ASSESSMENT OF RISKS AND CONSEQUENCES OF NON-NATIVE CRAYFISH INVASIONS IN MICHIGAN'S LOWER PENINSULA

By

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ABSTRACT

ASSESSMENT OF RISKS AND CONSEQUENCES OF NON-NATIVE CRAYFISH INVASIONS IN MICHIGAN'S LOWER PENINSULA

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Crayfish are critical components of freshwater ecosystems. In some ecosystems crayfish can comprise more biomass than all other benthic invertebrates combined. Despite their role as keystone species, crayfish are often an understudied organism. This is particularly true for Michigan, where the last comprehensive crayfish survey was reported in 1975. Since this time, many non-native species, including the rusty crayfish (*Orconectes rusticus*), have been introduced into the Great Lakes region that could alter crayfish communities across the state. Further, documenting crayfish species composition and distributions in Michigan is becoming increasingly critical due to the imminent invasion of the red swamp crayfish (*Procambarus clarkii*). The Michigan Department of Natural Resources (MDNR) has implemented legislation to help discourage the introduction of invasive crayfishes, but the lack of recent surveys prevents the determination of whether any non-native crayfish, other than rusty crayfish, are already established in the state. To fill these knowledge gaps we designed and implemented a stratified random survey of streams in Michigan's lower peninsula to assess the presence, distribution, and habitat associations of native and non-native crayfish species, as well as documented the expansion of rusty crayfish. Our results indicated that the distribution of most crayfish species are widespread throughout the state, while others have expanded their range, including the invasive rusty crayfish. Our results also suggest that rusty crayfish likely influence the habitat associations of some native crayfish species. Although no red swamp crayfish were discovered in Michigan, our assessment indicated risks associated with entry vectors red swamp crayfish could use to enter the state. Introduction would appear to be likely unless management actions are taken to prevent red swamp crayfish entry into Michigan via the pet, food, and bait trade.

To my family, blood and otherwise.
I could not have done this without the constant support and encouragement from my friends, family, and community. I am truly blessed to have such a wonderful network of passionate and caring individuals to surround myself with.
Go raibh maith agaibh, Gracias, Miigwech, Thank you.

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INTRODUCTION

Crayfish, sometimes called crawdads, crawfish, or mudbugs, are crustaceans in the order Decapoda, which includes shrimps, crabs, and lobsters. Crayfish are native to every continent except continental Africa and Antarctica, although they have been introduced to parts Africa (Mkoji et al. 1999, Foster and Harper 2007, Taylor et. al 2007). There are currently 640 described species of crayfish worldwide, consisting of three families; Astacidae, native in Europe, Western Asia, and the Pacific Northwest, Parastacidae, native to South America, Madagascar, Australia, New Zealand, and New Guinea, and Cambaridae, native throughout much of North America and the only family native to Michigan (Creaser 1931, Lippson 1975). Crayfish are often the largest macroinvertebrates found in aquatic and semi-aquatic habitats, and can account for more biomass than all other macroinvertebrates in an ecosystem (Lodge and Lorman 1987). Ecologically crayfish often act as important keystone species, affecting food web structure and habitat characteristics (Lodge et al. 1994, Dorn and Mittelbach 2004, Ilheu et al. 2007, Carreira et al. 2014)

In some locales crayfish are an important form of sustenance (Huner 1994, Foster and Harper 2007). In the South-central United States for example 60,000 tons of crayfish are farmed each year for consumptive purposes (Hobbs et al. 1989, Huner and Lindqvist 1995). The appetite for crayfish is found outside of North America as well. Europe has a traditions and culinary cultures that revere crayfish, especially in Scandinavia (Lodge et al. 2000). Crayfish have been introduced to Africa, China, and other areas of Europe in hopes of creating either sustainable protein sources or to support markets for increasing interest in crayfish as culinary delicacies (Barbaresi and Gherardi 2000, Li et a. 2005, Foster and Harper 2007).

Crayfish are not only sought after for food but also make popular pets among aquarium enthusiasts. Their bright color morphotypes and hardiness in tanks are appealing to many hobbyists around the world. This coupled with their relative ease to transport has meant that exotic crayfish have become available to buyers across the globe. The crayfish pet trade is suspected to have resulted in the introduction of several invasive crayfish species in Europe and South America including *Cherax destructor, Cherax quadricarinatus, Procambarus alleni, Procambarus clarkii,* and *Procambarus fallax* f. *virginialis* (Chucholl 2013, Loureiro et al 2015).

Recreational anglers are also drawn to crayfish as they are an effective bait for many popular sport and game fishes. Recreational anglers are thought to be one of the most influential vectors for the spread of invasive crayfish in North America (Olden et al. 2006, Chucholl 2013). The spread of the rusty crayfish across Michigan and Wisconsin is particularly associated with the angler behavior of gathering crayfish in one watershed and then traveling to fish in another watershed where unused crayfish might be released into the water (Olden et al. 2006).

Despite their economic importance and ability to drastically alter food webs, crayfish are an often overlooked group of organisms (Loughman 2007, Taylor et al. 2007, Jones et al. 2010, Swecker et al. 2010). This is especially true in Michigan where the last comprehensive survey took place prior to the 1960's (Lippson 1975). Michigan is reported as having at least eight native crayfish species; *Cambarus diogenes, Cambarus polychromatus, Cambarus robustus, Fallicambarus fodiens, Orconectes immunis, Orconectes propinquus*, *Orconectes virilis,* and *Procambarus acutus* (Creaser 1931, Lippson 1975, Hobbs and Jass 1988, Thoma et al. 2005). Michigan also currently harbors at least one invasive crayfish species, the rusty crayfish (*Orconectes rusticus*) which has been present in the state for at least 130 years (Faxon 1884, Creaser 1931, Lippson 1975). Like many other invasive species, the initial introduction of *O. rusticus* into Michigan is attributed to shipping canals, specifically those connecting the Ohio River and Maumee River watersheds in Ohio. However, subsequent expansion in Michigan is often attributed to bait release and natural dispersal (Creaser 1931, Olden et al. 2006). Michigan biologists are also concerned about the potential entry of another invasive crayfish, *P. clarkii*, the red swamp crayfish. *P. clarkii* is native to the Southcentral U.S. but has become invasive in several foreign countries and U.S. states including watersheds in Ohio that are near Michigan (Norrocky 1983, Hobbs et al. 1989).

In North America, many crayfish species are at risk due to human induced alterations to the environment, ongoing stream and wetland degradation, and introductions of nonindigenous organisms including other crayfish species (Loughman 2007, Taylor et al. 2007, Jones et al. 2010, Swecker et al. 2010). In Michigan, there is great uncertainty regarding crayfish populations due to potential expansions of the established rusty crayfish into new watersheds, the potential invasion of the red swamp crayfish, and a lack of understanding regarding the status of native crayfish. These sources of uncertainty all indicate that a statistically-robust, comprehensive survey for crayfish is necessary. By clarifying the current status of crayfish in Michigan, managers can make informed decisions regarding mitigation of potential introduction vectors and will build a base for further understanding the unique role of crayfish in Michigan's aquatic ecosystems.

The first chapter of this thesis will focus on updating the current knowledge of crayfish species in Michigan's Lower Peninsula. Although crayfish can be found in wetlands, fields, lakes and streams, this survey focused on streams and the crayfish inhabiting stream ecosystems (Hobbs 1988). Substrate data was collected in order to investigate any possible influence invasive crayfish, such as *O. rusticus,* might have on the substrate associations of native crayfish species where invasive and native crayfish co-occur. Looking at historical accounts of crayfish in Michigan also allowed me to compare the co-occurrence of obligate stream species, and how the presence of invasive crayfish might affect these assemblages (Lippson 1975).

