POTENTIAL IMPACTS OF *ORCONECTES RUSTICUS* (RUSTY CRAYFISH) ON WILD RICE IN THE 1854 CEDED TERRITORY

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INTRODUCTION

Orconectes rusticus (rusty crayfish) is the only confirmed species of invasive crayfish in the 1854 Ceded Territory in NE MN. *O. rusticus* prefer rocky substrate, but have been found to inhabit sand, silt, clay, and gravel. They prefer warmer temperatures, but can tolerate cool water and are usually found in shallow areas. *O. rusticus* do not possess an ability to burrow, and require clear and well- oxygenated water to survive. [1]

O. rusticus has been found to graze heavily on germinating aquatic vegetation, and are capable of displacing and reducing the diversity of native macrophytes. [1,2] Introductions of *O. rusticus* have shown detrimental impacts on macrophyte populations around the littoral zone of lake systems. In some instances, reductions of macrophyte populations by as much as 80% have occurred. [1] There have also been suggestions that the seed bank of these infested areas has potential for depletion as an infestation prevents new macrophyte populations from germinating and producing new seedbank stock. [2] Evidence of seedbank depletion incentivizes a need for control of rusty crayfish populations to avoid the need for manual planting and restoration of native aquatic flora.

The 1854 Treaty Authority has interest in the effects of *O. rusticus* on macrophyte communities that produce native wild rice (*Zizania palustris*) within the 1854 Ceded Territory. Wild rice is a culturally significant plant that provides sustenance in many forms to the Bois Forte and Grand Portage Chippewa bands. Wild rice typically grows best in shallow depths of 1-3 feet in areas containing soft, organic bottoms. In mid-June, wild rice reaches the "floating-leaf" stage at which point wild rice lays flat on the surface of the water and can form dense leafy mats. In July, wild rice begins to emerge vertically and can stand out of the water up to 6-8 ft. tall. In August and September ripe seed can be harvested or will fall in the water to germinate the next year. However, if conditions are not favorable wild rice can remain dormant for several years until ideal conditions are present. Wild rice is also at risk of being outcompeted by vegetation such as pickerelweed, water shield, and water lilies. [3]

Wild rice populations have been observed to decline in some areas infested with *O. rusticus*, but whether this impact is directly related to an infestation is unknown. The 1854 Treaty Authority conducted a study from 2013 to 2016 testing for the potential impacts of *O. rusticus* on wild rice. From 2013-15 the study focused on if *O. rusticus* negatively impacts wild rice populations, and in 2016 the study addressed the questions of which stages of wild rice growth can *O. rusticus* affect, and if wild rice is a preferred food source.

METHODS

Structures

Studying the effects of *O. rusticus* on wild rice was done by creating structures meant to either completely contain or exclude rusty crayfish and placing them in wild rice beds from early spring (before germination) to late summer (after maturation). The structures were placed in various lakes and locations around the Ely, MN and Isabella, MN area that had naturally occurring wild rice and documented populations of rusty crayfish. Structures that contained rusty crayfish are referred to as "enclosures". The purpose of an enclosure is to test for direct impacts of rusty crayfish on wild rice in forced conditions by stocking a pre-determined number of rusty crayfish inside the enclosure and monitoring the growth of wild rice inside compared to outside the structure. The structures meant to exclude crayfish are referred to as "exclosures", their purpose is to act as a barrier so wild rice growth inside can be monitored without any interference from rusty or native crayfish and compared to rice growth outside the structure.

The structures have gone through a series of transformations to successfully serve as either a cage or barrier. However, the basic design has remained the same being approximately 10 ft. x 10 ft. with a mesh netting protruding above the surface of the water, and a weight attached to the bottom of the netting to seal it to the substrate. Each side panel (shown below) is five feet wide and six feet tall. The most successful construction was in 2016 using mesh netting (¼" x ¼") on top sewn to plastic mesh skirting (¼" x ¼") on the bottom and with 2" diameter sand bags attached to the plastic netting, using rebar as the supports for the structures (see photos below). Bird netting was also attached to the top of the enclosures in 2016 to prevent crayfish from climbing out of the structures. Coordinates for the locations of the structures can be found in Table 1.

Preliminary design for the structures in 2016. The wood support was removed and the chains were replaced with 2" diameter sandbags.

Final structural design after installation, 2016.

In 2013 one exclosure was placed in Farm Lake (MN DNR lake I.D. # 38077900) and one exclosure was placed in White Iron Lake (MN DNR lake I.D. # 69000400) on 6/3. The exclosure in Farm Lake did not adequately deter rusty or native crayfish from entering the wild rice bed inside the structure and was thus removed from the study after approximately one month on 7/9. However, the exclosure placed in White Iron Lake did prevent a significant number of crayfish from entering inside and was left in place for the duration of the summer season and removed on 9/3.

In 2014 one exclosure was placed in Garden Lake (MN DNR lake I.D. # 38078200) and one exclosure was placed in Farm Lake. Both were installed on 6/6 when rice was already seen to be germinating, and removed on 10/8.

In 2015 one enclosure was installed in Dumbbell Lake (MN DNR lake I.D. # 38039300) on 5/19 and removed 9/1. An eight -inch small mouth bass was observed inside the enclosure on 6/11, suggesting a tear in the netting. A backpack electrofishing unit was used to remove the bass on 6/19. Wild rice was seen to be germinating in May, but heavy disturbance occurred during installation, possibly affecting wild rice growth on the perimeter and outside the enclosure. It was stocked with 75 rusty crayfish on 5/19 and 68 rusty crayfish on 7/16.

In 2016 three structures were placed in Dumbbell Lake. Two were enclosures (one enclosure containing an alternative food source), and one was an exclosure. The exclosure and the enclosure without an alternative food source were in the same wild rice bay in near proximity to each other, thus labeling this enclosure the "pair" enclosure. The enclosure containing the alternative food source was labeled the "food-plot" enclosure and was in the NE bay. All three of the structures were installed on 5/24 and removed on 8/16. The pair enclosure was stocked with 51 male rusty crayfish on 5/24, with a fluorescent latex orange tag injected under the exoskeleton in the ventral side of the tail used as an identification marker. The food- plot enclosure was stocked twice throughout the summer, once with 50 male rusty crayfish on 5/24 marked with a fluorescent yellow tag, and once with 50 male rusty crayfish on 7/18 with no tag to test for impacts on wild rice after emergence. The food-plot enclosure was stocked bi-weekly with one cucumber, one green pepper, and one head of lettuce attached to the inside of the mesh netting approximately one to two feet below the surface of the water (see photos below).

