Where Have All the Crayfish Gone? *Change in Occurrence of Invasive and Native Crayfish in the St. Louis River Estuary Over 14 Years*

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Abstract
The rusty crayfish, *Orconectes rusticus*, is invading lakes and rivers of Wisconsin, Minnesota, Michigan, and Ontario; negatively affecting native crayfish, invertebrate, and plant populations. In July 1999, we sampled crayfish at 84 locations in the St. Louis River Estuary (northwestern Wisconsin). Two rusty crayfish were captured in Superior Bay and 87 native crayfish throughout the estuary (three species: *O. virilis*, *O. propinquus*, and *O. immunis*). In 2013, the surveys were repeated; including measures of habitat, substrate, vegetation, pH, and dissolved calcium levels. In July and August, 2013, we sampled 148 locations, including the 84 sites sampled in 1999. Due to its observed invasiveness in northern Wisconsin, rusty crayfish populations were hypothesized to have expanded during the 14 years between samples. However, despite a greater sampling effort, no rusty crayfish were caught and only three native crayfish (all *O. virilis*) were captured during the 2013 sampling. Dissolved calcium concentrations and pH were found to be at levels acceptable to support crayfish populations. It is hypothesized that the decline may be due to; 1) disturbance: in June 2012 the estuary experienced a 1 in 500 year flood event, 2) predation: sport fish populations have been increasing over the past decade, 3) parasitism: rusty crayfish declines in some Northern Wisconsin lakes have been attributed to parasitization by a trematode identified as *Microphallus sp*., or 4) pollution: possibly an undetected pollution event occurred between sample events. Additional research will examine reasons for the observed declines.

Introduction

There are over 390 identified species of crayfish in North America (Lodge et al., 2000) and three species are common in the Great Lakes Basin including the native virile crayfish (*Orconectes virilis*), the native clear-water crayfish (*O. propinquus*), and the invasive rusty crayfish (*O. rusticus*) (Olden et al., 2006). Originating from the Ohio River Basin, the rusty crayfish has been identified as the most invasive crayfish species in North America (Clancy, 1997). The rusty crayfish has spread rapidly throughout the northern Midwest and through Wisconsin in particular where more than 86% of the watersheds have been colonized (Olden et al., 2006). The effects of rusty crayfish on native crayfish (Hill and Lodge, 1999; Peters and Lodge, 2012), vegetation (Lodge and Lorman, 1987; Lodge and Hill, 1994), and food webs (Lodge and Hill, 1994) are well documented. Rusty crayfish are currently displacing native crayfish species including *O. virilis* and *O. propinquus* in many northern Wisconsin lakes (Lodge, 1985; Hill and Lodge, 1999; Olden et al., 2006).

Rusty crayfish exhibit wide tolerances for climate and habitat. They can survive on a variety of substrates such as clay, silt, sand, gravel, or rock, but prefer cobble, carbonate substrates, gravel substrates, and woody habitats (Gunderson, 2008; Hill & Lodge, 1994). They can be found in both lentic and lotic habitats, usually at depths of less than 1 meter (Capelli, 1982; Crandall and Fetzner, 2003). Rusty crayfish prefer temperatures between 20 and 23°C;

however they can survive water temperatures ranging from 0 to 30°C (Mundahl and Benton, 1990). Currently, the only identified factor limiting establishment of rusty crayfish in permanent water bodies in the northern United States is water quality; low dissolved calcium concentrations (<2.5 mg/L) and low pH (<5.5) have been found to inhibit establishment of rusty crayfish in northern Wisconsin lakes (Olden et al., 2006).

The St. Louis River is the largest U.S. tributary to Lake Superior. At its confluence with Lake Superior, the river forms a freshwater estuary with over 4,000 ha of aquatic habitat, much of it shallow (<2m depth). The estuary can be divided into four zones; 1) the upper estuary is a system of braided channels and islands with a mixture of cobble and sandy substrates, 2) the mid-estuary includes sheltered bays and estuarine flats with a mixture of vegetated silt, sand, and clay substrates, 3) St. Louis Bay is an industrial harbor with boat slips and armored rocky shorelines, and 4) Superior Bay is an industrial harbor with boat slips, armored rocky shorelines, marinas, urban development, and sandy beaches. The St. Louis River Estuary is the largest U.S. port on the Great Lakes and there is significant interest in detecting and managing invasive aquatic species.