The second chapter of this thesis will focus on an assessment of entry vectors and risks associated with *P. clarkii.* The focus is based on *P. clarkii* due to the presence of carcasses found in popular fishing spots, eluding to the possibility they are being used as bait, and are thus being imported into the state by some means (MDNR 2013). With prior literature suggesting that *P. clarkii* could potentially cause ecological and economic issues in Michigan, I wanted to assess the potential pathways *P. clarkii* could use to enter Michigan. Potential entry routes surveyed included the pet, bait, and food trades, use in classrooms, and natural dispersion from known populations in Ohio as likely modes of introduction. Qualitative methods were then applied to determine the relative likelihood that any particular vector could result in the introduction of *P. clarkii* to Michigan. This study's findings will help form management decisions to prevent the introduction of *P. clarkii* as well as other non-native crayfish into Michigan, and mitigate the spread of any future introductions.

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Chapter 1:

Changes in distribution and substrate associations of Michigan's Lower Peninsula crayfishes

Introduction:

Crayfish are often a dominant component of freshwater biomes (Lodge et al. 1994, Huner and Lindqvist 1995, Charlebois and Lamberti 1996, Nyström et al. 2006). In some aquatic ecosystems, crayfish account for more biomass than all other macroinvertebrates combined; this, coupled with their ability to drastically alter food webs and habitat features, makes them powerful ecosystem engineers (Momot et al. 1978, Lodge and Lorman 1987, Hobbs et al. 1989, Momot 1995, Carreira et al. 2014). As a group, crayfish show several unique life-history traits, such as terrestrial burrowing, that allow them to thrive in a wide variety of aquatic habitats. These unique life histories have allowed different species to coexist by occupying distinct ecological niches depending on seasonal water cycles or habitat heterogeneity (Hobbs 1942, Hobbs 1981, Welch and Eversole 2006).

Michigan is home to eight native crayfish species, three of which are primarily found in permanent open water habitats such as streams and lakes (*Cambarus robustus*, *Orconectes propinquus*, and *Orconectes virilis*). Three are found primarily in subterranean burrows and are therefore rarely observed in open water as adults except in early summer and spring while releasing their young (*Cambarus diogenes*, *Cambarus polychromatus*, and *Fallicambarus fodiens*). Two are facultative burrowers, depending on variable conditions (*Orconectes immunis*, and *Procambarus acutus*) (Lippson 1975, Hobbs and Jass 1988, Thoma et al. 2005).

One non-native species of crayfish, the rusty crayfish (*Orconectes rusticus*), has been reported in Michigan for over 130 years with major range expansion occurring during the 20th Century (Faxon 1884, Lippson 1975). Initial *O. rusticus* range expansion is attributed to shipping canals connecting the Ohio River and Maumee River watersheds in Ohio, and subsequent spread in the region is believed to be primarily a result of bait bucket release by anglers or intentional release by lake managers seeking to manage macrophyte communities (Creaser 1931, Olden et al. 2006). *O. rusticus* lives primarily in streams and lakes and has been consistently observed to have negative impacts on populations of native stream and lake dwelling crayfish, including the northern crayfish (*O. virilis*) and northern clearwater crayfish (*O. propinquus*). This species is quite invasive as it competes for resources, exhibits less susceptibility to predation by native predators, and hybridizes with *O. propinquus* (Capelli and Munjal 1980, Capelli and Munjal 1982, Hill and Lodge 1993, Perry et al. 2001, Perry et al. 2002, Roth and Kitchell 2005). *O. rusticus* can affect native fish assemblages by more successfully preying on fish eggs than native crayfish, altering habitat through extensive macrophyte clipping when foraging, and disconnecting adjacent food webs (Capelli and Munjal 1982, Lodge et al. 1998, Dorn and Mittelbach 2004, Roth et al. 2007, Morse et al. 2013, Kreps et al. 2016).

Another non-native crayfish, the red swamp crayfish (*Procambarus clarkii*), has recently become a point of concern for Michigan natural resource professionals, including the Michigan Department of Natural Resources (MDNR). *P. clarkii* is native to the Southcentral U.S., and is capable of dispersing long distances both over land and in aquatic environments (Hobbs et al. 1989, Kerby et al. 2005, Banha and Anastácio 2014). *P. clarkii* is invasive in several foreign countries and U.S. states, including an area of Ohio that shares a watershed with Michigan (Norrocky 1983, Hobbs et al. 1989). Although there is no documented populations in Michigan, *P. clarkii* carcasses have been found at popular fishing sites. This raises concerns that they might be introduced into the wild via human mediated actions, such as bait bucket dumps (MDNR 2013). Like *O. rusticus, P. clarkii* also tends to be more aggressive than native crayfish and can affect food web structure and habitat characteristics (Lodge et al. 1994, Dorn and Mittelbach 2004, Ilheu et al. 2007, Klose and Cooper 2012, Carreira et al. 2014). Compared to native Michigan crayfishes, the life history of *P. clarkii* is most similar to that of the native white river crayfish (*Procambarus acutus*), a species that overlaps in range in some portions of the Southcentral United States. Despite being native to warmer climates, studies from Europe and the Pacific Northwest demonstrate that *P. clarkii* can become established in cold water streams and lakes, suggesting that Michigan is well within abiotic conditions necessary for invasion (Chucholl 2011, Taylor et al. 2015).

Across North America, many crayfish species are at risk due to human induced alterations to the environment, ongoing stream and wetland degradation, and introductions of nonindigenous organisms including other crayfish species (Loughman 2007, Taylor et al. 2007, Jones et al. 2010, Swecker et al. 2010). Despite these concerns, and evidence stating the importance of timely organismal studies addressing the status and trends of species composition and populations (Schloesser et al. 2006, Taylor et al. 2007), Michigan lacks recent information on statewide distributions and the status of native and invasive crayfish, the most recent survey having occurred in the 1960's (Lippson 1975). Concerns about the potential expansion of *O. rusticus*, possible invasion and establishment of *P. clarkii*, and effects of *O. rusticus* on native crayfishes since the last comprehensive survey underscore the need for a new survey. This study seeks to update our current understanding of the status and range of crayfish within Michigan's Lower Peninsula, document changes in the range of *O. rusticus* compared to historical data, and identify the possible presence of other non-native species such as *P. clarkii*.

Previous studies have shown that *O. rusticus* can outcompete native congeners for preferred shelter such as cobble or other substrates with large interstitial space, forcing native species to use less desirable habitat types (Capelli and Munjal 1982, Bergman and Moore 2003, Garvey et al. 2003). Consequently we have added an objective to explore patterns in habitat use by native crayfish when in sympatry with *O. rusticus.*

Methods:

Data Collection

Although crayfish occupy two general habitat types – open water habitats and burrows (Hobbs 1989) – this survey was constrained to streams. Because no crayfish species that occur in Michigan are limited to lake habitats, it was assumed that stream surveys would be sufficient to document the general distribution of crayfish species that might also occur in lakes (Lippson 1975). During the summers of 2014 and 2015, species presence and absence was assessed at 326 (20.5%) of the 1,590 unique stream segments in Michigan's Lower Peninsula (Figure 1.1) as defined by Michigan Department of Natural Resources'(MDNR) Stream Status and Trends Program (SSTP) (Seelbach et al. 1997, Wills et al. 2006). Stream segments were selected by stratifying the SSTP database by management unit and major watershed. A random sample of 20% of available stream segments were selected for collection from each stratum as an attempt to evenly distribute sampling effort across watersheds. A watershed was defined by the hierarchical structure found in the SSTP database, where watersheds are listed by streams and their tributaries directly connected to a Great Lake (Wills et al. 2006).