Alternative food source preparation. Vegetation was attached to netting using zip ties. One cucumber, green pepper, and head of lettuce were used.

Food source attached 1 - 2 feet below surface of the water and was evenly distributed around the inside netting of the enclosure. The red plants are water lilies growing naturally inside.

Observations

The aim in every year of the study was to check the structures biweekly. The main observation that was recorded was wild rice growth, and what was happening to wild rice on the inside of the structure as compared to the outside. In the exclosure studies the wild rice inside would ideally be unaffected by rusty or native crayfish and the wild rice outside would be exposed to the study area's normal crayfish population. In the enclosure studies the wild rice inside would be subject to high density populations of rusty crayfish, and the wild rice outside would be exposed to a normal population of rusty crayfish and would show unbiased rice growth. Observations included making note of wild rice growing inside and/ or outside the enclosure, and taking pictures of wild rice growth at each bi-weekly check. Wild rice density was measured at the end of each season, and is discussed in more detail below.

Water depth and water temperature were also recorded during the duration of each study. Wild rice can be particularly susceptible to changes in the water level, especially during the floating- leaf stage of growth, which usually occurs in June. Monitoring water temperature has also aided in monitoring climate change as conditions become more favorable to invasive species such as rusty crayfish. Other factors, such as human disturbance, beaver activity, flooding, storms, strong winds, and other weather patterns can also affect the growth of wild rice and were recorded if they were observed.

The number of rusty and native crayfish found both inside and outside the structures was also recorded throughout the duration of each season. In the enclosures, rusty crayfish were visually monitored on a bi-weekly basis. The ability of the rusty crayfish to lay below the sediment often made it difficult to get an accurate visual representation of the number of individuals remaining inside. Any crayfish visually observed in the surrounding area were also recorded. In the exclosures any crayfish visually observed inside or in the surrounding area were recorded. Trapping occurred both inside and outside of the structures several times throughout each of the seasons and is discussed more in depth in the sections below.

In 2016 two new unique goals of the study included 1) determining if wild rice was a preferred food source and 2) identifying which stages of wild rice growth *O. rusticus* can affect. Specific observations to answer these questions included recording the amount of the alternative food source that was depleted on a bi-weekly basis, the presence or absence of wild rice inside the enclosure, the rice density inside the enclosure as compared to outside, and observing and recording changes in wild rice growth with a new introduction of rusty crayfish when the rice was already in its emergent phase.

Crayfish Trapping

Modified minnow traps were baited with frozen fish (white sucker) and left for approximately a 24 -hour period to capture crayfish. Trapping crayfish served several purposes including 1) trapping in the enclosures established a re-capture rate, testing how many crayfish from the initial stocking remained inside 2) trapping outside of the enclosures and exclosures established normal catch per unit effort (CPUE) estimates for both native and invasive crayfish in the test area and enclosure escape rates and 3) trapping inside the exclosures tested the impenetrability of the barrier by testing how many gained access and at what size.

In 2013, crayfish were trapped eleven times in Farm Lake from early June until mid-July when the structure was removed. Trapping occurred nine times in White Iron Lake including three times in June, three times in July, twice in August, and once in September. Typically, one trap was set inside and one was set outside the exclosures. On 7/29 and 8/15 one trap was placed inside the exclosures and six to seven traps were placed outside the exclosures.

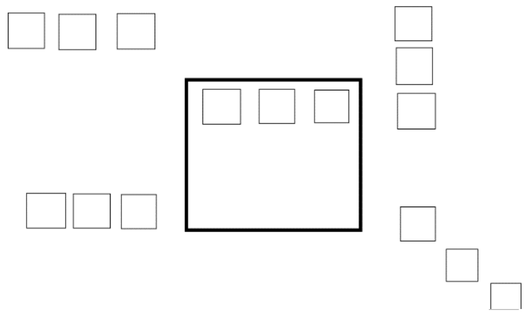
In 2014, crayfish were trapped eight times throughout the summer in both Farm and Garden lakes. Six trappings took place in June, and one took place in July and August. Typically, one trap was set inside and one was set outside the exclosures. On 8/20 one trap was set inside the exclosures and five were set outside in the nearby surrounding area.

In 2015, crayfish were trapped twice, and electro-shocked once. The trapping occurred once in July and once in September. The electro-shocking occurred in June, but was unsuccessful in surfacing any crayfish. Three traps were set inside the enclosure in each trapping event, and six traps were set outside in near proximity to the enclosure in August.

In 2016, crayfish were trapped a total of three times; once in mid-June, once in mid-July, and once in mid-August. The food- plot and pair enclosure each had two traps placed inside and two traps placed outside in each trapping event. The exclosure had one trap placed inside in each trapping event. Due to the exclosures proximity to the pair enclosure, the same outside CPUE was used for both structures with no additional external trapping taking place.

Wild Rice Density Measurements

Densities were measured inside and outside the structures to gauge differences in rice growth and survival. The general measurements of the rice involved the counting the number of stalks in a ½ m^2 plot. Three ½ m^2 plots were measured inside the structures, and a total of twelve ½ m^2 plots were measured outside of the structures (three progressing out from each corner, see illustration below). Starting in 2015 the outside densities were measured starting a ½ meter away from the structure to account for any disturbance during the installation of the structures. The densities were then averaged for the inside and the outside and compared to see if there were any significant differences.



Different ways density measurements can occur. Dark black line represents 10 ft. x 10 ft. structures. Lighter grey boxes represent density measurements in a ½ m² plot. They are arranged in some of the ways the density measurements took place. Three ½ m² plots were measured and averaged for the inside wild rice density and twelve ½ m² plots were measured and averaged for the outside wild rice density.

