
QUETICO PROVINCIAL PARK

ASSESSMENT OF NATIVE AND INVASIVE CRAYFISH

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Quetico Provincial Park acknowledges that the surveys summarized in this report were carried out on the traditional lands of the Anishinaabe people of Treaty Three.

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EXECUTIVE SUMMARY

Historically, Quetico Provincial Park has been home to one species of crayfish, the northern or virile crayfish (*Orconectes virilis*). In the past couple decades, non-native rusty crayfish (*Orconectes rusticus*) and northern clearwater crayfish (*Orconectes propinquus*) have arrived and invaded lakes along the southern border of Quetico Park. Surveys were conducted during the summers of 2014, 2015, 2016 and 2017 in seventeen lakes throughout the Park to collect data on the relative abundance, distribution, habitat use, and size of these species. The purpose of this project was to assess the risk of non-native crayfish to the aquatic ecosystems of Quetico Provincial Park and similar surrounding areas.

Crayfish were captured using modified minnow traps baited with cat food or by hand-capturing them along transects. Information on crayfish size, relative abundance, sex, species, and habitat distribution was collected. A total of 1465 traps were set and 51 transect surveys conducted. In traps and transects combined, 3173 crayfish were caught, consisting of 801 *O. virilis*, 1721 *O. propinquus*, 238 *O. rusticus*, and 413 unidentified invasive crayfish (either *O. propinquus* or *O. rusticus*).

The objective of these studies was to address the following questions:

1) Increase understanding of the factors affecting the abundance, habitat use, and biological characteristics of native *O. virilis* and invasive *O. rusticus* and *O. propinquus* in Quetico Provincial Park.

Abundance of both native and the non-native crayfish *O. propinquus* appears to be highly affected by the presence of crayfish specialist predators such as smallmouth bass and rock bass with higher densities of crayfish in lakes without the crayfish specialist predators. Only *O. rusticus* has been captured in high densities in the presence of crayfish specialist predators.

Both *O. virilis* and *O. propinquus* showed similar habitat use with both species caught at similar rates in cobble and macrophyte habitat.

O. rusticus were significantly larger than both *O. virilis* and *O. propinquus* with an average calculated total length ~15mm longer than the other species. Although *O. propinquus* was statistically larger than *O. virilis*, the differences was less than 2mm. This is likely biologically insignificant, especially to predator fish, given the lack of predator-prey size relationship found to date for adult fish. *O. virilis* had a wider observed range in sizes than *O. propinquus*.

2) Determine the distribution and dispersal ability of invasive *O. rusticus* and *O. propinquus*.

O. propinquus appears to have demonstrated the ability to disperse widely and quite rapidly over the past 25 years and are currently found in all the lakes surveyed in the south end of the park. Dispersal ability does not appear to be limited by water chemistry, physical barriers, direction of water flow, or presence of crayfish specialist predators. Based on these studies, *O. propinquus* appears to have the ability to disperse throughout the majority of Quetico lakes.

To date, there appears to be much less expansion of *O. rusticus* outside of the areas where it was identified in the late eighties and early nineties. While the factors affecting its dispersal ability are not completely understood, if it is calcium concentrations as suggested by other studies, their range and dispersal may be limited to high calcium lakes found in the southeast corner of

the park. This region is defined by lakes underlain by volcanic origin bedrock and the lakes immediately downstream from them.

3) Assess the likelihood for invasion and potential risk that invasive crayfish will cause in Quetico's aquatic ecosystems.

Although the potential risk for invasion of the majority of park lakes by *O. propinquus* appears high, the ecological impact risk may be low to moderate given the similarity of size and habitat use with native crayfish. The largest potential impacts may be the loss of a native species (*O. virilis*) and a potential shift in biomass/energy flow within the community if the crayfish abundance in the lakes increases.

Conversely, for *O. rusticus*, although the likelihood of invasion in Quetico lakes is less, the ecological risk of invaded lakes appears to be much higher. Besides the likely loss of the native species *O. virilis*, there is likely to be higher crayfish abundance in the lakes with a greater potential shift in biomass/energy flow and subsequent community impacts. These studies and others would predict a shift in size distribution of the crayfish population which may affect prey availability for smaller fish species as well as the young of larger species. In addition, there is the risk of reduced abundance and diversity of aquatic plants observed in other studies, which may have an impact on habitat availability for other species.

INTRODUCTION

Crayfish (*Anishinaabemowin* - *Zhaagheshii*) are an important part of freshwater ecosystems, making up the majority of benthic invertebrate biomass (Keller and Moore, 2000). They are omnivores, eating a variety of aquatic plants, fish eggs, and benthic organisms (Hanson and Chambers, 1995; Wilson et al. 2004), as well as an important source of food for larger fish species (Tetzlaff et al. 2011). However, little is known about the mechanisms by which crayfish are influenced by their habitat and other species or factors affecting their distribution and abundance in northwestern Ontario shield lakes.

Historically, Quetico Provincial Park has been home to one species of crayfish, the northern or virile crayfish (*Orconectes virilis*). In the 1980's, the Minnesota Department of Natural Resources identified non-native rusty crayfish (*Orconectes rusticus*) and northern clearwater crayfish (*Orconectes propinquus*) in Sucker and Birch Lake along the southern border of Quetico and by 1993, they had moved downstream into the east end of Basswood Lake in the area around Prairie Portage (Figure 1; Jackson 2015a).

There has been a great deal of concern over the potential impacts of invasions of *O. rusticus* over the past decades. They are typically larger and more aggressive than *O. virilis* and have been found to displace this native species as they move into lakes (Wilson et al. 2004). Due to their preference for aquatic vegetation as food, decreased macrophyte cover has been associated with the arrival of *O. rusticus* (Hanson and Chambers, 1995), which may have subsequent effects on benthic invertebrates and fish communities.

The effects of *O. propinquus* invasions are less well studied though their diet may not consist of as much aquatic vegetation as rusty crayfish (Saffran and Barton 1993). They have also been observed to temporarily displace *O. virilis* before *O. rusticus* become dominant (Lodge et al. 1986, Wilson et al. 2004).

Three factors with the potential to affect crayfish abundance, habitat use and risk of potential invasion by non-native crayfish were considered during these studies:

- 1) Presence of physical barriers to dispersal, particularly the distance and stream flow connecting waterbodies
- 2) Water chemistry, particularly the amount of calcium in water
- 3) The presence of crayfish specialist predators, particularly smallmouth bass and rock bass.

Invasive crayfish have been reported to expand at about 2 km/year within a lake environment, which is very similar to that reported for the movement of crayfish within Basswood Lake and downstream to Crooked Lake (Jansen et al. 2009, Jackson 2017). However, it was less clear about the ability of crayfish to move upstream and whether there may be barriers to upstream movement such as longer, low flow streams. This would greatly affect which lakes were at risk to being accessed by invasive crayfish. In 2016, studies focussed on looking on the ability of invasive crayfish in moving from a known source (i.e. Basswood Lake) into a number of connected lakes that varied in distances, flow of connecting streams and physical barriers (i.e. Nest, Dahlberg, South, West, Burke - Champaigne-Klassen, 2016). The use of baitfish by anglers has been prohibited in Quetico Provincial Park since the late seventies and the use of crayfish for bait has been illegal since the mid-2000's. Although it has been assumed that the original introduction of non-native crayfish into Quetico lakes has been due (directly or indirectly) to movement by anglers using crayfish for bait, these studies assume that movement between lakes is due to unassisted migration by crayfish.

Calcium has also been shown to influence crayfish populations because it is an important component in exoskeleton growth for crustaceans (Edwards et al. 2013). *O. rusticus* are thought to have a high requirement for calcium concentrations with different studies documenting minimum requirements of between 2-8 mg/L of calcium while *O. propinquus* and *O. virilis* can survive at lower thresholds (Latzka 2015, Capelli and Magnuson 1983). In Quetico, calcium concentrations above 8 mg/L are only found in lakes overlying volcanic bedrock, specifically lakes located in the south-west corner of the Park (Jackson 2016). Lakes on granite and sedimentary origin bedrock have much lower calcium concentrations (average 2.8 mg/L) and may be less susceptible to *O. rusticus* invasion. In 2017, surveys were conducted on lakes within the volcanic bedrock area of the park (i.e Sheridan, Crawford, That Man, Carp) to see if differences in water chemistry resulted in differences in species composition or density.

Several species of centrarchids that are present in Quetico Provincial Park are known to feed on crayfish, including smallmouth bass, rock bass and largemouth bass as well as pumpkinseed sunfish, green sunfish, northern (longear) sunfish and bluegill sunfish (Garvey et al. 2003). Bass, particularly smallmouth bass and rock bass, are crayfish specialists. Smallmouth bass were introduced to the area in the 1940s and by the 1970s they were wide spread throughout the Park. They have currently been reported from 175 lakes (72% of lakes with fish species information). Rock bass are native to Quetico and have been reported from over 55 lakes. In 2010, Broadscale Fisheries Monitoring (BsM) surveys conducted in Quetico found that over 50% of all smallmouth bass and over 60% of the rock bass caught with food in their stomachs had consumed crayfish (Jackson, 2015c). Comparatively, less than 20% of northern pike with food in their stomachs had consumed crayfish, and less than 10% of walleye. Smallmouth bass have also been shown to be effective at population control of invading rusty crayfish (Hein et al. 2006)

OBJECTIVES OF REPORT

The objective of this report is to address the following:

- 1) Increase understanding of the factors affecting the abundance, habitat use, and biological characteristics of native *O. virilis* and invasive *O. rusticus* and *O. propinquus* in the lakes of Quetico Provincial Park.
- 2) Determine the distribution and dispersal ability of invasive *O. rusticus* and *O. propinquus*.
- 3) Assess the likelihood for invasion and potential risk that invasive crayfish will cause in Quetico's aquatic ecosystems.

METHODS

Crayfish trapping and transect methodology is described in detail in the Quetico Park Crayfish Monitoring Protocol (Jackson 2015b). This protocol was used consistently during all years of the study, however in 2014 size data was not collected for the crayfish caught in Basswood Lake.


Crayfish_Monitoring_Protocol-2017.doc

STUDY SITES

Quetico Provincial Park is located between Thunder Bay and Fort Frances in the Province of Ontario, sharing a border with the Boundary Waters Canoe Area in Minnesota. The area is underlain by Canadian Shield bedrock in a transition zone between southern mixedwood forests and northern boreal forests. Numerous lakes and streams support 48 species of fish in cold and warm water habitats. Between 2014 and 2017, seventeen lakes were surveyed for crayfish (Figure 1): Basswood, Dalhberg, Nest, South, West, Burke, Crawford, Sheridan, Carp, Knife, That Man, Point and Sucker are located at the southern boundary of the Park and were the among the first to experience the arrival of *O. rusticus* and/or *O. propinquus*. Pickerel, Wolseley, Crooked, and Stannar Lakes are located along the north and western borders and only contain native *O. virilis*.

