesis that neither site nor date significantly contributed to the amount of percent of caught cravfish parasitized. This analysis resulted in p-values of 0.77 (sites) and 0.05 (dates). Therefore, I marginally fail to reject the null hypothesis for dates significantly contributing to percent of caught crayfish parasitized. Given this result, a post-hoc was still performed, due to proximity to the alpha, based on dates under the null hypothesis that no two dates compared have different means. This resulted in a marginally insignificant result of 0.07, which we fail to reject the null hypothesis with..

During collection, flow rate (Figure 7), pH (Figure 8), temperature (Figure 9), and GH (Figure 10) have notable distributions that can be seen in their respective plots. Ringwood Creek had a mean flow rate of 0.11 m/s (standard deviation of 0.03), GH of 36.67 ppm (standard deviation of 13.23), pH of 7.93 (standard deviation of 0.1), and 22.26 °C (standard deviation of 2.84). Rockaway River had a mean flow rate of 0.13 m/s (standard deviation of 0.02), GH of 60 ppm (standard deviation of 0), pH of 8.01 (standard deviation of 0.13), and temperature of 22.88 °C (standard deviation of 2.05). Ringwood 2 had a mean flow rate of 0.05 m/s (standard deviation of 0.01), GH of 36.67 (standard deviation of 13.23), pH of 7.78 (standard deviation of 0.16), and temperature of 22.93 °C (standard deviation of 2). All of which can be shown in Table 4. The abiotic factors were run under an ANOVA under the null hypothesis that there was now significant difference among site means for each factor. As shown in Table 5, flow rate (p-value of <0.001), GH (p-value of <0.001), and pH (p-value of 0.002) reject the null hypothesis while temperature (p-value of 0.79) fail to reject the null hypothesis.

Discussion:

The goal of this study was to create a deeper understanding of Faxonius rusticus presence in Northern New Jersey and the parasite loads harbored by these crayfish at these locations. My results suggest that rusty crayfish are certainly present in Northern New Jersey in the Rockaway River and tributary creeks in the Ringwood area. In addition, these are likely well-established populations that may have dispalaced native species given the lack of bycatch species throughout the experiment. I also found signs of parasitism/infection present within these crayfish at all the sites. However, my results suggest that the sites themselves were not key contributors to parasite load differences among locations. This is based on the large p-value of 0.77 from the ANCOVA performed on the data. However, the date did result in a p-value of 0.05 after running an ANCOVA and, after Post-Hoc analysis, specifically showed marginal insignificance at a 95% confidence interval from July 7 to July 20, which had a p-value of 0.07. July 7 had 5 crayfish caught on that day while July 20 only had 2, which means there was a site with no crayfish caught. This could be one potential explanation for this difference. Another potential natural influencer could have been weather. During that week of 2021, there was a large storm the day prior and could greatly affect the number of mobile crayfish at that particular time. With the storm surge, they could have been seeking refuge in cobble and/or foliage and waiting out the harsher water flow. For example, Ringwood Creek recorded a peak flow rate during July 20 of 0.15 m/s while July 7 was its valley at 0.07 m/s.

Abiotically, we can conclude that all three sites had similar abiotic conditions throughout the summer only for temperature, which had a p-value of 0.79. From a logical point of view, the 3 streams are in the same regional area and all three streams had the same general observational look, specifically tall foliage overhang allowing for plenty of sunlight with brush on the bank. The only difference in this general look is that Ringwood Creek had a cobblestone substratum while Ringwood 2 and Rockaway River were mud substratum. As for flow rate, general hardness (GH), and pH, all three resulted in p-values lower than or equal to 0.002. Therefore, the sites varied in some form on these factors. If you look at Figures 7-10, you will see that Flow Rate and pH shows a great significance due to Ringwood 2 (RW) while GH shows a great significance due to the Rockaway River.

Combining both, the data further supports the presence of *Faxonius rusticus* in Northern New Jersey. Although parasitism's presence seems uninfluenced based on site, this may be an artifact of small sample sizes and should still be considered in future studies. Meanwhile, the potential significance in time of year and how the environment during that time of year differs should continue to be looked into. Late July in the Northeast United States is very much known for mass amounts and large thunderstorms so it is possible that the post-influences of these storms may affect the crayfish and parasitism presence. For example, the larger flow rates may be preventing settling of sediment and microorganisms; this creates a natural barrier to avoid parasitism and may be a cause for the difference found among dates. In addition, visual confirmation of fungal plague presence in Northern New Jersey was recorded via the calcification picture in Figure 2, which was recorded on the primary indicator for those parasitized in this study.