RESULTS

Observations

Temperature (Figure 1) and water depth (Figure 2) were recorded every season and compared to the average growth of wild rice in the area surrounding the structures (Figure 3). Each year, in each lake system the average wild rice growth was relatively the same, as were the water depth and temperature. However, in 2015 in Dumbbell Lake, there was significantly less average undisturbed wild rice growth outside of the enclosure, with an average density of 3.75 stalks/ m². The 2015 water level in Dumbbell Lake was also significantly higher than any other year, and the location of the structure appeared to be in an area with a naturally low density of wild rice. Although no wild rice stalks were seen inside of the enclosure in 2015, there were also very few rice stalks found outside the enclosure, limiting evidence of rusty crayfish interference on wild rice growth. However, in 2016 a significantly higher density of wild rice growth was observed outside of the "pair" enclosure and virtually no wild rice growth inside the enclosure, giving stronger evidence that negative impact on wild rice growth from *O. rusticus* can occur.

Wild rice in general was observed to be more dense inside the exclosures, where less interference from native and/or rusty crayfish occurred. Wild rice also appeared to be less dense inside of the enclosures where rusty crayfish densities were maintained at a high level.

Wild rice growing inside the exclosure at White Iron Lake 2013.

In 2013, wild rice was seen to be growing very sparsely inside and outside the exclosure at White Iron Lake. There was not enough rice present to see a difference in density (see photo on right), rendering wild rice density results from this lake inconclusive. Due to the sparse growth, all the wild rice stalks were counted inside the exclosure, rather than the average of three plots. The exclosure in Farm Lake was removed before substantial wild rice growth comparisons could take place.

In 2014, substantial wild rice growth was observed inside and outside each of the exclosures at Farm and Garden lakes (see photos below). The wild rice growth inside the exclosures was comparable to the wild rice growth outside of the exclosures, including comparable density, growth rate, and height.

Farm Lake wild rice growth inside and outside the exclosure 2014.

Garden Lake wild rice growth inside and outside the exclosure 2014.

Wild rice growth inside and outside enclosure 2015.

In 2015, virtually no wild rice was observed to be growing inside of the enclosure (see photo on left) in Dumbbell Lake. However, very little wild rice was observed to be growing outside of the enclosure. Although rusty crayfish interference is suspected to be the cause of no growth inside of the enclosure, it is difficult to draw conclusions based on the low density of wild rice growing naturally outside the enclosure.

In 2016, wild rice was observed both inside and outside all three structures set up throughout the entire growing period on Dumbbell Lake. Wild rice was last observed inside the pair enclosure on June 8th and the enclosure remained vacant of wild rice growth the rest of the season (see photo on right), suggesting a negative impact from the stocked rusty crayfish (Figure 4). The wild rice inside the exclosure appeared to grow taller and more dense than wild rice outside of the exclosure throughout the study period (see photo on right), possibly indicating wild rice could temporarily survive pressure from external forces, such as rusty crayfish predation. The food plot enclosure had some surviving wild rice stalks, but the amount of emergent wild rice remained much less dense inside the enclosure than outside (see photo below). However, compared to the enclosure with no alternative food source, the wild rice had a

2016 pair enclosure on 8/4 with no remaining wild rice stalks and extremely dense rice growth in the surrounding area.

Exclosure wild rice growth inside compared to outside.

Wild rice growth inside and outside the pair enclosure 2016.

much higher density. The alternative food source stocked was also completely depleted during every bi-weekly check except during the first check after installation (6/8). Evidence of heavy consumption was still present, but some cucumber peels and lettuce leaves remained. Upon every other bi-weekly check, no alternative food was left. When the alternative food source stopped being stocked and 50 new rusty crayfish were put in to the food plot enclosure, no change in the emergent wild rice was observed, suggesting rusty crayfish do not have the potential to impact wild rice once it has reached the emergent phase.

Crayfish Trapping

Exclosures

The exclosure trapping occurred in 2013, 2014, and 2016. In all three years, rusty crayfish were found both inside and outside of the exclosures (Figure 5). During 2013 and 2014 native crayfish were also found inside and outside the exclosures in Farm, Garden, and White Iron lakes. The crayfish captured inside and outside the exclosures and in preferred rusty crayfish habitat each year were compared by calculating a catch per unit effort (CPUE) (Table 2) where CPUE is defined as the number of crayfish caught per trap night (1 trap night = 1 trap set for 1 night) (see equation below).

$$CPUE = \frac{Total\ Number\ of\ Crayfish\ Captured\ (Native\ and\ Invasive)}{Total\ Number\ of\ Nights\ traps\ were\ in\ the\ water \times Total\ Number\ of\ Traps\ Set}$$

In 2013, 98.8% of crayfish captured in Farm Lake were native species. The CPUE outside the exclosure in 2013 was 0.13 and inside the exclosure was 0.19. Due to the CPUE being higher inside than outside, the exclosure was removed on 7/9. Additional trapping events took place on 7/29-7/30 and 8/15-8/16 where six to seven traps were placed in a mix of wild rice and adjacent rocky/ woody areas. The CPUE for rusty crayfish in the additional trapping events was 4.19 and the CPUE for native crayfish was 0.88. Results from trapping in areas outside the exclosure in and near the wild rice beds indicated that native crayfish (mostly calico, *O. immunis*) were most abundant in the wild rice and mucky substrates, and rusty crayfish were most abundant on the edge of, or outside, the wild rice in areas of rocky substrate and/or wood cover.

In White Iron Lake in 2013 the CPUE outside the exclosure was 0.06 and inside was zero. Fifty percent of the crayfish captured were rusty crayfish. On 7/29-7/30 and 8/15-8/16 one trap was placed inside the exclosure and six were placed outside in a mix of wild rice and adjacent rocky/ woody areas. The six traps placed outside the exclosure had a CPUE of 0.62 for rusty crayfish and 0.21 for native crayfish species. The trends in the additional trapping results were similar to Farm Lake in 2014 with mostly native species (calico, *O. immunis)* found in the wild rice and mucky substrates and rusty crayfish found in areas with rocky substrate and/ or wood cover.

In 2014 at Farm Lake the CPUE outside of the exclosure was 0.55 and inside was 0.11. Rusty crayfish accounted for 91% of the CPUE inside and outside the exclosure. For native crayfish species, the CPUE inside the exclosure was 0.04 and outside was 0.02. For rusty crayfish, the CPUE inside the exclosure was 0.07 and outside was 0.53. In the additional trapping event that occurred on 8/20-8/21 the CPUE for native crayfish was 0.60 and for rusty crayfish was 11.00. This indicates that native crayfish were more likely to be inside the structure in the wild rice beds and mucky/ organic substrate areas. Similar to previous patterns, rusty crayfish had a much higher CPUE outside the exclosure in areas of rocky substrate and/or wood cover.