Stream sampling was conducted with dip nets, using standard dip netting protocols for crayfish collection (Olden et al. 2006). Dip netting was selected because it allowed a consistent sampling technique to be implemented across all streams regardless of substrate type. Technicians worked in pairs to sample stream segments and generally attempted to access streams from a road crossing, with one individual working upstream and the other downstream of the crossing. Technicians worked to catch as many crayfish as possible in a 20-minute period. This included netting to scoop individuals off substrate, lifting rocks or larger substrate with the foot or hand, and using hands and twigs to probe crayfish out of root structures or undercut banks. Collected crayfish were temporarily retained for identification and measuring until dip netting was completed at a site.

Once sampling at a site was complete, GPS coordinates at the center of each sampling unit were recorded. After exiting the stream each crayfish was identified by species along with the sex and carapace length measured in millimeters using digital calipers. Once crayfish data were recorded, all rusty crayfish were euthanized while native crayfish were returned to the area of stream they were collected.

Habitat sampling

Substrate characteristics were identified using a visual assessment of the sampling area. Substrate categories were based on a modified Wentworth scale and included clay (<1/256 mm), silt (>1/256 mm, <1/16 mm), sand (>1/16 mm, <4 mm), pebble (>4 mm, <64mm), cobble (>64 mm, <256 mm), boulder (>256 mm), woody material (roots, tree limbs, etc.), detritus, and living macrophytes (Wentworth 1922). Substrate classifications were rated on amount present in each sampling area based on a scale of 0%, 1-24%, 25-49%, 50-74%, and 75-100%.

Data Analysis

Crayfish presence/absence data were compared to Lippson’s 1975 dissertation (Lippson 1975) to determine any changes in the ranges of crayfish in Michigan. Any changes in the co-occurrence of obligate stream species (*C. robustus, O. propinquus, O. virilis*), as a result of increased *O. rusticus* ranges from previous reports, were also compared between studies. In an effort to make the Lippson’s data more applicable to management, I report current and past occurrences at the watershed scale, based on the United States Geological Survey (USGS) 8-digit Hydrologic Unit Code (HUC) and MDNR Fisheries Management Unit (FMU) (MDNR 2001, USDA/NRCS 2013). In order to convert Lippson's data, which was originally reported at the county, township, range, and section level, to watershed level we used the centroid of township, range, and section data available and converted these points to latitude and longitude points. Range maps were constructed using shapefiles published by USDA/NRCS - National Geospatial Management Center and the MDNR in ArcGIS version 10.1 (MDNR 2001, ESRI 2011, USDA/NRCS 2013). It should be noted that the number of samples for 1975 is unknown because reporting only listed locales where a crayfish was found, not all locations that were surveyed. This meant we could not report on the percentage of sites within a watershed that a species occurred.

The relationship of crayfish species to substrate characteristics was investigated using the generalized linear model (GLM) function in R (R version 3.0.2, R Core Team 2016). GLMs were performed for each species using the substrate classifications from the modified Wentworth scale as covariates. Each species acted as the dependent variable and was treated as a 1, present, or 0, absent. Covariates were rated as a 1 or 0, corresponding to whether or not a substrate class was present or absent for a sampling event. This allowed me to determine if the presence of a crayfish species was positively or negatively associated with individual substrate classes. In order to quantify any shifts in substrate associations based on the presence or absence of *O. rusticus*, GLMs were then run for each native species after separating samples where *O. rusticus* was present from samples where *O. rusticus* was absent. Results for each species were then compared to see if a species' substrate association differed between samples where a native species was found in the presence or absence of *O. rusticus*. It was determined that a substrate class was significantly associated with crayfish presence or absence based on p-values ≤ 0.05.

I ran an occupancy and detectability model using methods described in MacKenzie et al. (2002) to gain insight into the overall effectiveness of my methods at the chosen temporal and spatial scales. Detectability and occupancy models were fit to pooled data of both technicians from 22 stream segments that were visited in 2014 and again in 2015. This temporal-scale model tested whether a species would be detected at a location on every occasion that it was sampled. As well, spatial detectability and occupancy were modeled by comparing samples from the same stream segment, i.e. one sample from upstream compared to the other from downstream of the road crossing. This model tested whether crayfish assemblages were uniform throughout a stream segment. Samples conducted on the same segment but at different times were treated as individual sampling, allowing a sample size of 350 comparisons for the spatial model.

C. diogenes and *C. polychromatus* were combined for all analyses due to their low catch rates and difficulty in distinguishing young individuals. Because both species were formerly part of a species complex (Thoma et al. 2005), data for the two were likely combined during Lippson's survey and will be referred to as the '*diogenes* complex' in this paper (Lippson 1975, Thoma et al. 2005).

Results:

Current Range of Michigan's crayfish

Overall, each of Michigan's native crayfish were detected in more watersheds during this survey than in 1975, with the exception of for *O. propinquus* being found in the same number. (Table 1.1). For *O. rusticus*, there is evidence for a substantial expansion in range. I found *O. rusticus* in 24% of all samples. *Orconectes rusticus* were documented in 11 HUC 8 watersheds in

1975 (Lippson 1975) and 28 of the 37 HUC 8 watersheds during this study (Table 1.1; Figure 1.2). Every watershed that contained *O. rusticus* in 1975 also contained *O. rusticus* in this study (Figure 1.3).

Native species ranges appear to have changed in some regards since 1975. Despite the increase in watershed coverage compared to Lippson's study, all native species failed to be detected in at least some watersheds that they were detected in 1975. In terms of facultative burrowing crayfish, The *diogenes* complex was not reported in one watershed that it was reported in 1975, *F. fodiens* was not detected in two watersheds it was detected in 1975, and *O. immunis* was not detected in four watersheds that it was detected in 1975. For the obligate stream species, Lippson found *C. robustus* in two watersheds it was not detected in during this survey, *O. propinquus* was not detected in two watersheds it was formerly detected in 1975, and *O. virilis* was not detected in three watersheds that it was detected in 1975.

The most widely distributed native species in Michigan was *O. propinquus*, found in 32 of 37 watersheds and 45% of samples. The second most common native species found in this survey was *O. virilis*, found in 30 watersheds and 22% of samples. *C. robustus* was found in 17 watersheds, and 8% of samples, making it the least common obligate aquatic species in Michigan. *O. immunis* was found in 16 watersheds and 6% of samples. *P. acutus* was found in 3 watersheds and 1% of samples, making it the least common crayfish in Michigan, limited to a few southern watersheds. The *diogenes* complex was found in 18 watersheds and 6% of samples. *F. fodiens* was found in 10 watersheds and 2% of samples (Figure 1.3).

The co-occurrence between obligate aquatic species did not substantially change from Lippson (1975), except for the reduced occurrence of *O. propinquus* in areas occupied by *O. rusticus* (Table 1.2). When I compared the occurrence of *O. propinquus* within *O. rusticus* occupied sites, *O. propinquus* was found in 43% of samples that contained *O. rusticus* in 1975, but only 18% of samples in 2014-15. In contrast *O. virilis* was found in 16% of samples that contained *O. rusticus* in 1975, and 17% of samples in 2014-2015. *C. robustus* was found in 8% of samples that contained *O. rusticus* in 1975 and 5% of samples in 2014-2015.