As an ongoing global problem, there is a large need for data in the United States about the disease and a continually need for more environmental data in invaded/natural areas (Martín-Torrijos *et al* 2021; Butler *et al* 2020). By using this study, the scientific community now has a baseline knowledge of potential variations that may lead to larger pools of infections in various crayfish species, specifically for non-burrowing specimens, and a very valuable starting point for identifying the potential presence of Fungal Plague in Northern New Jersey, which has not been

done prior. For future studies, I strongly recommend building upon this data in New Jersey. Despite the small dataset, the data that has been collected shows a significant reason to collect more data and move toward molecular confirmation of soft-bodied calcifications found on individuals. This data, in turn, can be used to proliferate the mass confirmation of the fungal plague's presence in the Northeast United States and, overall, move to aiding the conservation efforts that are needed globally toward this deadly invasive species.

Conclusions:

This report is able to add data to a fairly barren pool of published data for *F. rusticus* presence in Northern New Jersey. In addition, it is the first report concerning potential *A. astaci* infection within this *F. rusticus* population. This will enable further investigation of *A. astaci* presence in Northern New Jersey and aid toward our understanding of this global pandemic as a whole.

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1				
	Mean	Standard Deviation		
Ringwood Creek (RC)	3	2.24		
Rockaway River (RR)	0.8	0.84		

Table 1: This table shows the mean and standard deviation of the number of crayfish caught each trip to each Site.

Table 2: This table shows the mean and standard deviation of the number of crayfish caught for during each date crayfish were caught.

1

	Mean	Standard Deviation
July 6, 2021	1.67	2.08
July 7, 2021	1.67	1.15
July 20, 2021	0.67	1.15
July 22, 2021	2	3.46
August 5, 2021	2	0

Table 3: The table above depicts the results of an ANCOVA test on Site and Date under the null hypothesis that neither would influence the percent of parasitized crayfish. As shown, Date rejects the null hypothesis with a p-value of 0.05 unde a 95% confidence interval.

	Test	Degrees	
	of	Of	
	Independence	Freedom	P-Value
Site	0.839	2	0.77
Date	0.911	4	0.05

Ringwood Creek 2 (RW)

1

	Mean	Standard Deviation
Flow Rate (RC)	0.11	0.03
GH (RC)	36.67	13.23
pH (RC)	7.93	0.1
Temperature (RC)	22.26	2.84
Flow Rate (RR)	0.13	0.02
GH (RR)	60	0
pH (RR)	8.01	0.13
Temperature (RR)	22.88	2.05
Flow Rate (RW)	0.05	0.01
GH (RW)	36.67	13.23
pH (RW)	7.78	0.16
Temperature (RW)	22.93	2

Table 4: The table above depicts the mean and standard deviation for the distributions of flow rate (m/s), GH (ppm), pH, and temperature (°C) at each site.

Table 5: The above table depicts the ANOVA results for the abiotic factors tested under the null hypothesis that the sites means for each factor do not differ. As seen, flow rate, GH, and pH all reject the null hypothesis.

	Degrees of Freedom	P-Value
Flow Rate	2	<0.001
GH	2	<0.001
pH	2	0.002
Temperature	2	0.79



Figure 1: The Figure below depicts the major waterways of Northern New Jersey. Each black dot represents a site used in this study with the two most northern dots being from tributary creeks and the most southern dot being from the Rockaway River (New Jersey Rivers and Lakes 2022).



Figure 2: This image depicts a calcification caused by fungal infection of the soft areas of the abdomen.



Figure 3: The figure above depicts the distribution of crayfish caught at each site throughout the capturing period.



Figure 4: This figure depicts the spread of percent of crayfish parasitized at each site throughout the capturing period.

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Crayfish Caught By Date



Figure 5: This graph depicts the distribution of crayfish caught based on dates when crayfish were collected.



Figure 6: This graph depicts the percentage of crayfish parasitized for each date crayfish were obtained.





Figure 7: This graph depicts the flow rate distribution in each site throughout the duration of the study in order to compare the means of each site.



Figure 8: The above figure depicts the distribution of pH in each site throughout the duration of the study in order to compare the means of each site.



Figure 9: The graph depicts the distribution of Temperature in each site throughout the summer in order to compare the means of each site



Figure 10: The above graph depicts the distribution of general hardness (GH) in each site in order to compare the means of each site.

GH per Stream