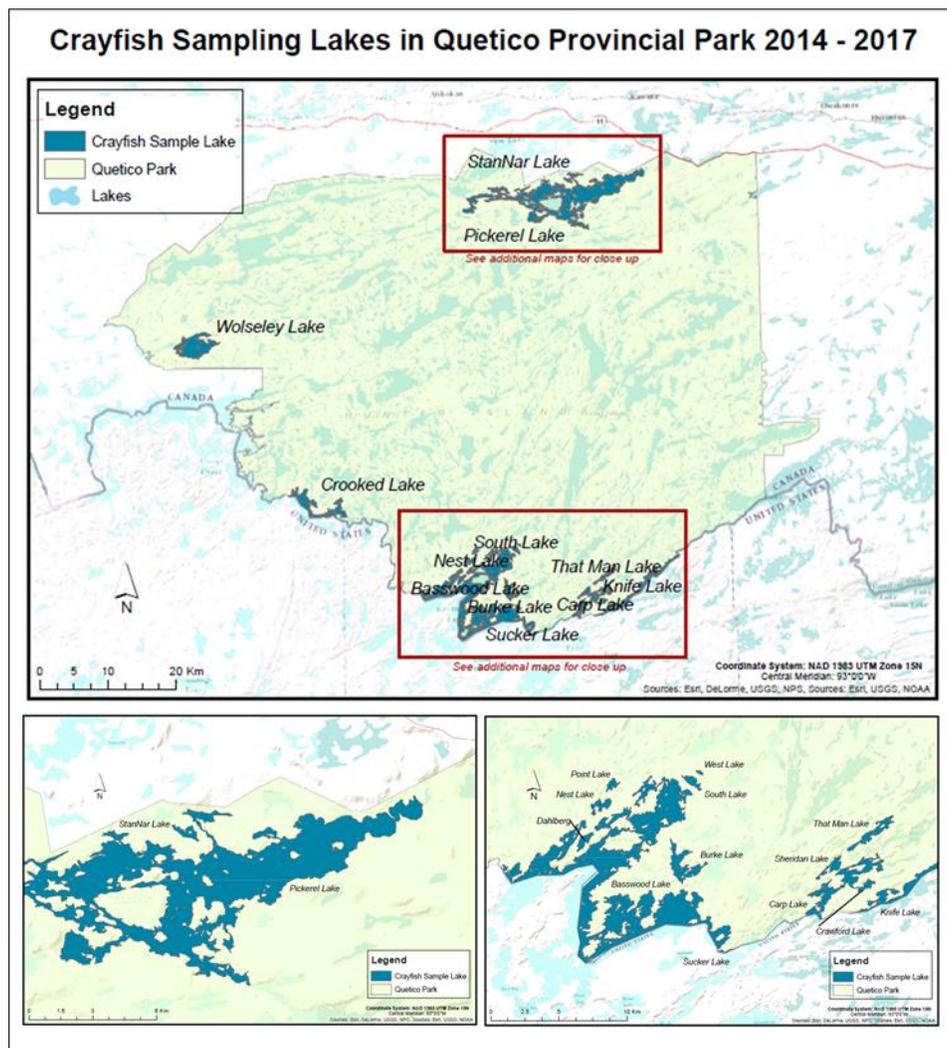


Figure 1. Location of lakes surveyed for crayfish between 2014-2017 in Quetico Provincial Park.

TRAP PLOTS

Modified minnow traps were used as a standardized method of crayfish collection. Though traps are known to select for large, aggressive, male crayfish (Wilson et al. 2004; Hein et al. 2006), they can still provide accurate assessments of species composition and relative abundance (Capelli, 1982), and assessments of population density and catch rates between sites (Jansen et al., 2009).

Minnow traps were modified to have entrance holes enlarged to 3.5 cm in diameter. Traps were baited with canned fish-based cat food and set in lines of three, spaced 3 m apart, and running perpendicular to the shore. Four lines of three traps, each approximately 10 m apart, constituted one plot for a total of twelve traps per plot. Plots were chosen by dividing the shoreline (including islands) into 50 m segments and randomly selecting from these segments. Maps of the plot locations are available in Appendix 3. Traps were set over night for a minimum of 12 hours. Set and lift time, bait type, observers, location, trap depth, habitat, water temperature, and the number, total length (tip of the rostrum to the tip of the central telson measured on the dorsal surface), carapace length (tip of the rostrum to the back of the carapace measured on the dorsal surface), and sex of each crayfish caught, and a note of any other organisms caught in the trap was recorded. Crayfish were then released at the site that they were trapped. Habitat was classified as cobble, macrophyte (aquatic vegetation), detritus (decaying leaf layer and fallen woody debris) or soft bottom (muck).

WADING TRANSECTS

Transect surveys allowed for an efficient assessment of the presence/absence of *O. rusticus* and *O. propinquus*. They also catch the less aggressive and smaller crayfish that are less susceptible to the traps (Wilson et al. 2004; Hein et al. 2006). Transect locations were selected using the same 50-segment method as the trap plots. Length was determined based on habitat and waypoints were collected at the start and end location. Transects were conducted near shore up to a depth of 1m. Observers, start and end time, habitat type and description, water temperature, number of crayfish, species, total length and carapace length were recorded.

SAMPLING PERIOD

Sampling dates for all lakes and years is provided in Table 1.

Table 1: Monitoring dates for crayfish surveys in Quetico Provincial Park

Year	Lake	Sample Date	Year	Lake	Sample Date
2014	Basswood	June 19-24	2017	Nest	July 6
2015	Crooked	August 17-19		Point	July 6
	Basswood River	August 19-20		Burke	July 7
	Basswood	August 20-21		Basswood	July 7 + 25
	Pickereel	August 29-30		Sucker	July 8
2016	Wolseley	June 28-30		Pickereel	July 11-13
	Dahlberg	July 22-24		Sheridan	July 20-22
	Nest	July 24-26		That Man	July 21
	South	July 27-28		Carp	July 22
	West	July 27-28		Crawford	July 22-24
	Burke	July 28-30		Knife	July 24

SPECIES IDENTIFICATION

The visual difference between *O. virilis* and invasive crayfish is fairly distinct, however it can be more difficult to distinguish between *O. propinquus* and *O. rusticus*. There has been confusion in the identification of *O. propinquus* vs *O. rusticus* in past Quetico Park surveys using a key developed in central Ontario (see Appendix 1). Studies conducted in 2017 used a visual key based on Roesler (2012) (Appendix 2) to correct this issue. Confirmatory surveys were conducted in 2017 through the southern Park lakes which found *O. propinquus* throughout North Bay in Basswood Lake, both *O. propinquus* and *O. rusticus* in Inlet Bay, and *O. rusticus* in Sucker Lake. Crayfish caught in 2016 in Dahlberg, Nest, South, West and Burke Lakes are now thought to be entirely *O. propinquus* based on review of photographs taken of captured species and 2017 surveys. Representative samples collected in 2017 from Crawford, Sheridan, Nest and North Bay of Basswood and sent to Trent University for genetic analysis confirmed species identification as *O. propinquus*.

STATISTICAL ANALYSIS

Crayfish carapace length as opposed to total length was used throughout the analysis because it is the more accurate measure to obtain in the field. All statistical analyses used a p-value of 0.05 as the cut-off for determinations of statistical significance. Populations with n values <20 were not included in statistical analyses; their average values are reported. Analyses that compared crayfish size used data collected using both the trap and transect methods while analyses that compared crayfish density (catch per trap) used only trap data. Catch per trap was calculated using only those traps that contained crayfish in order to normalize the data and remove traps that were not set in locations that did not contain a given species. Comparisons between two populations were conducted using two-sample t-tests for populations with equal variance if equality of variance was verified using an f-test statistic. Otherwise a t-test assuming unequal variance was used. Comparisons between three or more populations was conducted using a single-factor ANOVA. Populations were not the same size. Therefore a Tukey-Kramer post-hoc test was used to determine the location of significant differences between populations. Cohen's D statistic, which is a measure of the strength of the correlation, is also reported for all pair-wise comparisons. Statistical analysis was completed using MS Excel. Data summaries are available in Appendix 6.

For ease of interpretation, calculated total crayfish length is reported on the graphs in this report. This value was obtained using a regression equation, unique to each species, which relates crayfish carapace length to total length. Carapace length is easier and more reliable to obtain in the field compared to total length when working with live, sometimes difficult to handle, crayfish. Previous studies (Jackson 2017; Adair 2016; Champaigne-Klassen 2016; Jackson 2015a) have also found carapace length to be a good predictor of crayfish total length.

RESULTS

Individual lake results from the 2017 season are available in Appendix 5. Results and detailed discussion of findings from previous years can be found in Jackson (2015a), Adair (2016), Champaigne-Klassen (2016), and Jackson (2017). This report will focus on a synthesis of the data collected from 2014 through to 2017. A total of 1472 traps were set and 51 transect surveys conducted across 17 lakes in Quetico Park (Table 2). In traps and transects combined, 3173 crayfish were caught, consisting of 801 *O. virilis*, 1721 *O. propinquus*, 238 *O. rusticus*, and 413 unidentified invasive crayfish (either *O. propinquus* or *O. rusticus*).

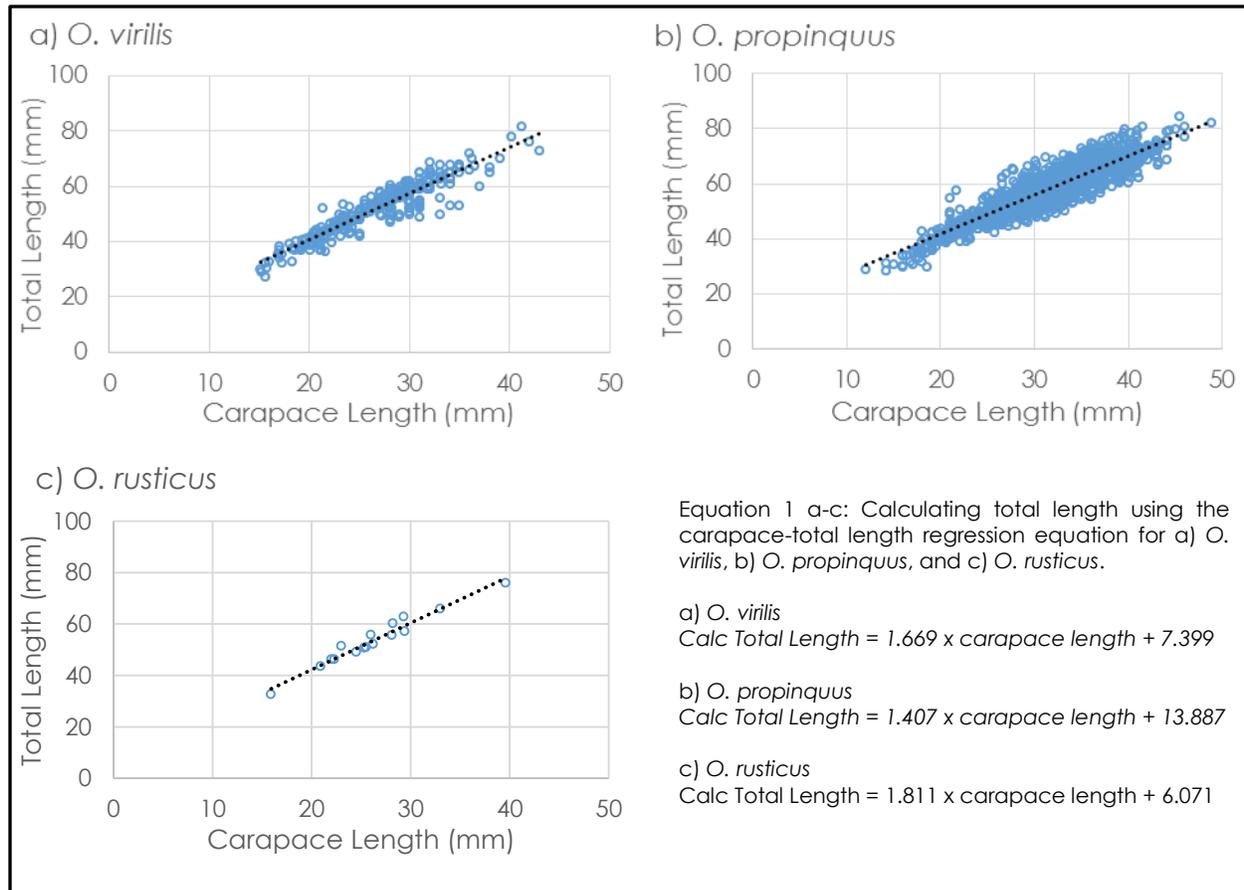
Table 2: Summary of crayfish monitoring efforts in Quetico Provincial Park from 2014 to 2017. "Invasive spp." refers to crayfish that were either *O. virilis* or *O. propinquus*.