When *C. robustus* was found we find similar co-occurrence across studies. *O. propinquus* was present in 62% of *C. robustus* samples in both 1975 and 2014-2015. *O. rusticus* was in 12% of *C. robustus* samples in 1975, and 16% of samples in 2014-2015. *O. virilis* was in 12% of *C. robustus* samples in 1975, and 18% of samples in 2014-2015.

When *O. propinquus* was found, it co-occurred with *O. rusticus* in 6% of collections in 1975 and 9% in 2014-2015. *O. virilis* occurred in 20% of samples in 1975, and 21% of samples in 2014-2015. *C. robustus* occurred in 6% of samples in 1975, and 11% of samples in 2014-2015.

Habitat Associations

GLM results (Table 1.3) for the substrate covariates and their effect on species presence were successfully calculated for all species except for *P. acutus* due to the small sample size. The burrowing species *F. fodiens* was positively associated with detritus, as was the *diogenes* complex. *O. immunis*, which is known to burrow but is more often found in slow waters with live vegetation, was found to be positively associated with live vegetation, and to a lesser extent clay and silt. This also agrees with the life history of *O. immunis* (Tack 1939, Lippson 1975, Taylor et al. 2015).

Substrate associations of obligate stream dwelling species also agreed with literature descriptions of their life history. *C. robustus* was associated with cobble and woody cover, *O. propinquus* with cobble, sand, and pebbles, and *O. virilis* with live vegetation and silt but showed a negative association toward sand (Lippson 1975, Hobbs 1988, Taylor et al 2015). The invasive *O. rusticus* showed associations with cobble and boulders, and a negative association with sand.

Evidence of the impact of rusty crayfish on native species was supported by an analysis that separated samples where *O. rusticus* co-occurred with native species from those where *O. rusticus* was absent. The analysis indicated shifts in substrate associations for some species when *O. rusticus* was present (Table 1.4). *C. robustus* associations shifted from cobble being the sole predictor in the absence of *O. rusticus*, toward woody debris being a more significant predictor when *O. rusticus* was present. *O. immunis* was positively associated with clay and silt in the absence of *O. rusticus*; however, when *O. rusticus* was present, *O. immunis* was associated with live vegetation, and showed a negative association with sand. *O. propinquus* shifted from being positively associated with cobble, sand, and wood in the absence of *O. rusticus* to being positively associated with pebbles and live vegetation and negatively associated with silt when *O. rusticus* were present. *O. virilis* showed relatively little change, as it remained positively associated with live vegetation when *O. rusticus* was both present and absent. However, *O. virilis* was negatively associated with sand in the absence of *O. rusticus* whereas sand showed no significant effect on *O. virilis* presence when *O. rusticus* was present.

Detectability

The detectability of crayfish at a temporal scale, comparing the same location at different times, and at a spatial scale, comparing samples from upstream and downstream locations within the same segment at the same time, was high for obligate aquatic species. No species had less than a 60% probability of detection and some had detectabilities over 90% (Table 1.6). The primary and secondary burrowing species showed lower detection probabilities, and temporal models for *F. fodiens* and *P. acutus* could not be run due to lack of data, despite having moderate spatial detectability (46% and 67%, respectively).

Discussion:

Ranges of Michigan crayfishes

Despite the broad distribution of native crayfish species across Michigan, there appears to be an ongoing expansion of the invasive *O. rusticus* from previous surveys (Creaser 1931, Lippson 1975). The increase in *O. rusticus* range and decrease in co-occurrence with *O. propinquus* suggest that *O. rusticus* are displacing *O. propinquus*. One method by which *O. rusticus* can affect *O. propinquus* is through hybridization, shifting the genetic and phenotypic population toward characteristics exhibited by *O. rusticus* (Capelli and Munjal 1980, Perry et al. 2001, Perry et al. 2002). Further pressure on native species could come from antagonistic interactions between the native species and *O. rusticus*. Previous work has shown that *O. rusticus* will outcompete both *O. propinquus* and *O. virilis* for habitat, especially in coarse substrates necessary for shelter, and exhibits lower susceptibility to predation by native predators (Capelli and Munjal 1982, DiDonato and Lodge 1993, Hill and Lodge 1994, Bergman and Moore 2003, Garvey et al. 2003, Roth and Kitchell 2005). My findings are consistent with these studies given the shift in *O. propinquus* associations away from preferred cobble and woody debris toward vegetation when *O. rusticus* is present (Lippson 1975, Hobbs 1988, Taylor et al. 2015). Excluding *O. propinquus* from preferred habitat would make them more susceptible to predation, or put them in competition with other native species such as *O. virilis* in areas where the three species co-occur.

Habitat associations of Michigan crayfishes

Previous studies indicate that *O. virilis* adults (in isolation) prefer rocky substrates, and macrophyte beds are important nursery habitat for young (Crocker and Barr 1968, Momot and Gowing 1983, France 1985). Although *O. virilis* is often considered a habitat generalist, it is vulnerable to exclusion from preferred habitat types as a result of competition, particularly with the congeners *O. propinquus* and *O. rusticus* (Lippson 1975, Peck 1985, Hobbs and Jass 1988, Taylor et al. 2015). In my study, *O. virilis* demonstrated an affinity for cobble and a negative association with sand in the absence of both *O. propinquus* and *O. rusticus*. However, in areas where *O. virilis* co-occur with either *O. propinquus* or *O. rusticus* I observed that *O. virilis* was positively associated with vegetation and was no longer associated with cobble (Table 1.5). This might indicate that *O. propinquus* could compete with *O. virilis* in areas where *O. rusticus* are pushing *O. propinquus* into vegetative habitats (Capelli and Munjal 1982). Further community change could arise if *O. rusticus* has a negative effect on macrophyte beds, thus eliminating the remaining refuge for *O. virilis* and *O. propinquus* (Lodge and Lorman 1987, Roth et al. 2007). Prior to *O. rusticus* invasion, *O. propinquus* and *O. virilis* likely lived abundantly in preferred habitat in the absence of the other, with *O. virilis* persisting in vegetative habitat when the two co-occurred (Peck 1985, Hill and Lodge 1994, Garvey et al. 2003). This still appears to be the case in many locations. However, when *O. rusticus* excludes *O. propinquus* from cobbles, areas where the three species overlap could result in the eventual removal of *O. virilis*.

C. robustus appeared largely unaffected by *O. rusticus* despite preferring large coarse substrates. This finding is consistent with Berrill (1978), suggesting that unknown differences in behavior or ecological roles might allow *C. robustus* to co-occur with *O. rusticus* and other members of *Orconectes spp*. However, a report by Daniels (1998) suggests that *O. rusticus* might be displacing *C. robustus* in an Ontario watershed. My data suggests that since 1975 the cohabitation of *C. robustus* and the other obligate aquatic species, including *O. rusticus*, is stable, further suggesting that there are determinants that influence the coexistence between *C. robustus* and *Orconectes spp.* other than substrate. Further research should be conducted to investigate possible differences in life history strategies or habitat requirements that allow co-existence despite overlapping resource needs.