On June 25, 1999, rusty crayfish were first discovered in the St. Louis River Estuary while conducting a zebra mussel inspection (Rodd, 1999). Four specimens were found including both adults and juveniles. Based on this sighting, surveys were conducted throughout the estuary in July 1999 and again in 2013. Given the rapid invasion of many Northern Wisconsin lakes and rivers (Wilson et al., 2004), an increase and spread of rusty crayfish populations is expected over the 14 years between surveys.

Methods

Surveys were conducted in July 1999 and July through August 2013. All surveys used modified cylindrical minnow traps (trap opening enlarged to 5 - 7 cm). Traps were baited, set out overnight in shallow water (0.5 - 2 m), and collected the following morning (12-18 hours later). Sites were selected haphazardly and the selection was stratified by estuary region (Superior Bay, St. Louis Bay, mid-estuary, upper estuary) with 21 sites selected in each region (Figure 1). In 1999, all traps were baited with fish (approx. 100 g per trap). In 2013, all trap sites were baited with beef liver (approx. 100 g) because it was recommended as the standard method to sample rusty crayfish in Wisconsin (pers. comm. C. Roesler, WI DNR). Due to the change in sampling methods, possible effect of bait on trap catch was examined using paired traps. Forty-two traps in the upper and mid-estuary regions baited with fish were paired (located within 2 m) with traps baited with beef liver. Paired traps were deployed and collected over the same time period. In 2013, the sampling program was expanded when few crayfish were caught at the prior sampling sites. Twenty-two additional sites were haphazardly selected in areas considered to be protected from disturbance. Therefore, in 1999 a total of 84 traps were set, and in 2013 a total of 148 traps were set. In 1999 four traps and in 2013 seventeen traps were not retrieved due to unknown causes.

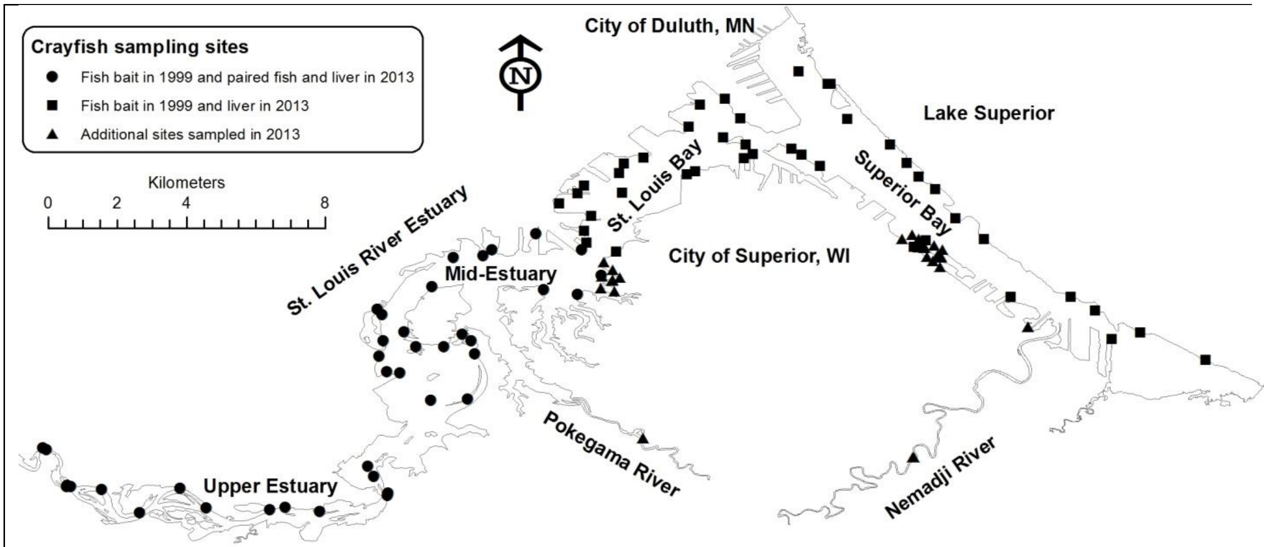

Figure 1. Crayfish sampling locations in the St. Louis River Estuary.