In Garden Lake in 2014 the CPUE outside of the exclosure was 0.28 and inside was 0.17. Fifty six percent of the crayfish captured were rusty crayfish. The CPUE for native crayfish species inside the exclosure was 0.13 and outside was 0.04. The CPUE for rusty crayfish inside the exclosure was 0.04 and outside was 0.24. This is again indicative of the fact that native calico crayfish are more likely to inhabit wild rice and mucky substrate areas. In the additional trapping event that took place on 8/20-8/21 the CPUE for native species was 0.20 and for rusty crayfish was 2.20. Rusty crayfish again were found in greater numbers outside of the exclosure in adjacent woody/ rocky habitat.

In 2016, at Dumbbell Lake the CPUE inside the exclosure was 0.667 and outside was 2.27. All the crayfish captured in Dumbbell Lake were the invasive *O. rusticus*. There were seven crayfish total trapped inside the exclosure, all less than 2” in size, suggesting they may have been very small to initially enter the exclosure Additional trapping events took place on 5/24, 6/24, 7/17, and 8/15. Ten traps were placed in nearby rocky and or wooded areas on 5/24, and on 6/24, 7/17, and 8/15 six traps placed in the same location. The CPUE for the additional trapping areas was 10.47. There were several more rusty crayfish captured in the rocky/wooded areas (CPUE= 10.47) than in the trapping that occurred adjacent to the structures in wild rice and mucky substrate (CPUE= 2.27). This is similar to our previous results indicating that rusty crayfish prefer rocky/ wooded areas. However, 46 rusty crayfish were captured in areas adjacent to the structures indicating they can inhabit mucky/organic habitat if a food source is present (for trapping events the food source present was frozen fish bait).

Enclosures

The enclosure trapping occurred in 2015 and 2016. Both years resulted in re-captures of the stocked rusty crayfish, and both years saw a rusty crayfish population on the outside of the enclosure (Figure 4). The enclosures were both placed in Dumbbell Lake and no population of native crayfish was observed.

In 2015, there was a trapping recapture rate of 7.7%. However, including crayfish that were visually observed throughout the summer, it is estimated that 26.5% of crayfish remained inside the enclosure throughout the summer study period. A total of four rusty crayfish were captured outside of the enclosure, suggesting this area may not have been optimal habitat for rusty crayfish to be present in.

In 2016 in the pair enclosure there was a recapture rate of 56.9% (47.1% still with a fluorescent tag). Including the crayfish that were visually observed throughout the summer, it is estimated that 74.5% of rusty crayfish remained inside the enclosure throughout the study period. A total of 41 crayfish were also found outside the enclosure area, suggesting this was a suitable habitat for rusty crayfish to naturally occur in. One fluorescently tagged crayfish was also captured outside of the enclosure, suggesting there were still ways for rusty crayfish to escape from the structure, and potentially ways for rusty crayfish to get into the structure to get to the baited trap inside.

In 2016 in the food plot enclosure there was a recapture rate of 2% (1% still with a fluorescent tag), and including crayfish that were visually observed it is estimated around 5% remained inside. No crayfish were captured outside of the enclosure in the immediate surrounding area. The area of this enclosure had substantially more “mucky” and bottomless substrate than the pair enclosure and the exclosure. The traps would often sink into the muck so the bait would be buried and the entrance to the trap was buried. The crayfish that were observed were also partially covered by the sediment (rusty crayfish can bury themselves to some degree even though they are not capable of deep or extensive burrowing. It is likely this habitat is not the usual habitat rusty crayfish would be found in. It is also likely more crayfish remained in this enclosure, but the re-capture methods were not conducive to the sediment type.

Wild Rice Density

Exclosures

In 2013 the wild rice density data was inconclusive. However, years 2014-2016 showed wild rice density was higher inside the exclosures than outside (Figure 7). This was especially true in 2016 when the wild rice inside the exclosure was 2.7 times as dense (123.34 stalks/m^2) as the rice outside the exclosure (45.84 stalks/m^2). In 2014 on Farm and Garden lakes it was approximately 1.5 times as dense (inside Farm= 62 stalks/m^2 outside Farm= 42 stalks/m^2; inside Garden= 54 stalks/m^2 outside Garden= 42 stalks/m^2). The density measurement in 2014 was taken in October, slightly past the harvestable time for wild rice, and many stalks appeared to have been blown down by the wind before the structures were removed, suggesting the wild rice may have had a slightly higher density than recorded. The higher density inside of the structures suggests rusty crayfish can have a negative impact on wild rice growth.

Enclosures

In 2015 and 2016 wild rice was observed at a higher density outside of the enclosures than inside (Figure 8), suggesting that rusty crayfish can negatively impact wild rice growth in forced conditions. In 2015, there were only two stalks that remained inside the enclosure, one of which was observed to be expired from (presumed) natural causes on 7/15, the other disappeared after it was emergent and 68 crayfish were re-stocked inside the enclosure. Although most of the rice disappeared in the enclosure in 2015, there was also very little rice detected outside the enclosure with an average of 3.75 stalks/ m^2.

In 2016, a more defined outcome was present in the pair enclosure. Only one emergent stalk remained inside the enclosure at the end of the growing season. Wild rice density outside the enclosure averaged approximately 76.8 stalks/m^2. The high density of rice in the surrounding areas suggests the poor wild rice growth inside the pair enclosure was due to rusty crayfish.

In the food plot enclosure, the average density inside the enclosure was 10 stalks/m^2 and the average density outside the enclosure was 23 stalks/ m^2. There was also a population of water lilies that covered approximately ¾ of the surface area inside of the enclosure. The higher density of rice in the surrounding area suggests lesser wild rice growth inside could be due to a combination of effects from rusty crayfish and competition from lilies.