I did not observe any negative effects of *O. rusticus* on native burrowing species. Generally these species tended to prefer silt or detritus substrates, which *O. rusticus* did not prefer in my study. The presence of these species is likely not influenced by *O. rusticus* due in part to their differing life histories and ecologies. Their ability to occupy temporary water bodies and burrowing behavior likely excludes them from much of the shelter competition faced by obligate aquatic species. However, there is a void in literature relating to the relationships between these species and *O. rusticus*. Further research could address potential issues that might arise where species overlap.

Detectability

Exclusively sampling streams likely resulted in the under-reporting of Michigan's burrowing despite their need to enter water during the spring to release young into the water (Lippson 1975, Hobbs and Jass 1988). Although I observed burrowing species in more watersheds than Lippson (1975) caution should be used when interpreting this result as a range expansion due to our lack of understanding regarding the methods used in Lippson (1975). I suggest conducting further surveys aimed at more accurately depicting the range, habitat associations, and status of burrowing species to gain a fuller understanding of burrowing crayfish populations in the state. Surveys could include ephemeral waterbodies, wet meadows, roadside ditches, burrows near streams and ponds, and any other wetland bodies. Little is known on the status of burrowing species in the state and no extensive work has been done since *C. polychromatus* was described, separating it as a species apart from *C. diogenes* (Thoma et al. 2005). Thus, its current range is unknown.

Overall my methods of dip netting appear to have sufficiently sampled streams for obligate aquatic species of crayfish. Dip netting allowed me to sample all substrate types regardless of flow. Dip netting also removed the possibility of sample bias related to habitat preferences and sex-specific behavior (Hill and Lodge 1994, Smily and Dibble 2000, Olden et al. 2006, Price and Welch 2009). Passive methods of capture such as trapping results in a catch bias toward males of more aggressive species and might result in different catch rates in different waterbodies based on predator densities (Collins et al. 1983, Dorn et al. 2005). Throw traps were not used in order to avoid possible issues related to sampling larger substrate types, such as cobble and boulders, with interstitial spaces that would allow crayfish to escape under the gear (Dorn et al. 2005). Seines were not used mainly for their inability to sample undercut banks, rooted structures, and heavy course substrates (Price and Welch 2009). Electroshocking has been shown to be a successful form of crayfish capture, however there was concern that this sampling method would not be effective in cobbles, boulders, or thick vegetation (Price and welch 2009). SCUBA and snorkeling methods were not used due to the shallow depths of some streams. Budget constraints and safety issues also played a role in choosing not to use SCUBA, snorkeling, and electroshocking. Other studies have stated that detection probabilities upwards to 88% for throw traps (Dorn et al. 2005), 68% for electroshocking, 38% for trapping, and dip netting as low as 32% (for one half hour) (Price and Welch 2009). My model showed that dip netting appeared to be an effective method of detecting crayfish in a stream. For obligate aquatic species, spatial or temporal detectability was never below 60%. The possibility exists that the discrepancy between my study and others reporting low dip net detectability is due to individuals skills or other tactics.

I did not find *P. clarkii* or any other non-native crayfish other than *O. rusticus* during the course of this survey. Based on the probability of detecting similar species such as *P. acutus* within this study, I am confident that had there been a population of *P. clarkii* persisting at a sampling location they would have been detected. Further reports of crayfish resembling *P. clarkii* not related to this study were investigated by myself and the MDNR and found to be *P. acutus* or other Orconectids during 2013-2015 (MDNR personal conversation). I suggest that management agencies act diligently to address risks that might result from the introduction of additional non-native crayfish such as the red swamp crayfish. Aside from habitat degradation and pollution, one of the largest threats to indigenous crayfish populations around the world is the introduction of non-native crayfish (Hobbs et al. 1989, Taylor et al. 2011). This is especially concerning given the risk posed by potential dispersal of the nearby population of *P. clarkii* in Ohio (Sandusky Bay area) into Michigan (Norrocky 1983, R.Thoma *personal conversation*). There are already signs that this species is migrating into neighboring watersheds and getting closer to entering Michigan's southeastern corner (*personal observation*). Despite the MDNR's current concern of *P. clarkii* introduction, it would be wise to consider any non-native crayfish species introduction a risk to the state's biodiversity.

Other concerns with regards to crayfish populations might lie in unforeseen behavioral and life history differences among native species. As more is learned about invasive species effects on native and endemic species, managers should be aware that several of Michigan's crayfish species are undergoing taxonomic review, resulting in new species and associated ranges. In particular the white river crayfish (currently known in Michigan as *P. acutus*) and the *diogenes* complex (currently known in Michigan as *C. diogenes* and *C. polychromatus*) have recently seen taxonomic review in other areas of their ranges separating them into additional species with more restricted ranges. (Hobbs and Hobbs 1990, Jezerinac 1993, Mazlum and Eversole 2005, Thoma et al. 2005). Considering the diversity in crayfish ecologies and the ongoing taxonomic revision, we should be hesitant of simply labeling crayfish as native within the state in regards to management actions. In the case of *P. acutus*, which is only indigenous to Michigan's southernmost watersheds, it would be advisable that precautions are taken to avoid introduction to watersheds that are naïve to *P. acutus* because of unforeseen effects that might result.

Our surveys were limited to the Lower Peninsula of Michigan. A similar survey focusing on Michigan's Upper Peninsula would be advisable considering the findings of this survey. It is reasonable to assume based on the findings of this study that rusty crayfish have also spread within the Upper Peninsula (Lippson 1975). Extending the substrate association models of this survey to the Upper Peninsula could give valuable insight into the regional effects of *O. rusticus* invasion.

APPENDIX

Table 1.1: Crayfish occurrence by Lower Peninsula HUC8 watershed (n=37). A comparison of occurrences reported in a 1975 survey and findings during 2014 and 2015 field sampling.

	C. diogenes		*C. robustus*		*F. fodiens*		*O. immunis*		*O. propinquus*		*O. rusticus*		*O. virilis*		*P. acutus*	
Watershed	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015
Sum	7	18	12	17	3	10	10	16	32	32	11	28	23	30	0	3
Au Gres-Rifle		x	x	x		x		x	x	x		x				
Au Sable			x	x					x	x	x	x	x	x		
Betsie-Platte		x							x	x		x	x	x		
Birch-Willow									x	x			x	x		
Black			x						x	x		x		x		
Black-Macatawa	x	x						x	x	x		x		x		x
Boardman-Charlevoix									x	x	x	x	x	x		
Cass		x					x	x	x			x	x			
Cheboygan									x	x	x	x	x	x		
Clinton	x		x	x				x	x	x		x		x		
Detroit				x		x	x		x	x		x		x		
Flint			x	x					x	x		x	x	x		
Huron		x		x					x	x		x		x		
Kalamazoo	x	x			x	x	x	x	x	x	x	x	x	x		
Kawkawlin-Pine		x		x				x		x			x			
Lake St. Clair								x		x			x			
Little Calumet-Galien								x	x	x		x		x		

Table 1.1 (cont'd)