In 2013 a set of environmental variables at the survey locations was measured. We used a YSI 6600 data sonde to measure water temperature, specific conductivity, dissolved oxygen, pH, and turbidity at 72 site locations. In addition, dissolved calcium concentration (LaMotte 3609-01) was measured at 16 sites. Presence of aquatic vegetation and substrate type was recorded for all sites.

Results

In 1999, four species of crayfish were collected during the survey (Table 1). Only two specimens of rusty crayfish were collected, both from St. Louis Bay. The trap that captured the two rusty crayfish was set along a rocky shoreline with no visible vegetation. Three species of native crayfish were collected during the survey. Overall, *O. propinquus* was most frequently captured (63 individuals, 71%), but was only found in the upper estuary. *O. virilis* was commonly found throughout the St. Louis River Estuary (23 individuals, 26%). One individual *O. immunis* (1%) was collected in the upper St. Louis River section.

In 2013 only three crayfish were collected during the survey. No crayfish were collected from the 84 locations sampled previously. All three specimens were *O. virilis* collected from two traps set at additional sites. The first trap that captured an adult male and female was deployed in a swampy area of Pokegama Bay with emergent and submergent vegetation and a clay substrate. The second trap captured a juvenile female crayfish and was set underneath a bridge along a rocky shoreline of the Nemadji River with no visible vegetation and a clay substrate.

Table 1. Crayfish caught in 1999 and 2013 in the St. Louis River Estuary										
Species	***O. rusticus***		***O. propinquus***		***O. virilis***		***O. immunis***		**Total**	
Year	**1999**	**2013**	**1999**	**2013**	**1999**	**2013**	**1999**	**2013**	**1999**	**2013**
Superior Bay	0	0	0	0	11	0	0	0	11	0
St. Louis Bay	2	0	0	0	3	0	0	0	5	0
Mid Estuary	0	0	0	0	0	0	0	0	0	0
Upper Estuary	0	0	63	0	9	0	1	0	73	0
Additional sites	NA	0	NA	0	NA	3	NA	0	NA	3
Total	**2**	**0**	**63**	**0**	**23**	**3**	**1**	**0**	**89**	**3**

Water quality was measured at 72 sites during the 2013 sampling. Water temperature ranged from 18.7 to 24.1°C, pH ranged from 7.42 to 8.25, conductivity ranged from 0.152 to 0.379 ms/cm, dissolved oxygen ranged from 6.62 to 9.86 mg/L, turbidity ranged from 5.4 to 22.1, dissolved calcium ranged from 48 to 76 ppm.

Discussion

An increase in rusty crayfish over the 14 years between sampling events was expected. However, we did not collect any rusty crayfish at any of our sample locations despite an increased sample effort. In addition, we detected a 97% reduction in native crayfish sampled. No native crayfish were detected at our prior sampling locations, including in the upper estuary where *O. propinquus* previously had been most abundant. All three crayfish collected in 2013 were located in Pokegama Bay and the Nemadji River.

Dissolved calcium concentrations below 2.5 ppm and water pH below 5.5 have been shown to restrict rusty crayfish in some Northern Wisconsin lakes (Olden et al., 2006). However, our calcium measurements throughout the estuary were much higher, ranging from 48-76 ppm, so it is unlikely low dissolved calcium concentration is the reason for inhibition of rusty crayfish establishment. Our pH measurements ranged from 7.42 to 8.25, also well above pH values expected to limit rusty crayfish colonization. Additional water quality parameters do not indicate any reason to suspect that water quality is responsible for the observed reduction in rusty crayfish colonization or the reduction of native crayfish populations.

Conclusion

Despite an increased sampling effort we caught 97% fewer crayfish in 2013 than in 1999. Several potential reasons have been identified; 1) disturbance: in June 2012 the estuary experienced a 1 in 500 year flood event (Kotz et al., 2014), 2) predation: sport fish populations have been increasing over the past decade including small-mouth bass (*Micropterus dolomieu*) which are known predators of crayfish (Wilson et al., 2004), 3) parasitism: rusty crayfish declines in some northern Wisconsin lakes have been attributed to parasitization by a trematode identified as *Microphallus sp*. (pers. comm. C. Roesler, WI DNR), and 4) pollution: possibly an undetected pollution event occurred between sample events. More study is needed to examine the cause of the observed crayfish decline.

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