Year	Lake	Number of		Number of Crayfish Caught			
		Traps	Transects	<i>O. virilis</i>	<i>O. propinquus</i>	<i>O. rusticus</i>	Invasive spp.
2014	Basswood	150		2			323
			34	1			59
2015	Basswood (west)	35					24
	Basswood (Inlet Bay)	22		4		225	
	Basswood River	12		3			2
			2	7			4
	Crooked	70		1			
	Pickereel	12	3	3			1
2016	Stannar	118		607			
	Wolseley	120		13			
	Pickereel	24		27			
	Nest	142			1408		
	Dahlberg	142			136		
	South	65			25		
	West	6			6		
	Burke	142			15		
2017	Crawford	142			60		
			1		2		
	Sheridan	143			34		
			1		8		
	Pickereel	120		128			
	That Man		1				
	Carp		1		3		
	Knife		1		5		
	Burke		1		3		
	Nest		1		4		
	Point		1		3		
Basswood		3		6	13		
Sucker		1		3			
Total:		1465	51	801	1721	238	413

CRAYFISH SIZE

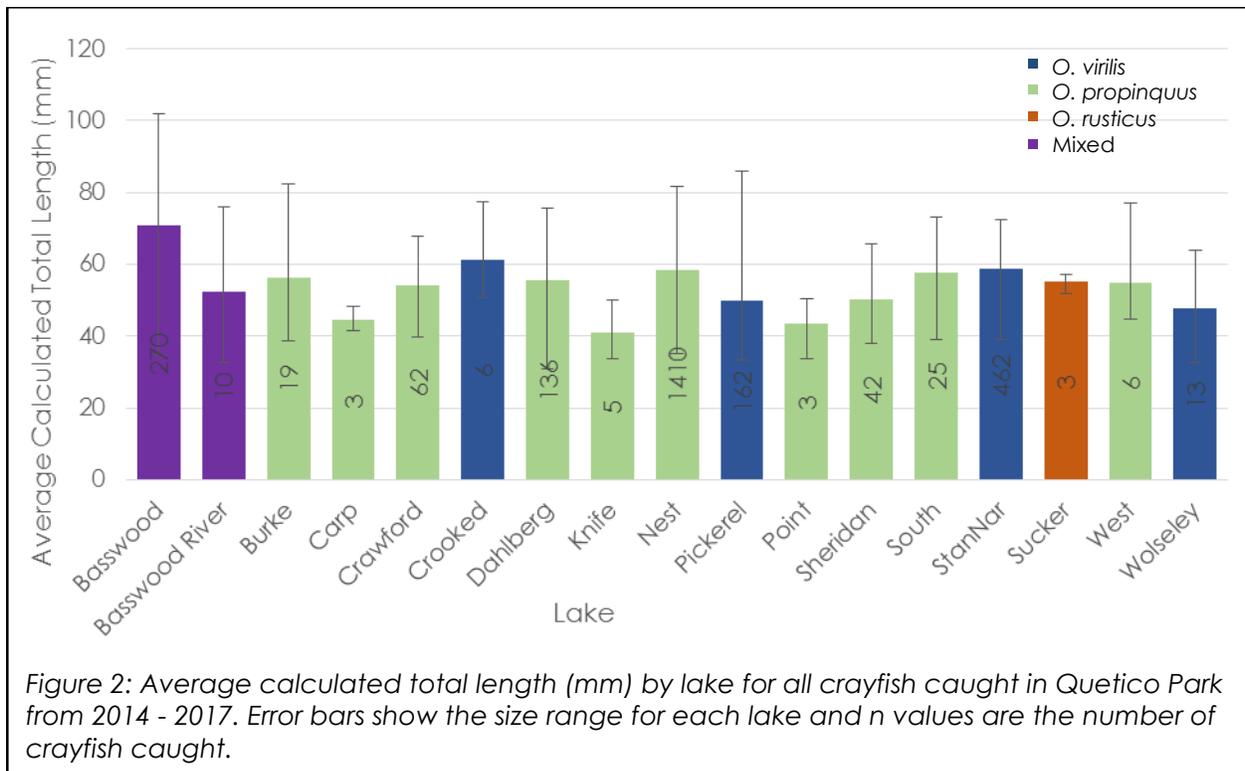
Relationship Between Carapace Length and Total Length

Carapace length was found to be a good predictor of crayfish total length for all three species of crayfish (regression test statistic after removing outliers: *O. virilis* = 1.1E-115, *O. propinquus* = 0, *O. rusticus* = 6.2E-11). Calculated total length (CTL) was determined using Equations 1 a-c.

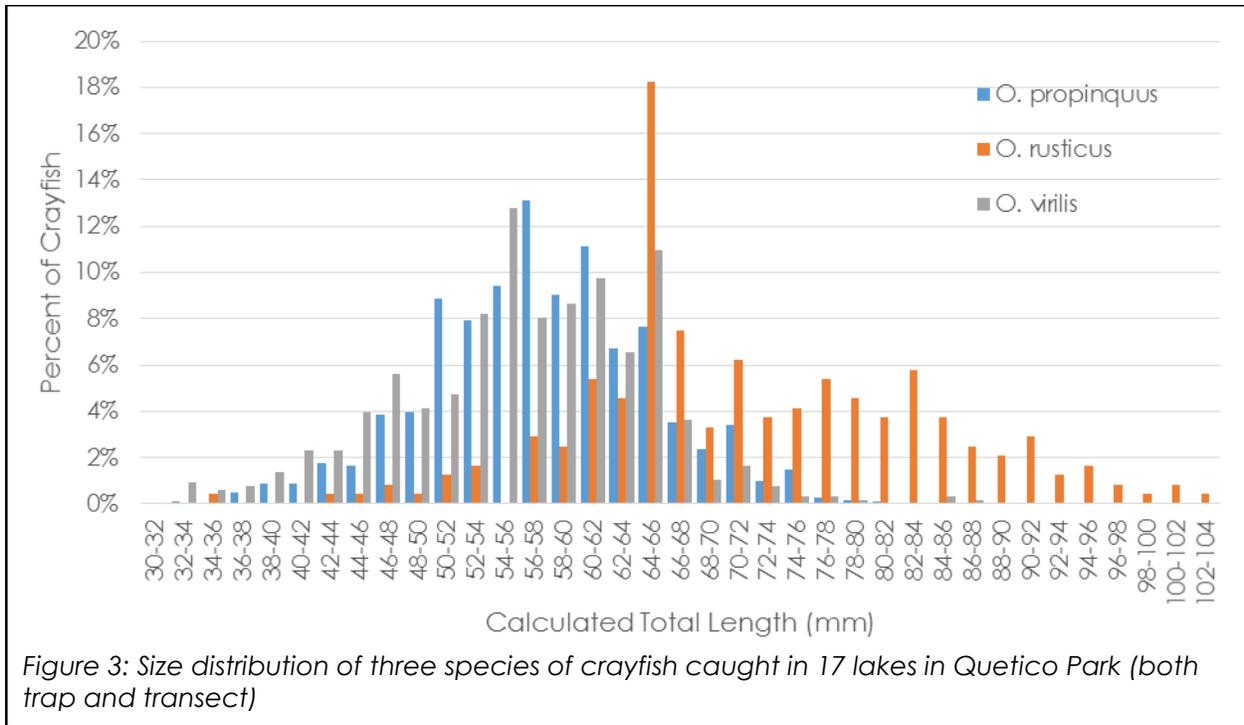


Between Lakes

Regardless of species, crayfish carapace size varied from 12.0 mm (30.8 mm CTL) in Dahlberg Lake to 53 mm (102.1 mm CTL) in Basswood Lake. On average, the smallest crayfish were caught in Knife Lake, and the largest in Basswood. However, small sample sizes from Basswood River, Burke, Carp, Crooked, Knife, Point, Sucker, West, and Wolseley lakes make it difficult to estimate the actual size of crayfish in these populations (Figure 2).



Across all lakes, *O. virilis* carapace length ranged from 15.1 mm (32.6 mm CTL) to 47.0 mm (85.8 mm CTL) with an average of 29.2 mm (59.3). *O. propinquus* ranged from 12.0 mm (30.8 mm CTL) to 48.8 mm (82.6 mm CTL) with an average of 31.1 mm (57.7 mm CTL). *O. rusticus* ranged from 15.9 mm (34.9 mm CTL) to 53 mm (102.1 mm CTL) with an average of 36.4 mm (72.0 mm CTL). *O. rusticus* was significantly ($q(3,\infty)=14.06$, $d=1.36$) larger than *O. propinquus* which was significantly ($q(3,\infty)=7.59$, $d=0.36$) larger than *O. virilis* on average. However, due to the large difference in sample sizes, the effect size between *O. rusticus* and *O. propinquus* is quite large. A histogram (Figure 3) also shows that *O. virilis* and *O. propinquus* had calculated total lengths that peaked between 54-62 mm while *O. rusticus* tend to be larger.



The majority of lakes contained only one crayfish species, allowing for between lake comparisons of size within a single species. Only lakes with a sample size of >20 crayfish caught in traps were considered. Five lakes contained only *O. propinquus*: Crawford, Dahlberg, Nest, Sheridan, and South, which had crayfish with average crayfish carapace length equal to 28.6, 29.6, 31.6, 25.9 and 31.2 mm respectively (CTL = 54.1, 55.5, 58.4, 50.3, and 57.8 mm respectively). Nest Lake had significantly larger crayfish than all other lakes except South while Sheridan had significantly smaller crayfish compared to all other lakes except Crawford. Note however that the large sample size of Nest Lake compared to all other lakes make the effect size high for many comparisons.