	C. diogenes		*C. robustus*		*F. fodiens*		*O. immunis*		*O. propinquus*		*O. rusticus*		*O. virilis*		*P. acutus*	
Watershed	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015	1975	2015
Lone Lake-Ocqueoc			x			x			x	x		x		x		
Lower Grand		x	x	x				x	x	x			x	x		
Manistee		x		x				x	x	x	x	x	x	x		
Maple		x		x				x	x	x			x	x		
Muskegon		x				x		x	x	x	x	x	x	x		
Ottawa-Stony							x	x	x			x		x		x
Pere Marquette-White		x							x	x				x		
Pigeon-Wiscoggin									x	x		x	x	x		
Pine									x	x		x		x		
Raisin	x	x	x	x		x	x	x	x	x			x	x		
Saginaw																
Shiawassee	x	x			x		x	x	x	x		x	x	x		
St. Clair				x			x	x	x	x		x	x	x		
St. Joseph		x	x	x							x	x				
St. Joseph	x	x		x		x	x		x	x	x	x	x	x		x
Thornapple		x				x	x		x	x			x	x		
Thunder Bay			x	x		x			x	x	x	x	x	x		
Tiffin			x	x							x	x				
Tittabawassee			x	x					x	x	x	x	x	x		
Upper Grand	x	x			x	x	x		x	x		x	x	x		

Table 1.2: Relative co-occurrence (%) of Michigan Stream dwelling crayfish in samples of *C. robustus* (n=56 for this study), *O. propinquus* (n=317 for this study), and *O. rusticus* (n=169 for this study) for years 1975 and 2015 and the amount of change between years. It should be noted that the number of samples for 1975 in unknown.

	Survey Species					
	C. robustus		*O. propinquus*		*O. rusticus*	
Co-occurring Species	1975	2015	1975	2015	1975	2015
C. robustus			6	11	8	5
O. propinquus	62	62			43	18
O. rusticus	12	16	6	9		
O. virilis	12	18	20	21	16	17

Table 1.3: Table of results from the generalized linear model for looking at the associations of crayfish species presence or absence based on habitat presence.

	Log Odds Estimate	Standard Error	Z value	Pr(>|z|)	
C. diogenes					
(Intercept)	-3.02236	0.50559	-5.978	2.26E-09	***
Clay	0.49764	1.08059	0.461	0.6451	
Silt	0.23180	0.39514	0.587	0.5575	
Sand	0.48498	0.37487	1.294	0.1958	
Pebble	-0.09066	0.41013	-0.221	0.8251	
Cobble	-0.37145	0.37762	-0.984	0.3253	
Boulder	-0.99492	1.04101	-0.956	0.3392	
Wood	0.11760	0.45400	0.259	0.7956	
Detritus	0.79800	0.41003	1.946	0.0516	.
Live Veg.	0.07449	0.35474	0.210	0.8337	
C. robustus					
(Intercept)	-3.92190	0.52969	-7.404	1.32E-13	***
Clay	0.27983	1.09870	0.255	0.7999	
Silt	0.61516	0.38021	1.618	0.1057	
Sand	0.01253	0.33778	0.037	0.9704	
Pebble	0.43735	0.34104	1.282	0.1997	
Cobble	1.87120	0.38232	4.894	9.86E-07	***
Boulder	0.21929	0.49049	0.447	0.6548	
Wood	1.02243	0.45457	2.249	0.0245	*
Detritus	-0.66981	0.76906	-0.871	0.3838	
Live Veg.	-0.43792	0.37725	-1.161	0.2457	
F. fodiens					
(Intercept)	-3.81380	0.9158	-4.164	3.12E-05	***
Clay	-14.2002	1792.729	-0.008	0.9937	
Silt	-0.15030	0.6672	-0.225	0.8217	
Sand	-1.64770	0.8549	-1.927	0.0539	.
Pebble	-1.22120	1.0906	-1.120	0.2628	
Cobble	0.12200	0.7556	0.161	0.8717	
Boulder	0.18450	1.1363	0.162	0.8710	
Wood	0.38600	0.8428	0.458	0.6469	
Detritus	1.58500	0.7161	2.213	0.0269	*
Live Veg.	0.29500	0.6454	0.457	0.6476	

Table 1.3 (cont’d)

	Log Odds Estimate	Standard Error	Z value	Pr(>|z|)	
O. immunis					
(Intercept)	-3.04334	0.50987	-5.969	2.39E-09	***
Clay	1.55439	0.83135	1.87	0.0615	.
Silt	0.65297	0.39219	1.665	0.0959	.
Sand	-0.31845	0.37754	-0.843	0.3989	
Pebble	-0.11887	0.43456	-0.274	0.7844	
Cobble	-0.41273	0.37695	-1.095	0.2736	
Boulder	-0.43342	0.76665	-0.565	0.5719	
Wood	-0.04362	0.51709	-0.084	0.9328	
Detritus	0.22757	0.46078	0.494	0.6214	
Live Veg.	0.91842	0.33132	2.772	0.0056	**
O. propinquus					
(Intercept)	-0.68886	0.24571	-2.804	0.0051	**
Clay	-0.26311	0.60546	-0.435	0.6639	
Silt	-0.24872	0.20313	-1.224	0.2208	
Sand	0.56532	0.18310	3.088	0.0020	**
Pebble	0.55130	0.19454	2.834	0.0046	**
Cobble	0.41070	0.17585	2.335	0.0195	*
Boulder	-0.21753	0.30516	-0.713	0.4759	
Wood	0.36188	0.25259	1.433	0.1519	
Detritus	-0.02444	0.26491	-0.092	0.9265	
Live Veg.	-0.12829	0.18162	-0.706	0.4799	
O. rusticus					
(Intercept)	-1.21013	0.28556	-4.238	2.26E-05	***
Clay	-1.46467	1.06438	-1.376	0.1688	
Silt	-0.24881	0.23922	-1.040	0.2983	
Sand	-0.53533	0.21492	-2.491	0.0127	*
Pebble	-0.21895	0.23047	-0.950	0.3421	
Cobble	0.80353	0.20450	3.929	8.52E-05	***
Boulder	0.53955	0.31314	1.723	0.0849	.
Wood	0.45334	0.29125	1.557	0.1196	
Detritus	-0.24622	0.33870	-0.727	0.4673	
Live Veg.	0.01958	0.21238	0.092	0.9265	

Table 1.3 (cont'd)

O. virilis					
(Intercept)	-1.75705	0.29940	-5.869	4.40E-09	***
Clay	-0.08618	0.80143	-0.108	0.9143	
Silt	0.52043	0.23735	2.193	0.0283	*
Sand	-0.42305	0.22155	-1.909	0.0562	.
Pebble	0.30795	0.23681	1.300	0.1935	
Cobble	0.11457	0.21449	0.534	0.5932	
Boulder	0.16904	0.36510	0.463	0.6434	
Wood	0.27966	0.30559	0.915	0.3601	
Detritus	0.27772	0.30154	0.921	0.3570	
Live Veg.	0.72777	0.20521	3.546	0.0004	***

Table 1.4: Table of results from comparing the generalized linear models looking at the associations of crayfish species presence or absence based on habitat presence in areas where *O. rusticus* were either present or absent.