DISCUSSION

The combined data from observational study, wild rice density measurements, and crayfish trapping suggest that rusty crayfish do have the ability to negatively impact wild rice, especially in forced conditions. In 2013 and 2014, more native crayfish were found in the study areas that had a softer, more organic sediment bottom. The rusty crayfish were mainly found in their preferred habitat of a rocky and/or sandy bottom, with a minority being caught and/ or observed in the wild rice beds. Native crayfish species and rusty crayfish are found in rocky/ wooded habitat, however calico crayfish (*O. immunis*) have also been found in areas such as ponds and ditches so they may prefer softer substrate. It is still believed, however, that rusty crayfish will outcompete native crayfish, which potentially limited their habitat options in this study to the wild rice beds and organic/ mucky substrate. Native calico crayfish that were trapped inside the wild rice beds in our study did not appear to have a detrimental effect on wild rice growth. However, further study is needed to see impacts of native crayfish on wild rice in forced conditions with competition from rusty crayfish. Areas with naturally occurring wild rice growth in transitioning sediment types between sandy/ rocky and mucky/organic are also areas of concern and may be at a greater risk of being impacted by rusty crayfish. Areas with naturally occurring wild rice with greater numbers of rusty crayfish and/ or no alternative food source available are also at a higher risk from suffering detrimental impacts on the native wild rice population.

Evidence from the food plot enclosure in 2016 also suggests that wild rice may not be a preferred food source for rusty crayfish. The alternative food source was depleted upon every bi-weekly check, and there was a small amount of emergent wild rice left, even after re-stocking occurred. Hypotheses for why wild rice growth was less inside the food plot enclosure included negative effects from rusty crayfish and/or competition from the lily population that had become the dominant vegetation type inside the enclosure.

Rusty crayfish also appear to have the largest impact on wild rice when it is in the germinating and submerged vegetation phases of growth. In 2016, submerged wild rice was observed growing both inside and outside the pair enclosure. However, by June 23rd, rice outside the enclosure had reached the floating leaf stage but rice within the enclosure had disappeared and was not observed in any stage of growth inside the enclosure the remainder of the study. In the food plot enclosure where an alternative food source was presented and maintained, no effect was observed on wild rice growth and the rice reached the emergent phase. Even when additional rusty crayfish were added after emergence of the rice no negative effects were noted. However, in 2016 the equipment was realized to be compromised (holes were observed in the upper mesh netting) upon the retrieval of the food plot enclosure, and retention of the stocked crayfish was believed to be only about 5%, which renders these results inconclusive. There is also evidence from the enclosure in 2015 that rusty crayfish may have the ability to effect emergent wild rice when one emergent stalk of wild rice was presumed to be affected (removed) after the stocking of 75 rusty crayfish. However, there was no other suitable food source inside the enclosure, creating an extremely forced and high pressure survival situation. Therefore, if this emergent wild rice stalk was impacted from the stocked rusty crayfish it would be an effect from extreme conditions and rendering the results inconclusive. The effect rusty crayfish may have on emergent wild rice requires further study.

Exclusion of rusty crayfish was also observed to be a positive condition for native populations of wild rice. Comparing average wild rice density measurements in the exclosure studies in 2014 and 2016 (2013 was inconclusive), wild rice always appeared to be denser inside the exclosure than outside the exclosure. This conclusion was especially supported by the exclosure study in Dumbbell Lake in 2016 when the CPUE of crayfish inside the exclosure was low (0.67) in comparison to the CPUE outside the exclosure (2.27) suggesting much less crayfish interference inside the exclosure than outside, and the average wild rice density inside the exclosure compared to outside the exclosure was the highest from 2013-2016 (2.7 times more dense) and reached upcoming growth phases (floating-leaf and emergent) sooner. Most of the crayfish interference observed in the other exclosures in 2013 and 2014 in Farm, White Iron, and Garden lakes was from native crayfish, or a mix of native and rusty crayfish. The average wild rice densities measured in 2014 in Farm and Garden lakes were approximately 1.5 times higher inside the exclosures than outside. The difference in the average wild rice densities inside vs. outside the exclosures between 2014 (1.5 times denser) and 2016 (2.7 times denser) could be due to more trapping events (eight trapping events in 2014 and three trapping events in 2016) resulting in the removal of more crayfish, compromised equipment (about 3" diameter holes 1 foot below the surface of the water) that occurred in both structures in 2014 allowing more crayfish to escape, from higher crayfish interference inside the exclosures in Garden Lake in 2014 (CPUE inside = 0.17 CPUE outside = 0.28), or from having more native crayfish present in the wild rice beds which are thought to be less detrimental to wild rice growth than *O. rusticus*. However, the related results of the exclosure study in Dumbbell Lake in 2016 having a low interference from crayfish inside the exclosure, the highest ratio of average wild rice growth inside the exclosure compared to outside (2.7 times denser), and reaching growth phases sooner shows that rusty crayfish have the potential to be detrimental to wild rice growth, and that wild rice growth improves with the exclusion of rusty crayfish.

TABLES

Table 1. Structure summary for 2013-2016 including type of structure and coordinates for locations of structures in each lake system.

Lake	Year	Structure Type	UTM Zone 15T	
			Easting	**Northing**
Farm Lake	2013	Exclosure	596649	5305098
White Iron Lake	2013	Exclosure	588706	5303361
Farm Lake	2014	Exclosure	595108	5307651
Garden Lake	2014	Exclosure	595291	5308190
Dumbbell Lake	2015	Enclosure	630633	5275136
Dumbbell Lake	2016	Exclosure	630591	5275093
Dumbbell Lake	2016	"Pair" Enclosure	630582	5275099
Dumbbell Lake	2016	"Food Plot" Enclosure	629016	5276440

Table 2. Catch per unit effort (CPUE) (# of crayfish caught/ trap night) of crayfish captured inside and outside the exclosures and in preferred rusty crayfish habitat ("Additional Trapping").

	CPUE Inside Exclosure- *O. rusticus*	CPUE Inside Exclosure- Native	Total CPUE Inside	CPUE Outside Exclosure- *O. rusticus*	CPUE Outside Exclosure- Native	Total CPUE Outside	CPUE Additional Trapping- *O. rusticus*	CPUE Additional Trapping- Native	CPUE Additional Trapping Total
White Iron Lake 2013	0	0	0	0.03	0.03	0.06	0.62	0.21	0.83
Farm Lake 2013	*0.00	0.19	0.19	0	0.13	0.13	4.19	0.88	5.08
Garden Lake 2014	0.04	0.13	0.17	0.24	0.04	0.28	2.20	0.20	2.40
Farm Lake 2014	0.07	0.04	0.11	0.53	0.02	0.55	11.00	0.60	11.60
Dumbbell Lake 2016	0.67	NA	0.67	2.27	NA	2.27	10.47	NA	10.47

FIGURES

Figure 1. Water temperature measurements in degrees Fahrenheit from May to October for Farm, Garden, White Iron, and Dumbbell lakes in 2013-2016.