Table 3: Statistical significance (*q*-values) of pairwise comparisons of calculated *O. propinquus* carapace length (mm) between lakes. *q*-values highlighted in orange show significant difference. i.e. $q > q_{test} (k=5, df=\infty) = 3.63$. *D* values in brackets represent the effect size (Cohen's *D*)

	N↓ Average →	Crawford 28.6	Dahlberg 29.6	Nest 31.6	Sheridan 25.9	South 31.2
Crawford	62	-				
Dahlberg	136	1.30 (0.182)	-			
Nest	1410	4.49 (0.618)	4.27 (0.343)	-		
Sheridan	42	2.66 (0.563)	4.15 (0.654)	7.12 (1.143)	-	
South	25	2.11 (0.469)	1.39 (0.246)	0.41 (0.075)	4.09 (0.942)	-

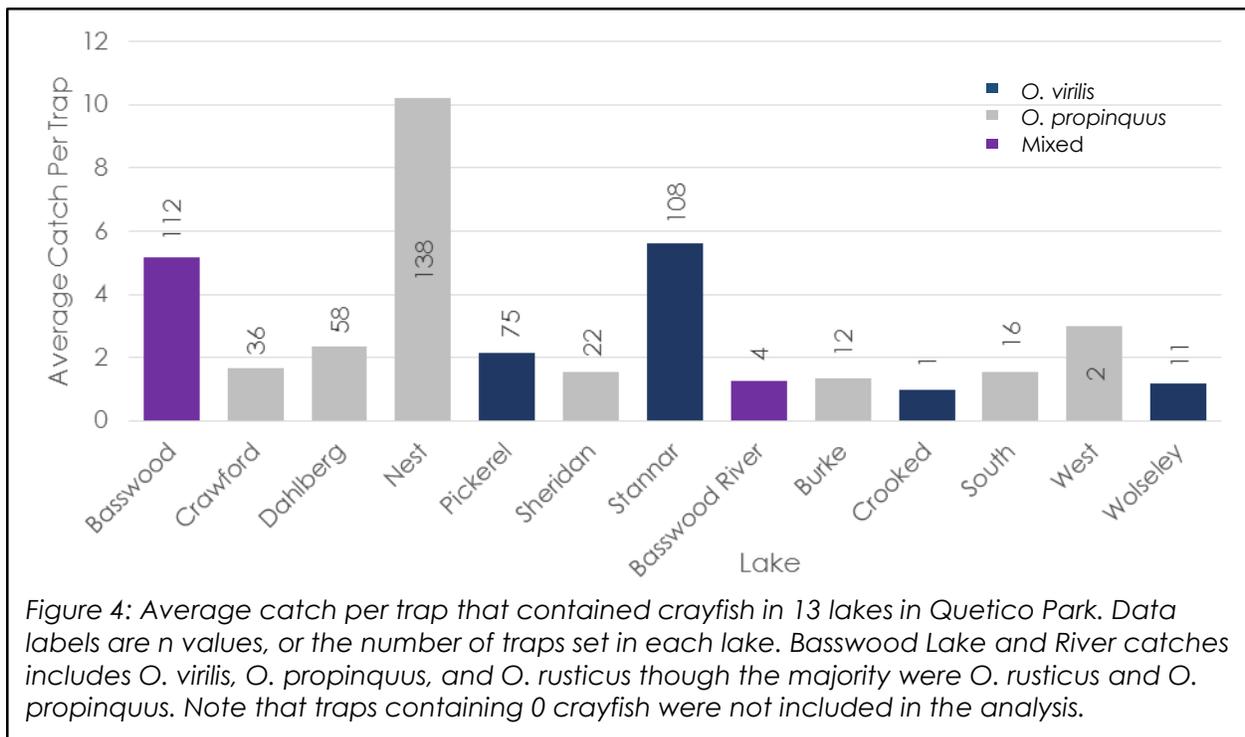
Two lakes contained only *O. virilis* with a sample size >20. Average carapace length of crayfish caught in Stannar Lake was 30.7 mm (CTL = 58.5 mm), significantly ($p = 1.9E-24$, $d = 1.15$) larger than the average total length of 25.3 mm for crayfish in Pickerel (CTL = 49.0mm).

Basswood Lake was analyzed separately because it was the only lake containing all three crayfish species. Sample sizes are highly variable, making it difficult to compare between species. Average carapace size of *O. rusticus* was 72.2 mm (CTL = 136.8 mm), for *O. virilis* was 70.8 mm (CTL = 125 mm), and for *O. propinquus* was 59.8 mm (CTL = 98.0 mm). These values are higher than the average across other lakes.

CRAYFISH DENSITY

Between Lakes and Species

Crayfish density also varied between lakes (Figure 4). Nest and Stannar had the highest catches per trap with catch per trap in Nest Lake almost twice that in Stannar. In the majority of lakes less than 2 were caught per trap. In all species, the majority of traps contained 0 crayfish and the number of traps containing a given number of crayfish decreased exponentially as the number of crayfish caught increased (Figure 5). Due to a low sample size, the density of *O. rusticus* was not compared. Average catch per trap of *O. propinquus* (5.7 crayfish per trap, $n = 298$) was significantly ($p = 2.57E-5$, $d = 0.365$) higher than the catch per trap of *O. virilis* (3.9 crayfish per trap, $n = 201$).



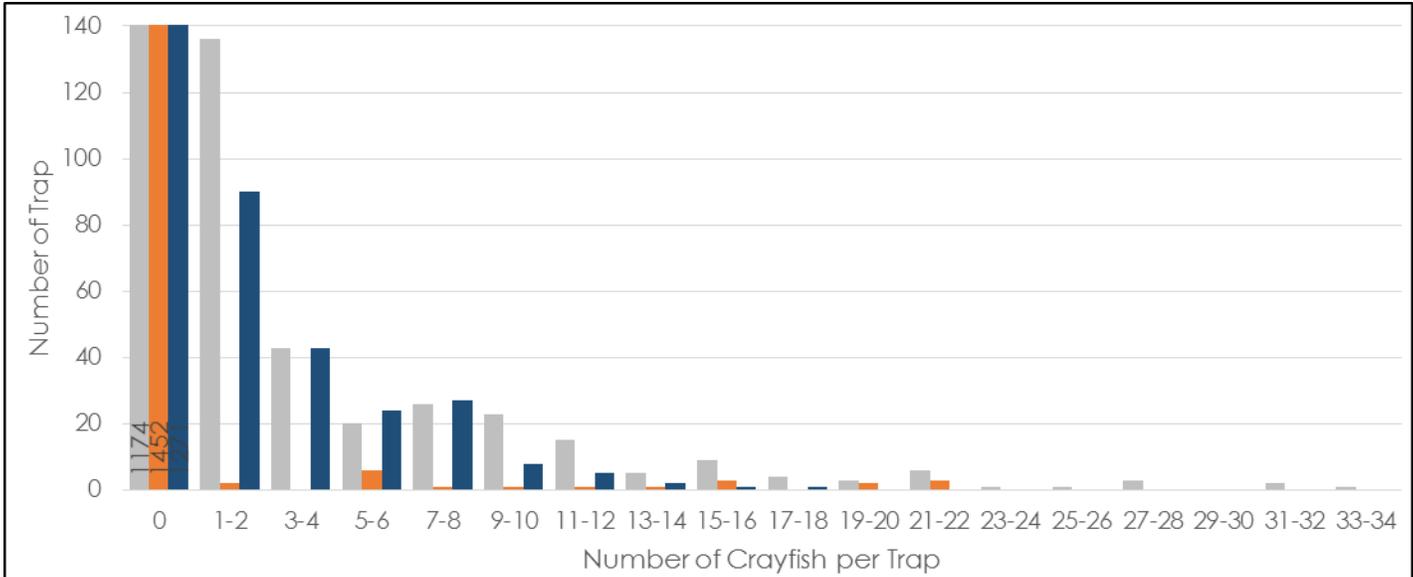


Figure 5: The number of traps in each catch per trap group for three species of crayfish across all lakes. Total number of traps set = 1472. Vertical axis has been cut and the number of traps containing no crayfish is labeled on the graph.

Although calcium values are not available for many of the surveyed lakes, conductivity has been found to be highly correlated with calcium concentrations ($p < 0.01$; $r^2 = 0.98$). Calcium concentration explains almost all the variation in conductivity measurements in the lakes sampled (Jackson 2016). Conductivity values were available for 7 of the lakes with crayfish trap catches. Comparing combined crayfish catches with estimated calcium levels did not show a significant relationship between the highest catch occurring in the lake and the highest calcium level (Figure 6).

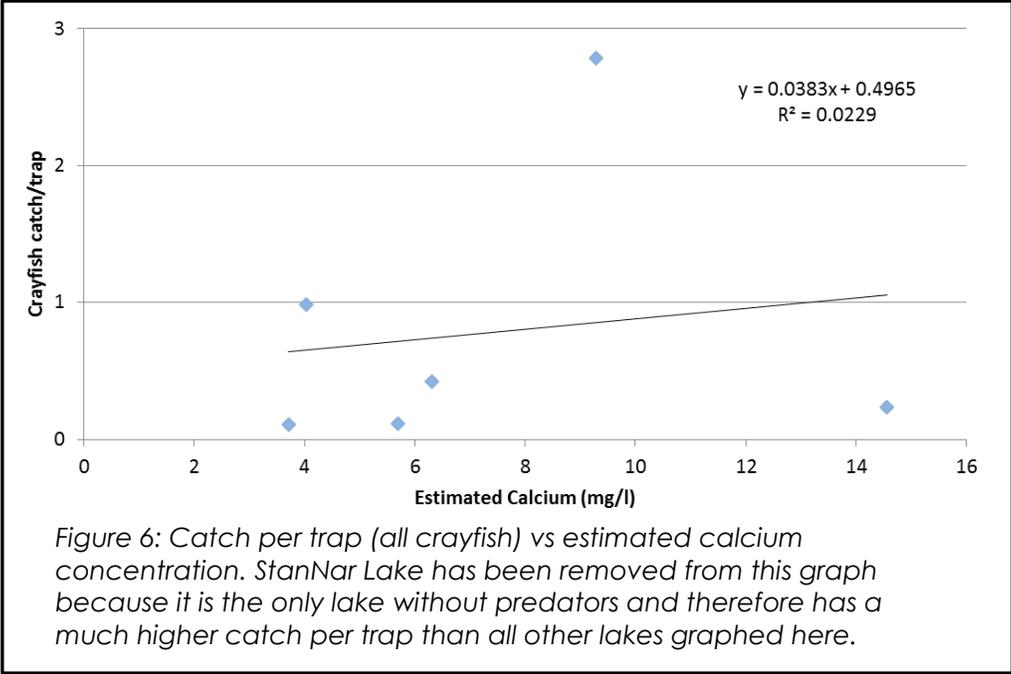


Figure 6: Catch per trap (all crayfish) vs estimated calcium concentration. StanNar Lake has been removed from this graph because it is the only lake without predators and therefore has a much higher catch per trap than all other lakes graphed here.

Presence of crayfish specialist predators (i.e. one or more of smallmouth bass, rock bass and/or largemouth bass) appears to have much more of an influence on the number of crayfish caught than water chemistry. Combined catch of all crayfish (in traps that contained crayfish) from lakes with crayfish predators was significantly lower than those lakes without (3.0 crayfish/trap vs. 8.2 crayfish/trap respectively ($p=2.7E-29$, $d=0.879$)). In lakes with crayfish predators, only 29% of the traps caught crayfish ($n=1210$ traps) while in lakes without crayfish predators, 94% of the traps had crayfish ($n=263$ traps).