	O. rusticus absent						*O. rusticus* present				
	log Odds	St. Error	z-value	Pr(>|z|)			log Odds	St. Error	z-value	Pr(>|z|)	
C. robustus											
(Intercept)	-3.50138	0.58507	-5.985	2.17E-09	***		-5.63183	1.64960	-3.414	0.00064	***
Clay	0.00599	1.11658	0.005	0.996			-10.92822	2399.54524	-0.005	0.99637	
Silt	0.52840	0.43137	1.225	0.221			0.34707	1.01208	0.343	0.73165	
Sand	-0.19151	0.38396	-0.499	0.618			-0.00602	0.84271	-0.007	0.9943	
Pebble	0.34612	0.39382	0.879	0.379			0.66801	0.81644	0.818	0.41324	
Cobble	1.89233	0.40201	4.707	2.51E-06	***		2.66085	1.37524	1.935	0.05301	.
Boulder	0.33856	0.56282	0.602	0.547			0.07626	1.17330	0.065	0.94818	
Wood	0.73108	0.55486	1.318	0.188			2.16820	0.93593	2.317	0.02052	*
Detritus	-1.31609	1.05541	-1.247	0.212			1.23934	1.34902	0.919	0.35825	
Live Veg.	-0.47356	0.40754	-1.162	0.245			-0.79612	1.14850	-0.693	0.48819	
O. immunis											
(Intercept)	-3.92607	0.65399	-6.003	1.93E-09	***		-1.3396	0.9455	-1.417	0.1565	
Clay	2.19619	0.86785	2.531	0.0114	*		-16.3868	10754.013	-0.002	0.9988	
Silt	1.25115	0.49569	2.524	0.0116	*		-0.3317	0.7371	-0.450	0.6527	
Sand	0.13039	0.44310	0.294	0.7685			-1.8396	0.846	-2.175	0.0297	*
Pebble	0.73636	0.49993	1.473	0.1408			-17.7997	1583.4986	-0.011	0.9910	
Cobble	-0.47871	0.48714	-0.983	0.3258			-0.8581	0.7394	-1.160	0.2459	
Boulder	-0.35175	1.06822	-0.329	0.7419			-1.0214	1.2246	-0.834	0.4042	
Wood	-0.08399	0.65928	-0.127	0.8986			0.1245	1.0042	0.124	0.9013	
Detritus	0.64834	0.52055	1.245	0.2130			0.1925	1.3187	0.146	0.8840	
Live Veg.	0.75822	0.40566	1.869	0.0616	.		1.3891	0.6782	2.048	0.0405	*

Table 1.4 (cont'd)

	O. rusticus absent						*O. rusticus* present				
	log Odds	St. Error	z-value	Pr(>|z|)			log Odds	St. Error	z-value	Pr(>|z|)	
O. propinquus											
(Intercept)	-0.4007	0.2943	-1.362	0.1734			-1.8328	0.6818	-2.688	0.007182	**
Clay	-1.0155	0.6504	-1.561	0.1184			17.2236	1455.3977	0.012	0.990558	
Silt	-0.3305	0.2401	-1.377	0.1686			-1.4640	0.6518	-2.246	0.024701	*
Sand	0.5107	0.2216	2.305	0.0212	*		0.1753	0.5170	0.339	0.734622	
Pebble	0.3155	0.2373	1.330	0.1837			1.7424	0.5234	3.329	0.000872	***
Cobble	1.0250	0.2120	4.835	1.33E-06	***		-0.5528	0.5071	-1.09	0.275695	
Boulder	0.3467	0.4002	0.866	0.3863			-1.5520	1.0834	-1.432	0.152005	
Wood	0.6489	0.3040	2.134	0.0328	*		0.1035	0.6586	0.157	0.875085	
Detritus	-0.0723	0.2982	-0.242	0.8084			0.1855	0.7941	0.234	0.815276	
Live Veg.	-0.3362	0.2088	-1.610	0.1073			1.2469	0.5282	2.361	0.018248	*
O. virilis											
(Intercept)	-1.4478	0.3434	-4.217	2.48E-05	***		-2.8994	0.7118	-4.073	4.64E-05	***
Clay	-0.2082	0.8192	-0.254	0.79938			-13.1144	1455.3977	-0.009	0.9928	
Silt	0.4708	0.2746	1.715	0.08639	.		0.5189	0.5510	0.942	0.3463	
Sand	-0.6828	0.2522	-2.708	0.00678	**		0.4477	0.5168	0.866	0.3863	
Pebble	0.2480	0.2777	0.893	0.37183			0.7199	0.5157	1.396	0.1627	
Cobble	0.1458	0.2425	0.601	0.54764			0.3246	0.4995	0.650	0.5158	
Boulder	0.2044	0.4346	0.470	0.63817			0.1792	0.7167	0.250	0.8026	
Wood	0.2289	0.3481	0.658	0.51073			0.3079	0.6740	0.457	0.6479	
Detritus	0.1871	0.3350	0.559	0.57650			0.3161	0.7618	0.415	0.6782	
Live Veg.	0.6098	0.2305	2.645	0.00816	**		1.1946	0.4826	2.476	0.0133	*

Table 1.5: GLM output for substrate co-variate effect on *O. virilis* presence when *O. propinquus* and *O. rusticus* were absent compared to when either *O. propinquus* or *O. rusticus* were present in samples.

O. propinquus and *O. rusticus* Absent							*O. propinquus* or *O. rusticus* Present				
	Log Odds	St. Error	z-value	Pr(>|z|)			Log Odds	St. Error	z-value	Pr(>|z|)	
(Intercept)	-1.00942	0.48259	-2.092	0.03647	*		-2.24817	0.40800	-5.510	3.58E-08	***
Clay	-0.29002	0.94696	-0.306	0.75940			-13.6716	645.0638	-0.021	0.983091	
Silt	0.07936	0.38762	0.205	0.83777			0.82572	0.32392	2.549	0.010798	*
Sand	-1.03264	0.38007	-2.717	0.00659	**		0.05314	0.29874	0.178	0.858821	
Pebble	0.08298	0.43878	0.189	0.85000			0.49541	0.29806	1.662	0.096488	.
Cobble	0.88867	0.36859	2.411	0.01591	*		-0.14185	0.27702	-0.512	0.608610	
Boulder	-0.85208	0.84215	-1.012	0.31164			0.60818	0.42596	1.428	0.153352	
Wood	0.36344	0.52393	0.694	0.48789			0.33260	0.39432	0.843	0.398955	
Detritus	0.49211	0.43765	1.124	0.26083			-0.16418	0.46367	-0.354	0.723273	
Live Veg.	0.15807	0.32723	0.483	0.62906			0.93026	0.27516	3.381	0.000723	***

Table 1.6: Detectability of crayfish species during 2014-2015 Stream surveys over time and space. ψ being occupancy and p being probability of detection.

	C. diogenes	*C. robustus*	*F. fodiens*	*O. immunis*	*O. propinquus*	*O. rusticus*	*O. virilis*	*P. acutus*	
Spatial Detectability									n=350
ψ	0.289285	0.131765	0.040238	0.120536	0.547923	0.283343	0.354053	0.012857	
p	0.222222	0.607143	0.461538	0.533333	0.826498	0.852071	0.609272	0.666667	
Temporal Detectability									n=22
ψ	0.181818	0.142045	-	0.181818	0.682630	0.230114	0.371212	-	
p	0.500000	0.800000	-	0.500000	0.965517	0.888889	0.857143	-	

Figure 1.1: 2014-2015 Sample locations (n=343).