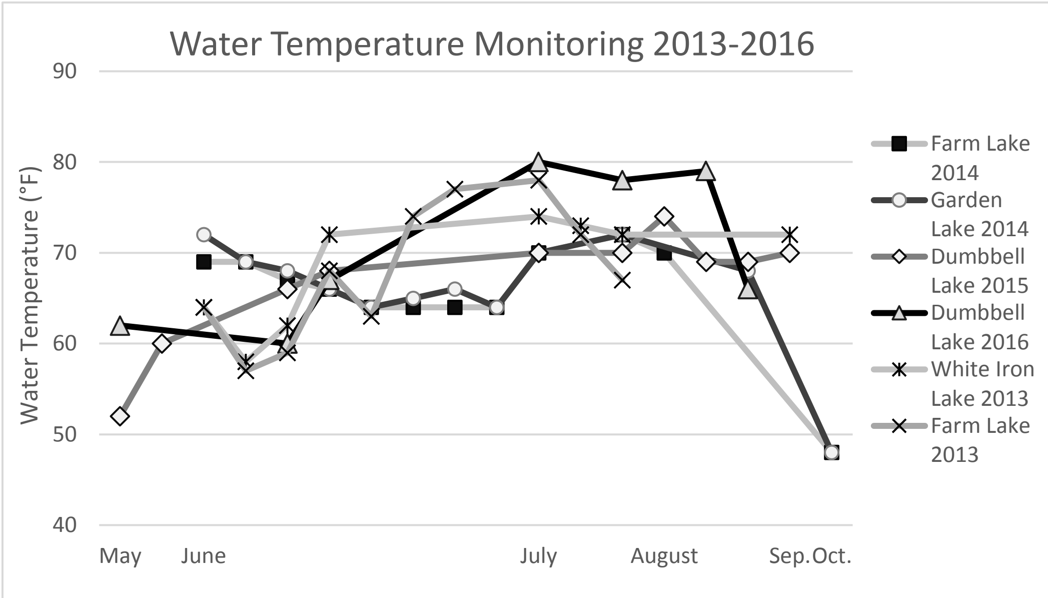


Figure 2. Water depth measurements in inches from May to October for Farm, Garden, White Iron, and Dumbbell lakes in 2013-2016. Higher water levels potentially affect wild rice growth, especially in the floating -leaf stage of growth which usually occurs in June. The water depth gauge was placed outside the structures on the shallower side within a 10 foot radius of (one of) the structures.

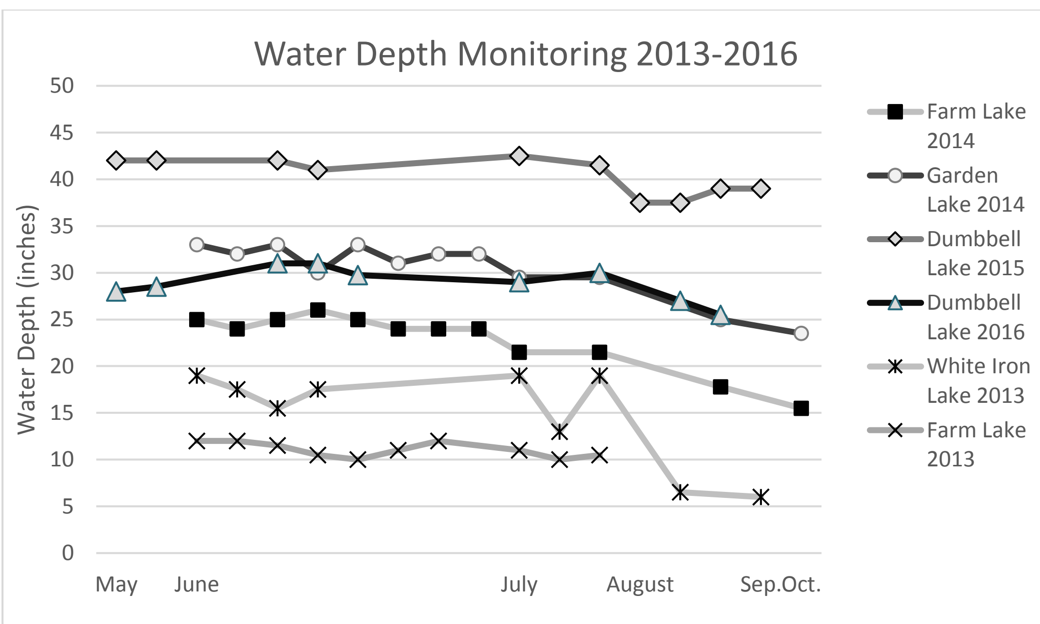


Figure 3. Average wild rice growth outside of the structures. This represents wild rice growth devoid of any interference from the study. The low density of average wild rice growth in Dumbbell lake in 2015 is potentially correlated with higher water levels that occurred that year (Figure 2) or the placement of the structure/ density measurements in an area with naturally low wild rice density.

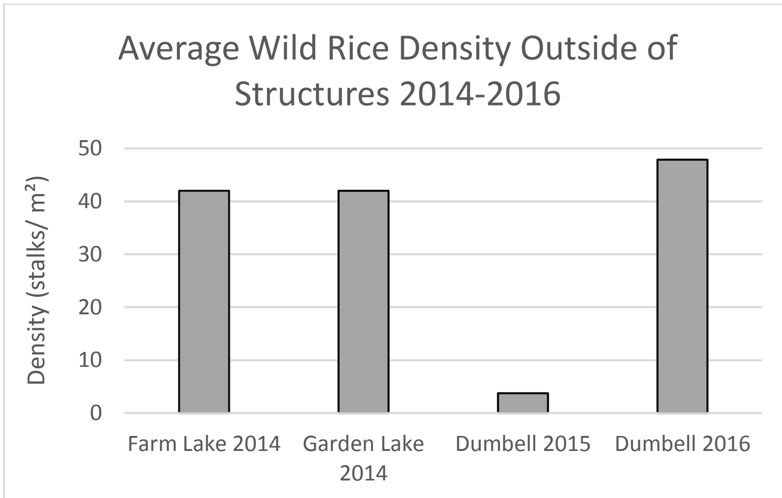


Figure 2. Monitoring of wild rice growth both inside and outside the structures in 2016 at Dumbbell Lake. On June 23rd, wild rice was no longer observed inside of the pair enclosure.