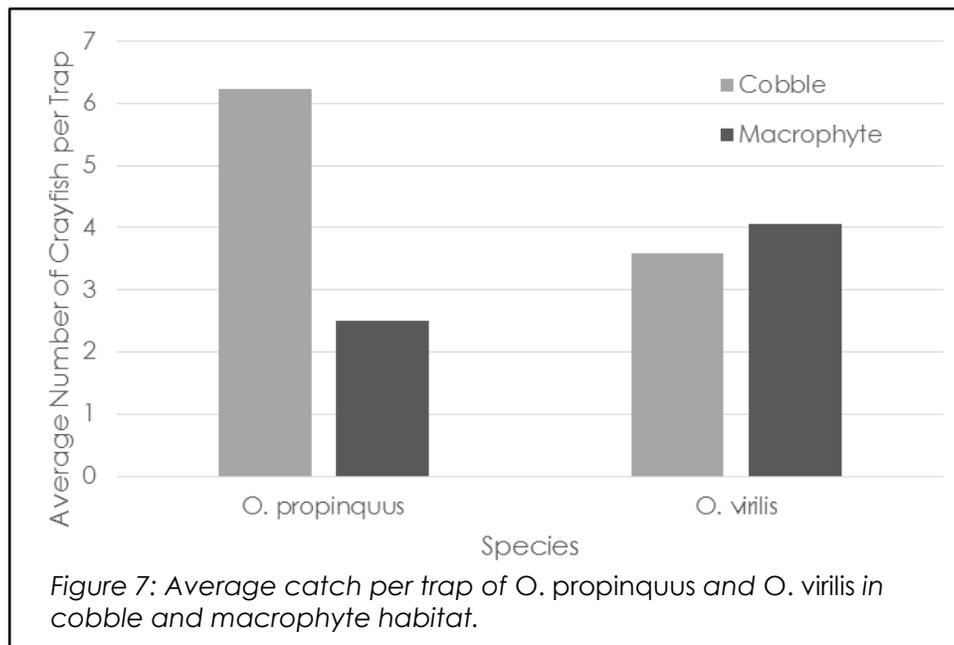
HABITAT USE

All Crayfish

Average catch per trap for all crayfish in soft bottom, detritus, cobble, and macrophyte habitats was 4.1, 9.5, 5.2, and 3.6 crayfish per trap respectively. However, habitat sampling was highly variable between lakes. For example, traps were only set in soft bottom habitat in Stannar Lake which had a high overall catch per trap regardless of habitat. Only cobble and macrophyte habitats were consistently sampled across all lakes.

Between Species

Therefore, to determine if *O. virilis* and *O. propinquus* are found at higher densities in certain habitats, only macrophyte and cobble habitats were compared (Figure 7). Catch per trap was not significantly different between macrophyte and cobble habitat for *O. virilis*. However, significantly more ($p = 1.99E-10$, $d = 0.757$) *O. propinquus* were caught in cobble (average 6.2 crayfish/trap) compared to macrophyte habitat (average 2.5 crayfish/trap) (Table 4).



DISCUSSION

Crayfish caught in Quetico Park fall within their expected size ranges (Roesler 2012). *O. virilis* are reported to have larger maximum carapace lengths than both *O. rusticus* and *O. propinquus*. A handful of very large *O. virilis* were captured in Quetico and size distributions show that average size peaks above that of *O. propinquus*. However, invasive crayfish, particularly *O. rusticus* are known to be large and aggressive, with average sizes much larger than *O. virilis* – as observed in Quetico. *O. propinquus* was only slightly larger than *O. virilis*.

Crayfish trapping in Quetico Park has revealed a strong 'lake effect' on crayfish size, density and distribution across habitats, regardless of species. Part of this lake effect is likely the presence of crayfish specialist predators. Crayfish predators can decrease the number of crayfish caught in traps by either reducing the mobility of crayfish (they spend more time hiding in refugia), or by reducing crayfish numbers through predation. Smallmouth, largemouth, and rock bass as well as sunfish species are known to feed on crayfish, especially small and young-of-the-year crayfish (Tetzlaff et al 2011; Hein et al. 2006; Keller and Moore 2000). Tetzlaff et al. (2011) showed that invasive rusty crayfish populations can be limited by predation. Lakes sampled throughout Quetico that are known not to contain crayfish predators (Nest, Stannar) have significantly higher crayfish catches per trap and crayfish size regardless of species. Only *O. rusticus* in the area near Prairie Portage in Basswood Lake was able to maintain high densities in the presence of crayfish predators. *O. propinquus* and *O. virilis* catches per trap in Basswood Lake were low. The most widespread of the crayfish specialist predators is the non-native smallmouth bass. No data is available on crayfish abundance in Quetico lakes prior to smallmouth bass introduction and expansion over the past 75 years but these surveys suggest that native crayfish density was likely higher in many lakes prior to its arrival.

Increased crayfish size and density in Basswood Lake might be partially explained by lake productivity. Compared to other lakes located on granitic bedrock, Basswood Lake has higher levels of calcium and productivity (as measured by total dissolved solids), likely due to being located immediately downstream from the area of volcanic origin bedrock although it is not as high as lakes located directly on volcanic origin rock (Appendix 4). France (1983) showed correlations between phytoplankton production and chlorophyll concentrations, both measures of lake productivity, and crayfish growth and maximum size. Basswood had larger average crayfish sizes, regardless of species, than any other lakes sampled.

Habitat use also shows a weak relationship with the presence/absence of predators. Hill and Lodge (1994) found that predation influences habitat selection by crayfish. When predation pressure is high crayfish seek shelter in cobble habitat. However, with an absence of crayfish specialist predators such as in Stannar and Nest Lakes, crayfish are free to select habitat based on food availability, which would be higher in macrophyte and detritus habitat. Adair (2016) reported that in Stannar Lake, *O. virilis* preferred macrophyte habitat over all other habitat types. Similarly, Champaigne-Klassen (2016) observed that *O. propinquus* used cobble and detritus habitat equally in Nest Lake. The remainder of lakes sampled in Quetico had crayfish specialist predators. Here it is expected that cobble would be preferred (Hill and Lodge 1994; Keller and Moore 2000). However, in Sheridan, Burke and Dahlberg Lakes a significantly higher catch per trap was found in macrophyte habitats and in Pickerel Lake cobble and macrophyte habitats were equally preferred. It is possible that even with high levels of predation, crayfish must still spend a large amount of time foraging in macrophyte areas in Quetico lakes. Overall, *O. virilis* were found to utilize cobble and macrophyte habitats equally while *O. propinquus* were caught only slightly more often in cobble.

Calcium also has the potential to influence the density of crayfish populations and the spread of invasive *O. rusticus* because calcium is critical for exoskeleton growth in crustaceans (Edwards et al. 2013). Calcium levels in waterbodies has been shown to restrict *O. rusticus* establishment or limit abundance, with studies from Wisconsin showing that they require minimum levels ranging from 2.5 - 7 mg/L (Capelli and Magnuson 1983) to 8 mg/L (Latzka 2015) while *O. virilis* can survive much lower levels. Although calcium values are not available for many Quetico lakes, conductivity has been found to be highly correlated with calcium concentrations ($p < 0.01$; $r^2 = 0.98$) with calcium concentration explaining almost all the variation in conductivity measurements in the surveyed lakes (Jackson 2016). Surveys of Quetico lakes showed that dissolved calcium concentrations range from 1.7 to 18.4 mg/L depending largely on the type of bedrock beneath the lake (Jackson 2016). Lakes with volcanic bedrock had significantly higher calcium concentrations than those on either sedimentary or granitic rock. Surveys in Quetico during 2017 were conducted in lakes underlain by calcium-rich volcanic bedrock (i.e. Sheridan, Carp, That Man, Sucker). However, *O. rusticus* were only observed in Sucker Lake, one of the lakes where they were originally observed in the eighties. *O. propinquus* on the other hand were found in all lakes surveyed at the south end of the Park. It is not clear at this time why *O. rusticus* appear to be the dominant crayfish in Sucker Lake and the area of Basswood Lake near Prairie Portage but have not expanded to other lakes, regardless of calcium levels, like *O. propinquus*.

Assuming that the results of these surveys are due to unassisted crayfish migration (i.e they weren't moved between lakes by anglers), they suggest that *O. propinquus* have the ability to eventually access all lakes within the Park with no evidence that water chemistry, physical barriers, flow direction or crayfish specialist predators limit their movement or establishment in lakes. Furthermore, assuming that *O. virilis* were originally present in all lakes in Quetico, *O. propinquus* has been shown to completely replace *O. virilis* populations. *O. virilis* was not found in any of the lakes sampled in 2016 and 2017 containing *O. propinquus*. It is not known why *O. rusticus* are not also expanding their ranges. However, studies in Wisconsin have shown that even after initial introduction, dominance by *O. rusticus* can take over a decade to occur. However, once a critical density was achieved, there was a rapid increase in *O. rusticus* and decline in *O. propinquus* and *O. virilis* (Wilson et al. 2004). It may be *O. propinquus* has characteristics that allow it to invade more rapidly in Quetico lakes although it may not exclude eventual movement into other waterbodies by *O. rusticus*.

If calcium concentrations are a factor in dispersal ability, it may allow some informed predictions of future *O. rusticus* distribution. Based on analysis of 59 lakes with conductivity data, 44% of the lakes are predicted to have calcium levels less than 2 mg/L that would not support *O. rusticus* (while only 5% have values above 8 mg/L that would support abundant populations. However, lakes with high calcium levels are restricted to certain locations in the park where the bedrock geology is dominated by volcanic origin rocks or in lakes immediately downstream from these areas such as Basswood Lake (Figure 8). Lakes in the south east corner of the Park would be predicted to be at highest risk from *O. rusticus* invasion and lakes downstream of them which show higher levels of calcium than expected being at medium risk (Figure 9). Current data somewhat supports this theory with known *O. rusticus* populations currently found in areas with calcium levels above 8mg/L. However, to date, surveys in calcium rich lakes have not shown that rusty crayfish have moved into them in spite of being found in lakes adjacent to them for ~25 years.

These surveys provide some information on the potential ecological impacts if non-native crayfish invade Quetico lakes. If the invasion is limited to *O. propinquus*, as appears to be occurring at this time, changes might include complete replacement of native crayfish but similar size distribution of the population. Habitat use might be similar however, there is some evidence for increases in crayfish abundance within lakes. Predators therefore should be able to prey on *O. propinquus* as they did *O. virilis* especially given the lack of observed predator-prey size relationship observed in data collected for adult fish to date (Jackson 2017b). Higher crayfish densities may cause other changes in the ecosystem if energy directed to crayfish biomass changes flow to other species.

O. rusticus on the other hand does have the potential to disrupt Quetico's aquatic ecosystems. They are larger (on average 15mm larger), than *O. virilis* and exist in high densities near Prairie Portage in Basswood Lake even in the presence of crayfish specialist predators. Decreased macrophyte cover has also been associated with the arrival of *O. rusticus* (Wilson *et al.* 2004) and they have been shown to be more effective predators of fish eggs than *O. virilis* (Morse *et al.* 2013). Though *O. rusticus* in Quetico are currently known in similar locations as they were found in the early 90's, they have frequently been shown to displace both *O. virilis* and *O. propinquus* in other locations (DiDonato 1993; Olden *et al.* 2006). It is not entirely known why *O. rusticus* have not spread throughout Basswood Lake or into neighbouring water bodies.

SUMMARY OF CONCLUSIONS

The objective of these studies was to address the following questions:

1) Increase understanding of the factors affecting the abundance, habitat use, and biological characteristics of native *O. virilis* and invasive *O. rusticus* and *O. propinquus* in Quetico Provincial Park.

Abundance of both native and the non-native crayfish species *O. propinquus* appear to be highly affected by the presence of crayfish specialist predators such as smallmouth bass and rock bass with higher densities of crayfish in lakes without the crayfish specialist predators. Only *O. rusticus* has been captured in high densities in the presence of crayfish specialist predators.

Both *O. virilis* and *O. propinquus* showed similar use of habitat with no strong preferences across lakes between cobble habitat or macrophyte habitat with either species.

O. rusticus were significantly larger than both *O. virilis* and *O. propinquus* with an average total length ~15mm longer than the other species. Although *O. propinquus* had statistically larger average length than *O. virilis*, the differences was less than 2mm which is likely biologically insignificant, especially to predator fish given the lack of predator-prey size relationship found to date for adult fish. *O. virilis* had a wider observed range in sizes than *O. propinquus*.

2) Determine the distribution and dispersal ability of invasive *O. rusticus* and *O. propinquus*.

O. propinquus appears to have demonstrated the ability to disperse widely and quite rapidly over the past 25 years and are currently found in all the lakes surveyed in the south end of the park. Dispersal ability does not appear to be limited by water chemistry, physical barriers, direction of water flow or presence of crayfish specialist predators. Based on these

studies, *O. propinquus* appears to have the ability to disperse throughout the majority of Quetico lakes.

To date, there appears to be much less expansion of *O. rusticus* outside of the areas where it was identified in the late eighties and early nineties. While the factors affecting its dispersal ability are not completely understood, if it is calcium concentrations as suggested by other studies, their range and dispersal may be limited to high calcium lakes found in the southeast corner of the park defined by volcanic origin bedrock and the lakes immediately downstream from them.

3) Assess the likelihood for invasion and potential risk that invasive crayfish will cause in Quetico's aquatic ecosystems.

Although the potential risk for invasion of the majority of park lakes by *O. propinquus* appears high, the ecological impact risk may be low to moderate given the similarity of size and habitat use with native crayfish. The largest potential impacts may be the loss of a native species (*O. virilis*) and a potential shift in biomass/energy flow within the community if the crayfish abundance in the lakes increases. The impact of this on other components of the aquatic ecosystem is unknown at this time.

Conversely, for *O. rusticus*, although the likelihood of invasion in Quetico lakes is less, the ecological risk of invaded lakes appears to be much higher. Besides the likely loss of the native species *O. virilis*, there is predicted to be higher crayfish abundance in the lakes with a greater potential shift in biomass/energy flow and subsequent community impacts. These studies and others would also predict a shift in size distribution of the crayfish population which may affect prey availability for smaller fish species including pumpkinseed, bluegill, rock bass, northern (longear) sunfish as well as young of larger species. In addition, there is the risk of reduced abundance and diversity of aquatic plants observed in other studies with potential impacts on habitat of other species.

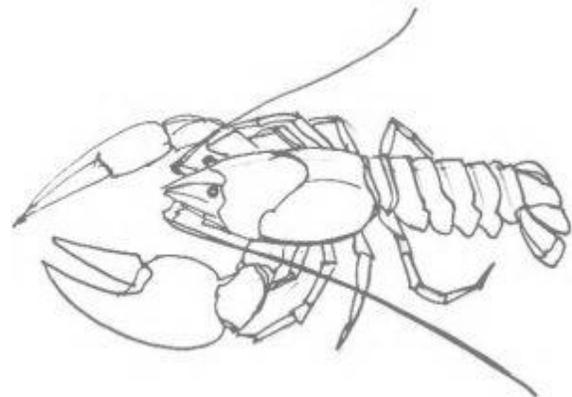
FUTURE RESEARCH

The threat of *O. rusticus* movement warrants some monitoring of their location in the southern lakes of Quetico Park. At its simplest, this would only require several transect surveys throughout Basswood and neighbouring lakes to determine if *O. rusticus* are moving away from Prairie Portage. Additional work could investigate the lake effect observed through previous year's studies. Chemistry profiles, full species counts and measures of the density of crayfish specific predators for each lake already trapped and any lakes surveyed in the future would allow for a better understanding of how environmental characteristics influence the spread of *O. rusticus* and interspecific competition between crayfish species.

Longer term monitoring of lakes currently with native crayfish but at high risk to be invaded by non-native crayfish would provide better understanding of the ecological risk of non-native crayfish invasion to lake ecosystems in Quetico. This would be even more effective if it was tied to long term monitoring of the lake ecosystem including the fish community. At this time, This Man Lake, located in the volcanic bedrock zone in the southeast corner of the park, is part of the BsM monitoring program as a trend lake and has been assessed in 2010 and 2016 with plans to continue on a five year cycle. It is not known to have non-native crayfish at this time although it is in the area at high risk for invasion by both *O. propinquus* and *O. rusticus*. It would appear to be an excellent site to consider for long term monitoring.

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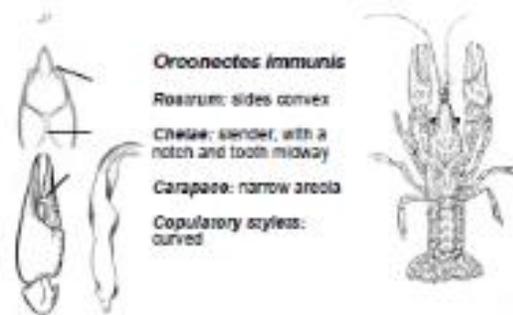
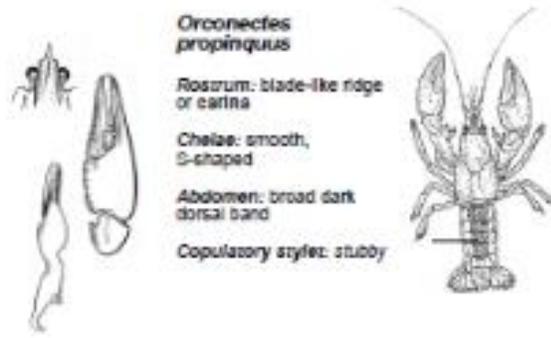
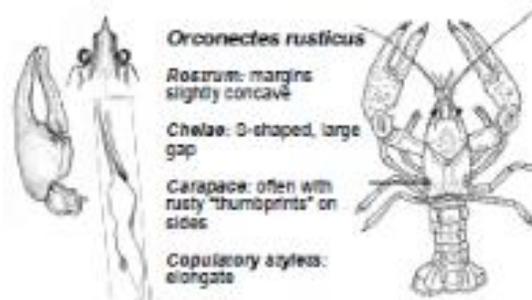
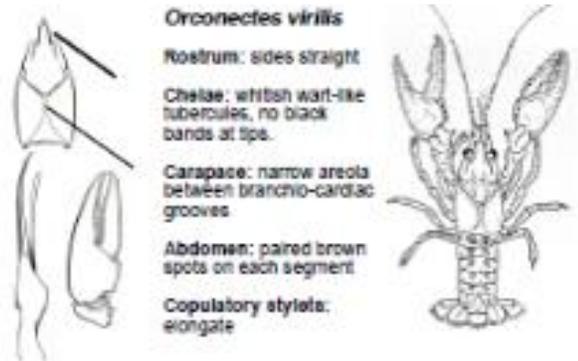
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APPENDIX 1:

IDENTIFICATION OF CRAYFISH SPECIES FOUND IN QUETICO PROVINCIAL PARK

(only used prior to 2017) (<http://pinicola.ca/crayfishontario/index.htm>)

(note: lack of visible rostral carina and presence of black bands on claws typical of *O. propinquus* found in Minnesota and Quetico caused identification between *O. propinquus* and *O. rusticus* using this key)



APPENDIX 2:

SIMPLIFIED VISUAL CRAYFISH KEY FOR QUETICO PP – 2017

(adapted from Roesler 2012)

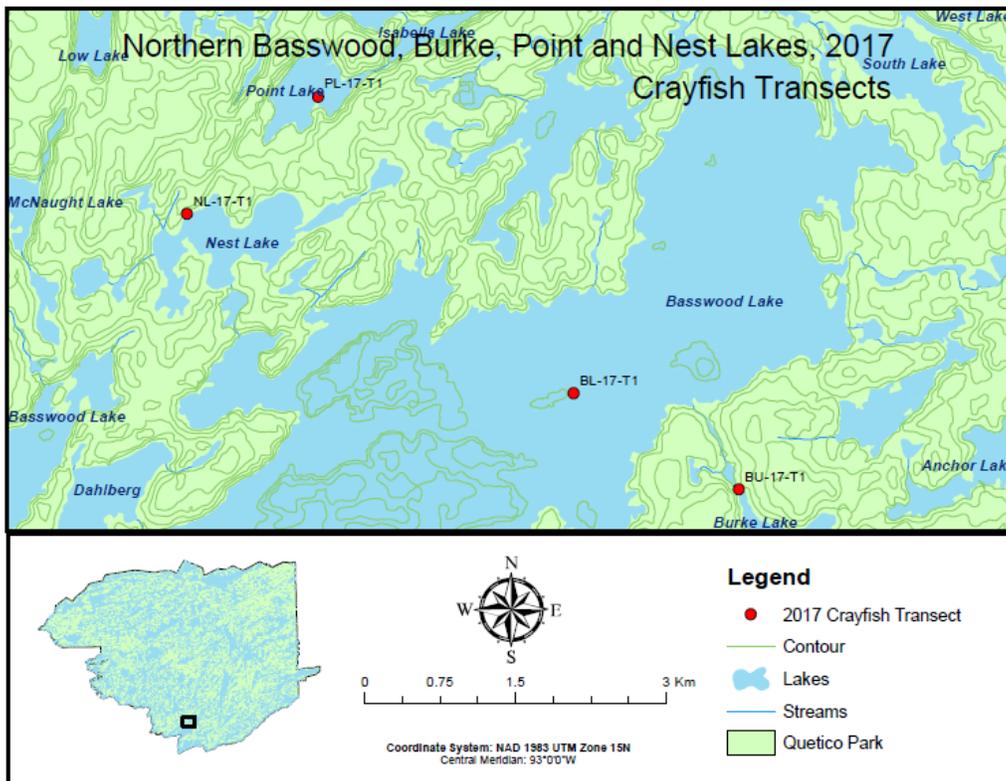
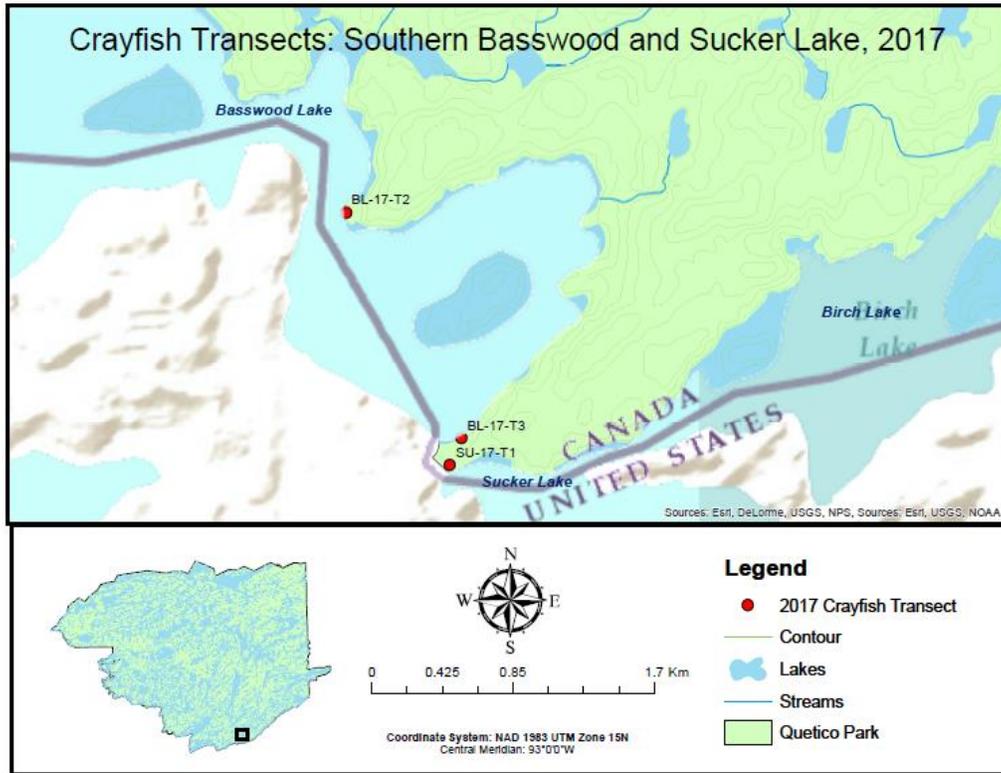
For medium to large-sized, live or fresh specimens