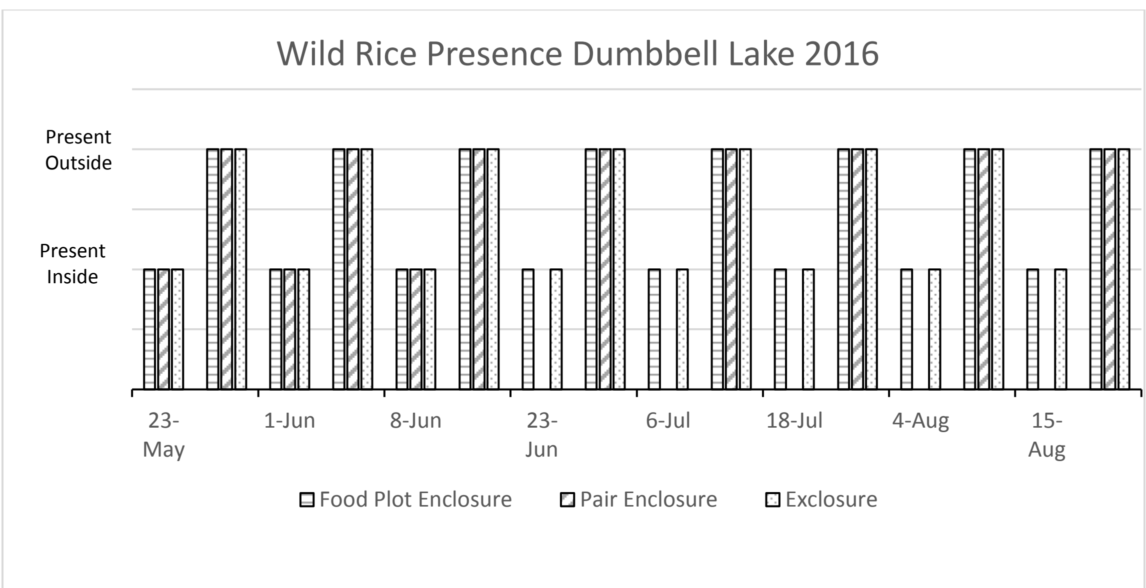


Figure 3. Exclosure crayfish trapping summary depicting native and rusty crayfish inside and outside the exclosures in 2013, 2014, and 2016. In Dumbbell Lake in 2016 all crayfish captured inside the exclosure were less than two inches in size. The total crayfish trapped inside vs. outside of the structures shows the effectiveness of each of the structures.

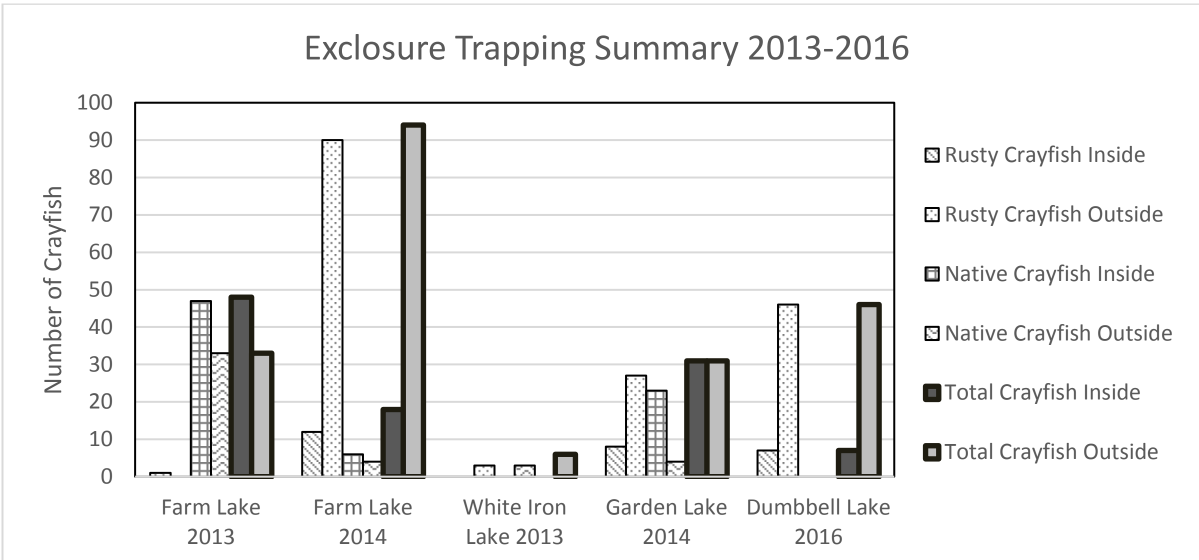


Figure 4. Enclosure crayfish trapping summary. Enclosures were placed in Dumbbell Lake which is only occupied by rusty crayfish. In 2016, some of the crayfish stocked inside the enclosures were marked with a fluorescent tag which tested the effectiveness of the enclosures. In 2015, an estimated 26.5% of crayfish remained inside the enclosure (total crayfish inside/ number of crayfish stocked). In 2016, an estimated 74.5% of crayfish remained inside the pair enclosure and an estimated 5% remained inside the food plot enclosure. The total crayfish outside represents the crayfish present in the area surrounding the structures.