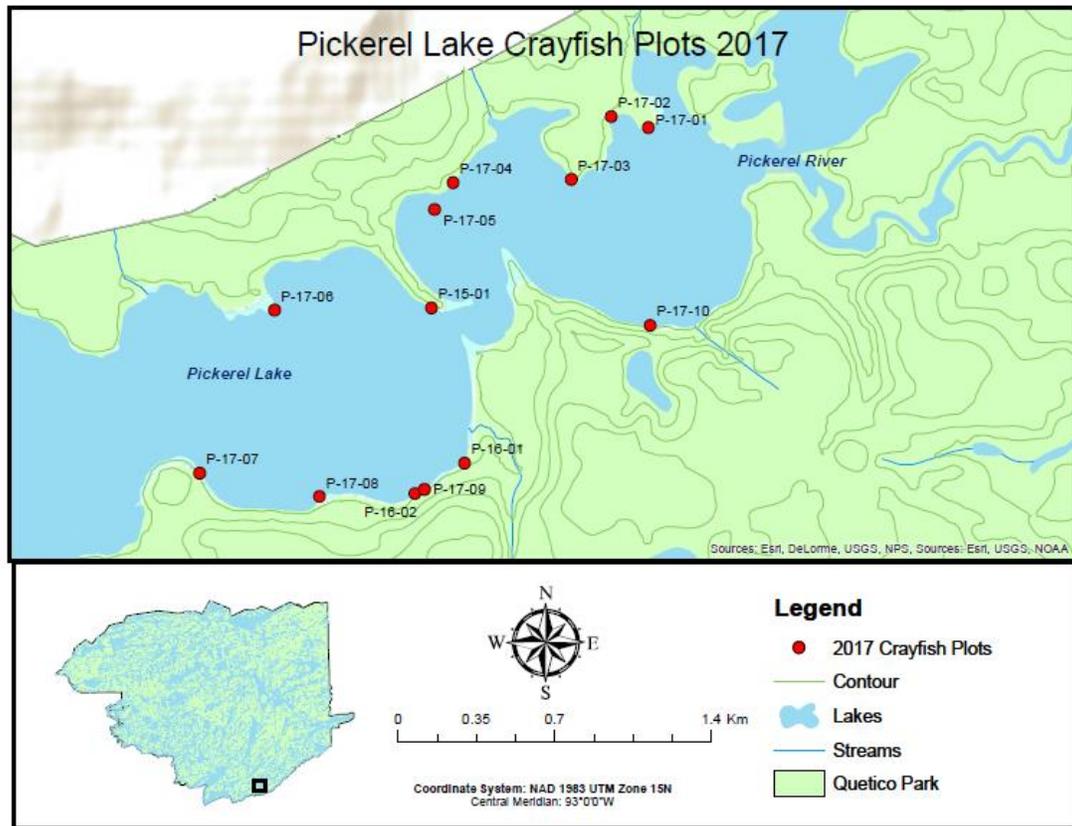
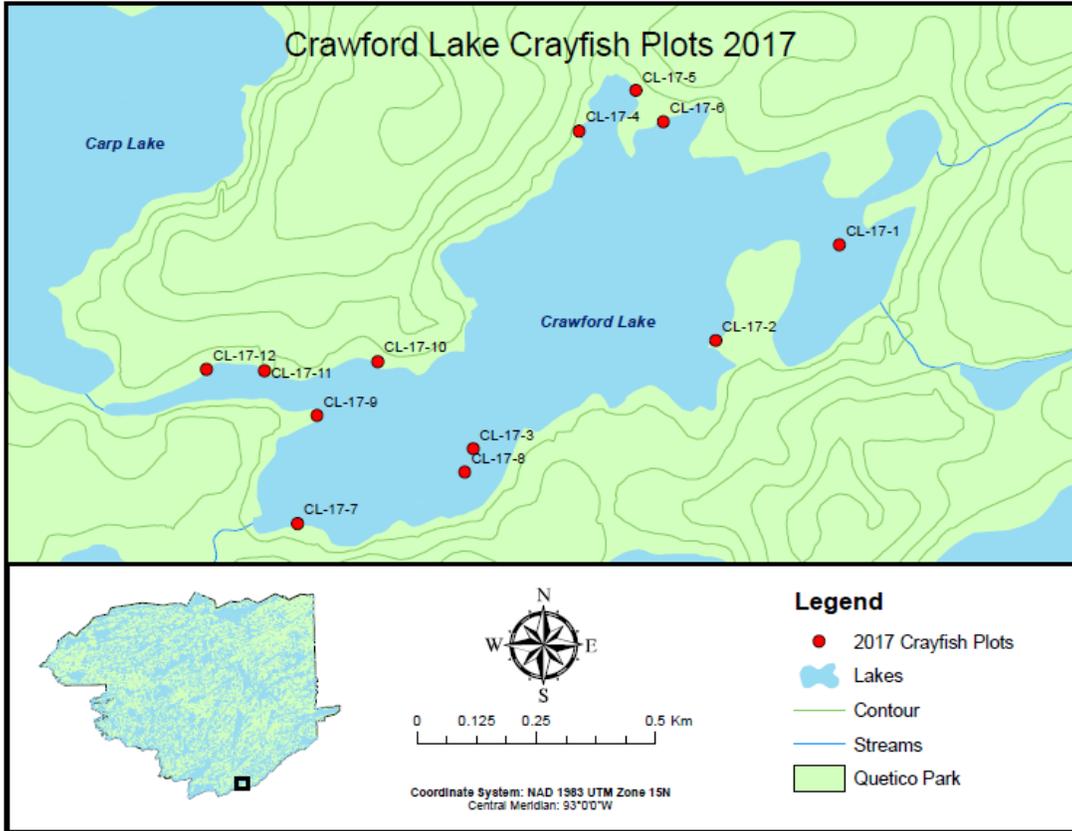
1. - Tuft of fibers present adjacent to claw hinge – *Orconectes immunis* (calico crayfish) (not currently identified from park – voucher any suspected sample)
 - No tuft of fibers present adjacent to claw hinge – 2

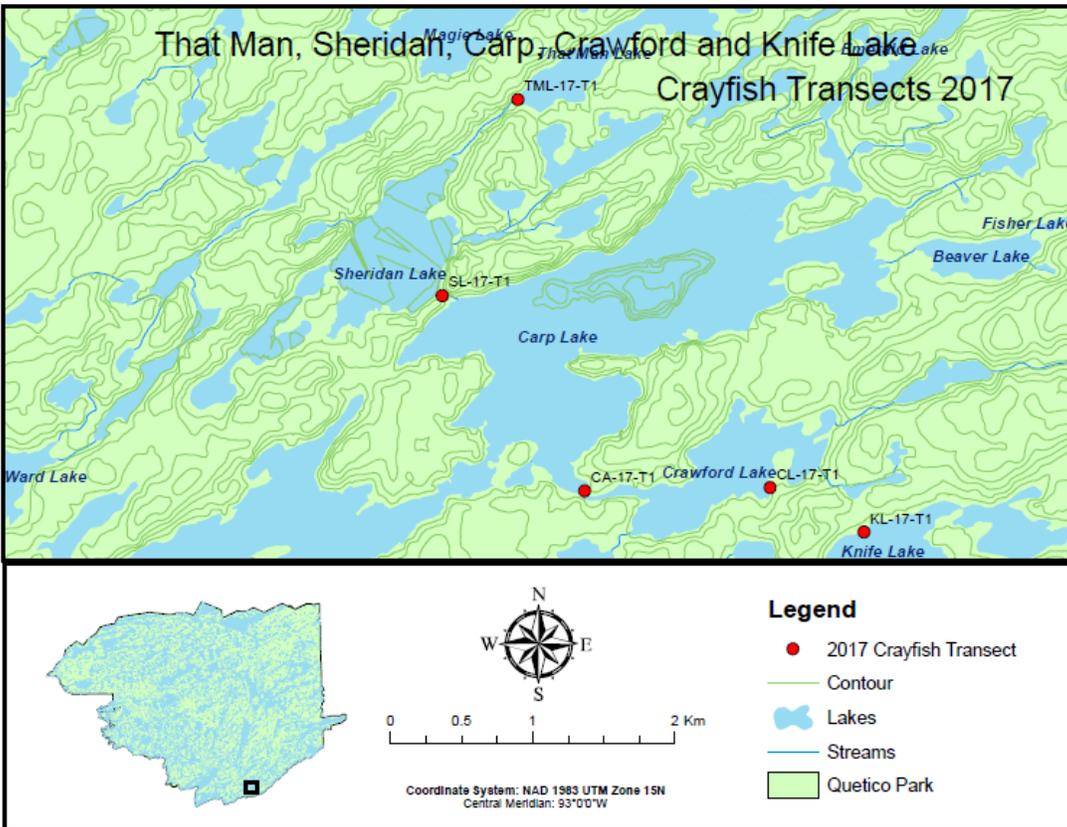
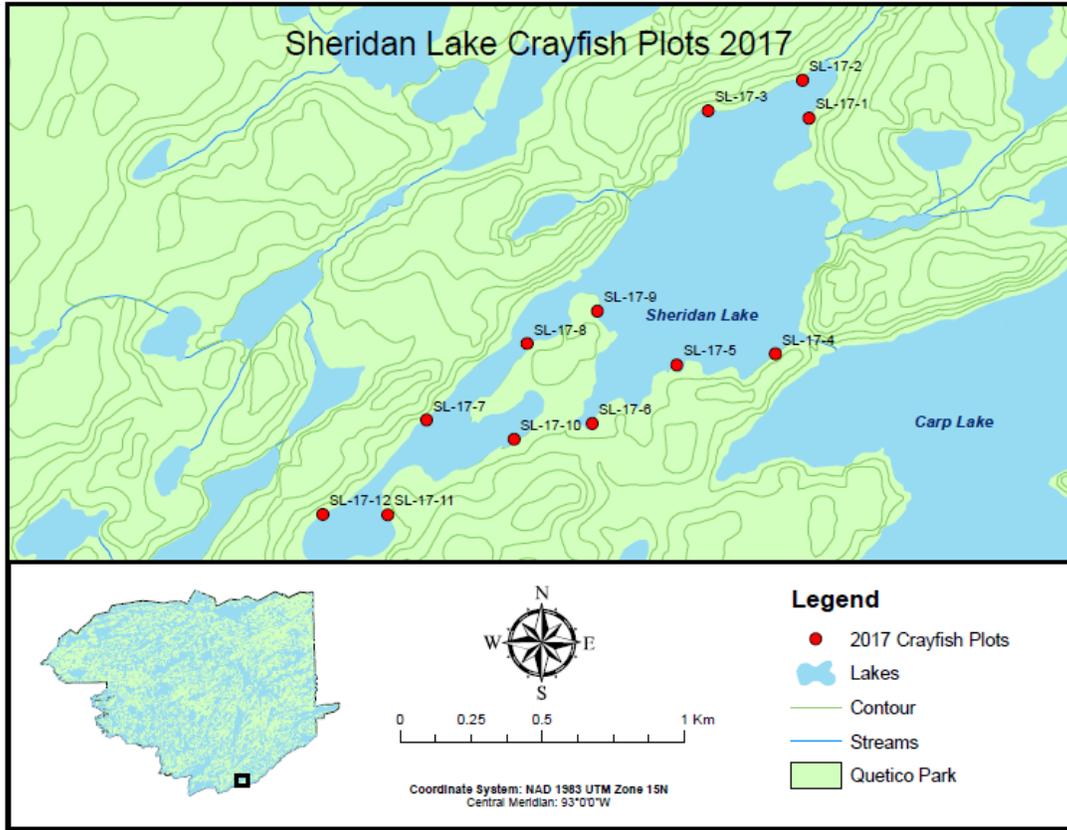
2. - Dark band on top of tail, brown or grey body color; claw tips orange/red with black rings; rostral carina present but not typically visible (can be felt with pen tip) – *Orconectes propinquus* (northern clearwater crayfish - invasive)
 - Without dark band on top of tail – 3

3. - Claw tips orange/red without black bands; two to four rows of angular spots on tail – *Orconectes virilis* (virile crayfish - native to Quetico)
 - Claw tips orange/red with black bands; rust colored bands on tail segments; usually with rust colored spots on sides – *Orconectes rusticus* (rusty crayfish – invasive)

APPENDIX 3: CRAYFISH PLOT LOCATIONS







APPENDIX 4: LAKE CHARACTERISTICS

Lake	Basswood	Crooked	Dahlberg	Nest	South	West	Burke	Crawford	Sheridan	Carp	Knife	That Man	Point	Sucker	Pickereel	Wolseley	StanNar	
	2014, 2015, 2017	2015	2016	2016, 2017	2016	2016	2016, 2017	2017	2017	2017	2017	2017	2017	2017	2015, 2016, 2017	2016	2016	
Year Sampled																		
<i>O. virilis</i>	x	x													x	x	x	
<i>O. propinquus</i>	x		x	x	x	x	x	x	x	x	x	x	x	x				
<i>O. rusticus</i>	x																	
Catch per trap (all crayfish)	2.8	0.0	0.9	9.8	0.3	1.0	0.1	0.4	0.2	NA	NA	NA	NA	NA	1.0	0.1	5.1	
Area (ha)	4840	3500	69	75	60	35	268	45	80	466	608	156	41		5754	1307	39	
Secchi Depth (m)	5.3			4.6				5.2		4.7		6.3	7	5	3	4.3	2.6	3.4
Conductivity (uS/cm)	58.3							40.1	43.2	85	119.6				79.8	31.6	30	16.5
Bedrock Type	granitic*	granitic*	granitic	granitic	granitic	granitic	granitic	sedimentary	volcanic	volcanic	sedimentary	volcanic	granitic	volcanic	granitic	sedimentary	granitic	
Ca Concentration (mg/L)				6					15.2		13				2.9			
Calculated Ca (from cond)	9.3							5.7	6.3	14.6	21.4				13.5	4.0	3.7	1.0
Crayfish Specialist Predators	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	
Crayfish Specialists	Smallmouth Largemouth Rock Bass	Smallmouth Largemouth Rock Bass	Smallmouth Rock Bass		Smallmouth Largemouth	Smallmouth Largemouth	Smallmouth Largemouth Rock Bass	Largemouth Rock Bass	Smallmouth	Smallmouth	Smallmouth Rock Bass	Smallmouth	Smallmouth	Smallmouth	Smallmouth Largemouth Rock Bass	Smallmouth	Smallmouth Largemouth Rock Bass	

* these lakes are on granitic bedrock but immediately downstream from volcanic origin bedrock

Lake	Known Fish Species
Basswood	Blackchin Shiner, Black Crappie, Blacknose Shiner, Bluegill, Burbot, Central Mudminnow, Cisco, Iowa Darter, Lake Trout, Lake Whitefish, Largemouth Bass, Northern (Longear) Sunfish, Northern Pike, Pumpkinseed, Rock Bass, Shorthead Redhorse, Shortjaw Cisco, Smallmouth Bass, Walleye, White Sucker, Tadpole Madtom, Yellow Perch
Crooked	Black Crappie, Blacknose Shiner, Bluegill, Bluntnose Minnow, Burbot, Cisco, Finescale Dace, Iowa Darter, Johnny Darter, Lake Trout, Lake Whitefish, Largemouth Bass, Log Perch, Northern (Longear) Sunfish, Mimic Shiner, Mottled Sculpin, Northern Pike, Redbelly Dace, Rock Bass, Silver Lamprey, Smallmouth Bass, Spottail Shiner, Walleye, White Sucker, Tadpole Madtom, Yellow Perch
Dahlberg	Bluegill, Rock Bass, Smallmouth Bass, Yellow Perch
Nest	Bluntnose Minnow, Green Sunfish, Lake Trout, Northern Pike, Pumpkinseed, Yellow Perch
South	Largemouth Bass, Northern Pike, Smallmouth Bass, Walleye, White Sucker
West	Largemouth Bass, Northern Pike, Smallmouth Bass, Walleye, White Sucker
Burke	Cisco, Lake Trout, Largemouth Bass, Northern Pike, Rock Bass, Smallmouth Bass, Walleye
Crawford	Bluegill, Largemouth Bass, Pumpkinseed, Rock Bass, Tadpole Madtom, Yellow Perch
Sheridan	Cisco, Deepwater Sculpin, Lake Trout, Northern Pike, Slimy Sculpin, Smallmouth Bass, Walleye, White Sucker
Carp	Cisco, Lake Trout, Lake Whitefish, Mimic Shiner, Northern Pike, Smallmouth Bass, Walleye, White Sucker, Yellow Perch
Knife	Burbot, Cisco, Lake Trout, Lake Whitefish, Northern Pike, Rock Bass, Smallmouth Bass, Walleye, Yellow Perch
That Man Lake	Black Crappie, Blacknose Dace, Blacknose Shiner, Bluntnose Minnow, Common Shiner, Fathead Minnow, Green Sunfish, Iowa Darter, Lake Trout, Northern Pike, Pearl Dace, Smallmouth Bass, Walleye, White Sucker
Point	Northern Pike, Small Mouth Bass,
Sucker	Bluegill, Burbot, Cisco, Lake Whitefish, Largemouth Bass, Mimic Shiner, Northern Pike, Rock Bass, Smallmouth Bass, Walleye, White Sucker, Yellow Perch
Pickereel	Blacknose Shiner, Blackchin Shiner, Bluntnose Minnow, Burbot, Cisco, Deepwater Sculpin, Johnny Darter, Lake Trout, Log Perch, Mimic Shiner, Northern Pike, Rainbow Smelt, Slimy Sculpin, Smallmouth Bass, Spottail Shiner, Trout Perch, Walleye, Whitefish, White Sucker, Yellow Perch
Wolseley	Black Crappie, Blacknose Shiner, Bluegill, Bluntnose Minnow, Burbot, Cisco, Common Shiner, Deepwater Sculpin, Iowa Darter, Johnny Darter, Lake Trout, Lake Whitefish, Largemouth Bass, Log Perch, Mimic Shiner, Mottled Sculpin, Northern Pike, Pumpkinseed Sunfish, Rock Bass, Sauger, Smallmouth Bass, Spottail Shiner, Tadpole Madtom, Trout Perch, Walleye, White Sucker, Yellow Perch
Stannar	Blacknose Shiner, Darter spp., Northern Pike, Pumpkinseed, Walleye, White Sucker, Yellow Perch

APPENDIX 5:

RESULTS OF 2017 SURVEYS

For a detailed methodology of the statistical analysis performed on 2017 data see Adair (2016). In 2017, trap plots were set July 11-13 on Pickerel Lake, July 20-22 on Sheridan Lake, and July 22-24 on Crawford Lake. Walking transects were conducted on July 6 on Nest and Point Lakes, July 7 on Burke and Basswood Lakes, July 8 on Sucker Lake, July 21 on Sheridan Lake, July 22 on Carp Lake, and July 24 on Crawford and Knife Lakes.

Consistent with previous surveys (Jackson 2017; Adair 2016; Champaigne-Klassen 2016; Jackson 2015a) carapace length was found to be a good predictor of crayfish total length across all lakes sampled in 2017 (regression test statistic Crawford Lake $p = 2.88E-4$, Sheridan Lake $p = 4.05E-21$, and Pickerel Lake $p = 5.25E-53$). Carapace length is an easier and more reliable measurement to collect in the field and therefore was used for all statistical analysis. However, for ease of interpretation, calculated total length is displayed in graphs.

O. virilis caught in Pickerel Lake ranged in size from 36 – 79 mm with an average size of 49 mm. *O. propinquus* found in Sheridan Lake which had an average crayfish size of 53 mm and a range of 35 – 72. *O. propinquus* caught in Crawford Lake had a range of 38 – 71 mm and an average size of 56 mm (Figure 5-2).

An average of 0.99 *O. virilis* were caught per trap on Pickerel, 0.24 *O. propinquus* in Sheridan and 0.42 in Crawford. Males (average carapace length = 26.8 mm) were significantly ($p = 0.003$, $d = 0.577$) larger than females (average carapace length = 23.8 mm) in Pickerel Lake. Though average carapace length for male crayfish was larger than females in both Sheridan and Crawford Lakes, these differences were not significant. Catch per trap containing crayfish was not significantly different for male vs female crayfish in Pickerel and Crawford Lake. Though significantly ($p = 0.016$, $d = 0.859$) more males (1.42 crayfish per trap) compared to females (1 crayfish per trap) were caught in Sheridan, the effect size in this comparison was large.

In all lakes, the number of crayfish caught per trap containing crayfish was not significantly different between cobble and macrophyte habitat. There was also no significant difference between the size of crayfish in these habitats in both Pickerel and Sheridan Lake. Crawford Lake had significantly ($p = 0.041$, $d = 0.569$) larger crayfish in macrophyte (mean carapace length = 30.7 mm) compared to cobble (mean carapace length = 28.2mm). However, in this case the effect size was also large, and the p-value is barely significant.

In all lakes, no relationship was found between the depth of the trap and the number of crayfish caught, or the depth of the trap and the size of crayfish caught.