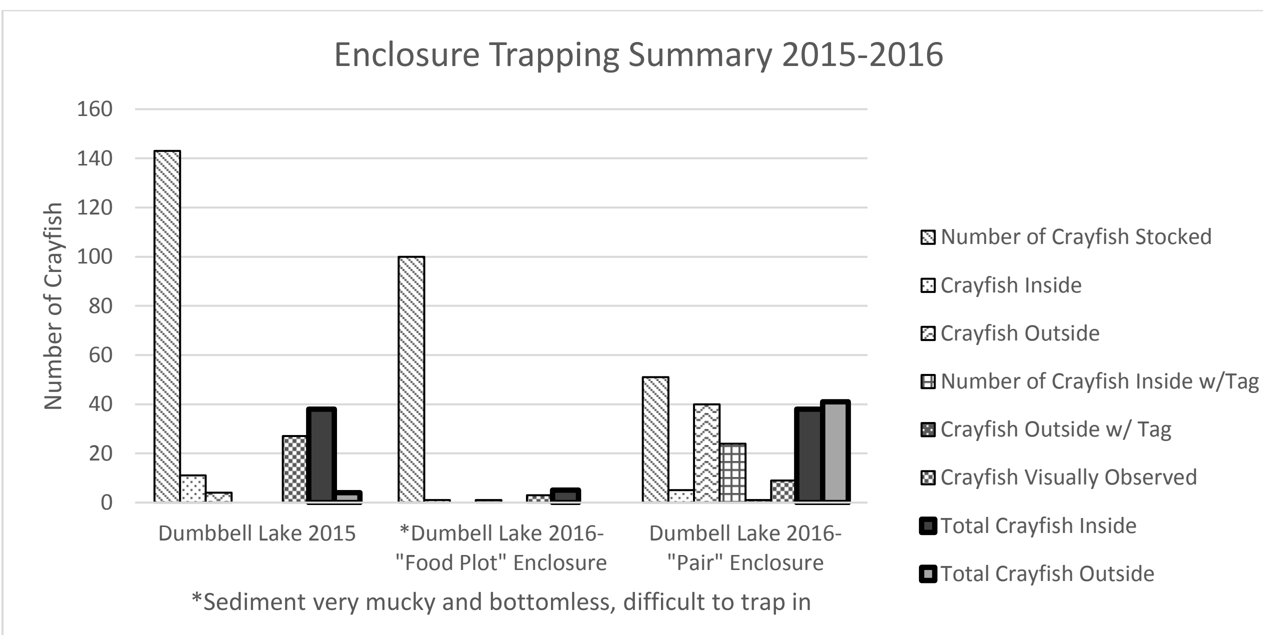


Figure 5. Wild rice density inside vs. outside the exclosures in 2014 and 2016 measured as stalks per meter squared (m²). In both years, the wild rice inside the exclosure (with little interference from rusty crayfish) is denser than the wild rice outside of the exclosure (subjected to interference from rusty crayfish).

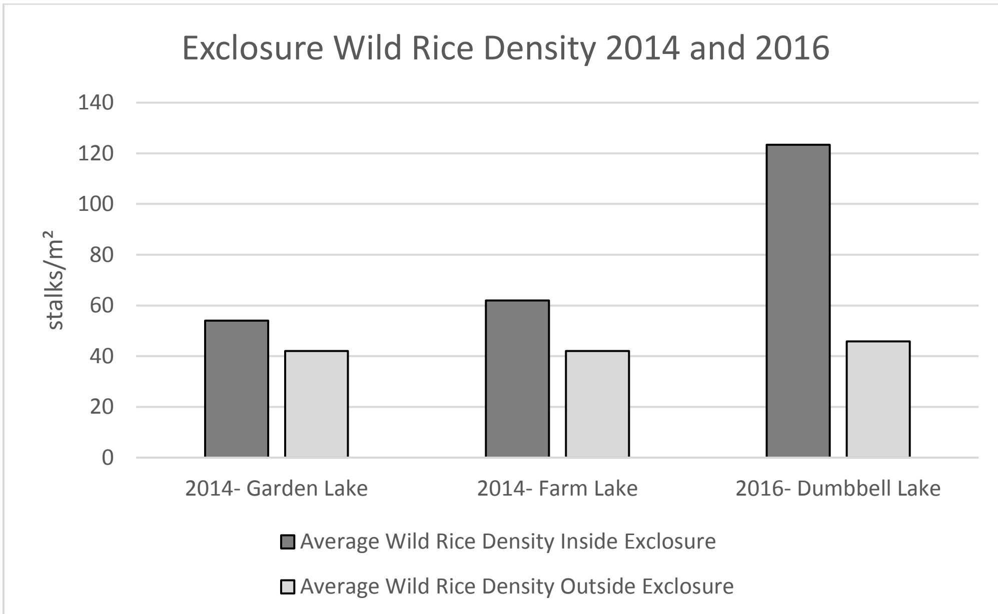
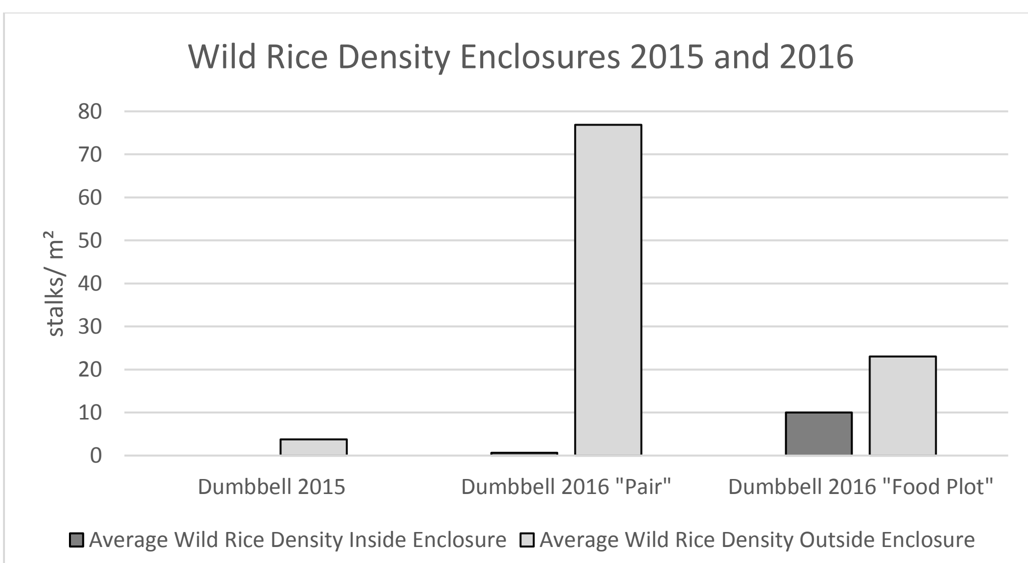


Figure 6. Wild rice density inside vs. outside the enclosures in 2015 and 2016 measured as stalks per square meter (m²). In the enclosures with no alternative food source there is virtually no wild rice left inside, even though in 2016 the wild rice surrounding the pair enclosure was abundant. With an alternative food source available (Dumbbell 2016 "Food Plot") we see a significant rise in the amount of wild rice remaining inside the enclosure.



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