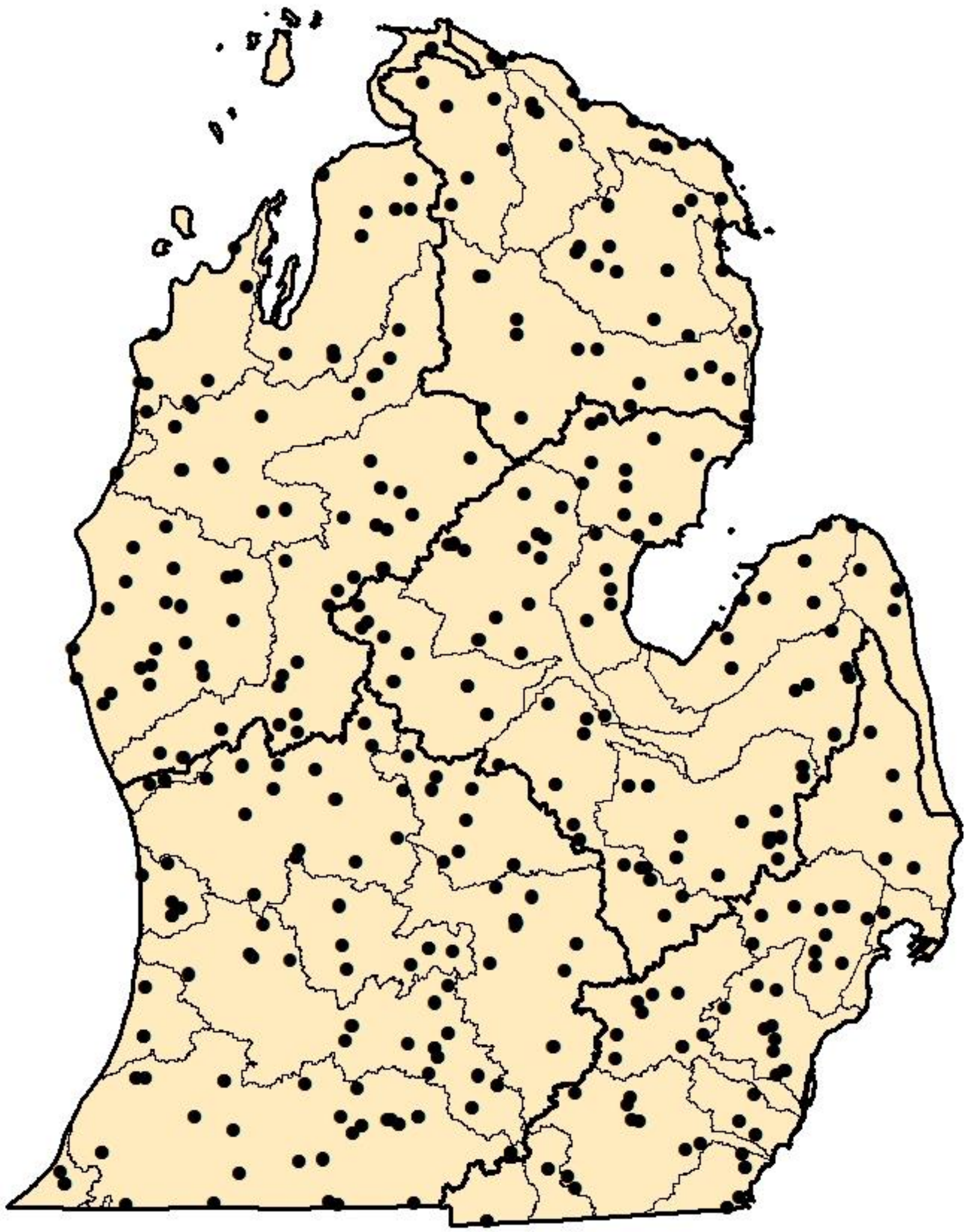


Figure 1.2: *O. rusticus* detection by watershed, 1975. Black dots are sites where *O. rusticus* was detected, hollow dots are where *O rusticus* was not detected, but other crayfish were.

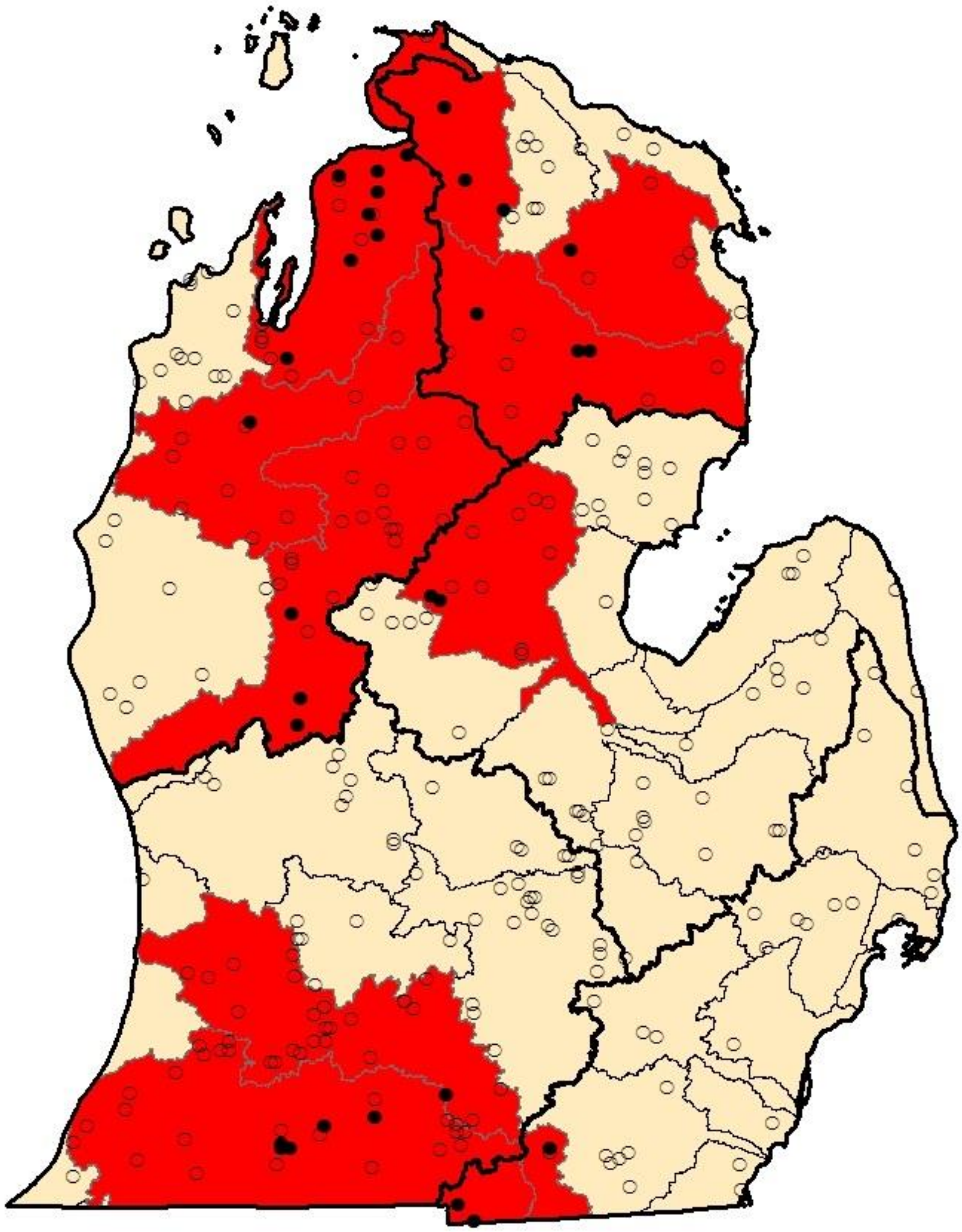


Figure 1.3: *O. rusticus* detection by watershed, 2015. Black dots are sites where *O. rusticus* was detected, hollow dots are where *O. rusticus* was not detected. Shading represents percentage of sites in a watershed that contained a species and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%.

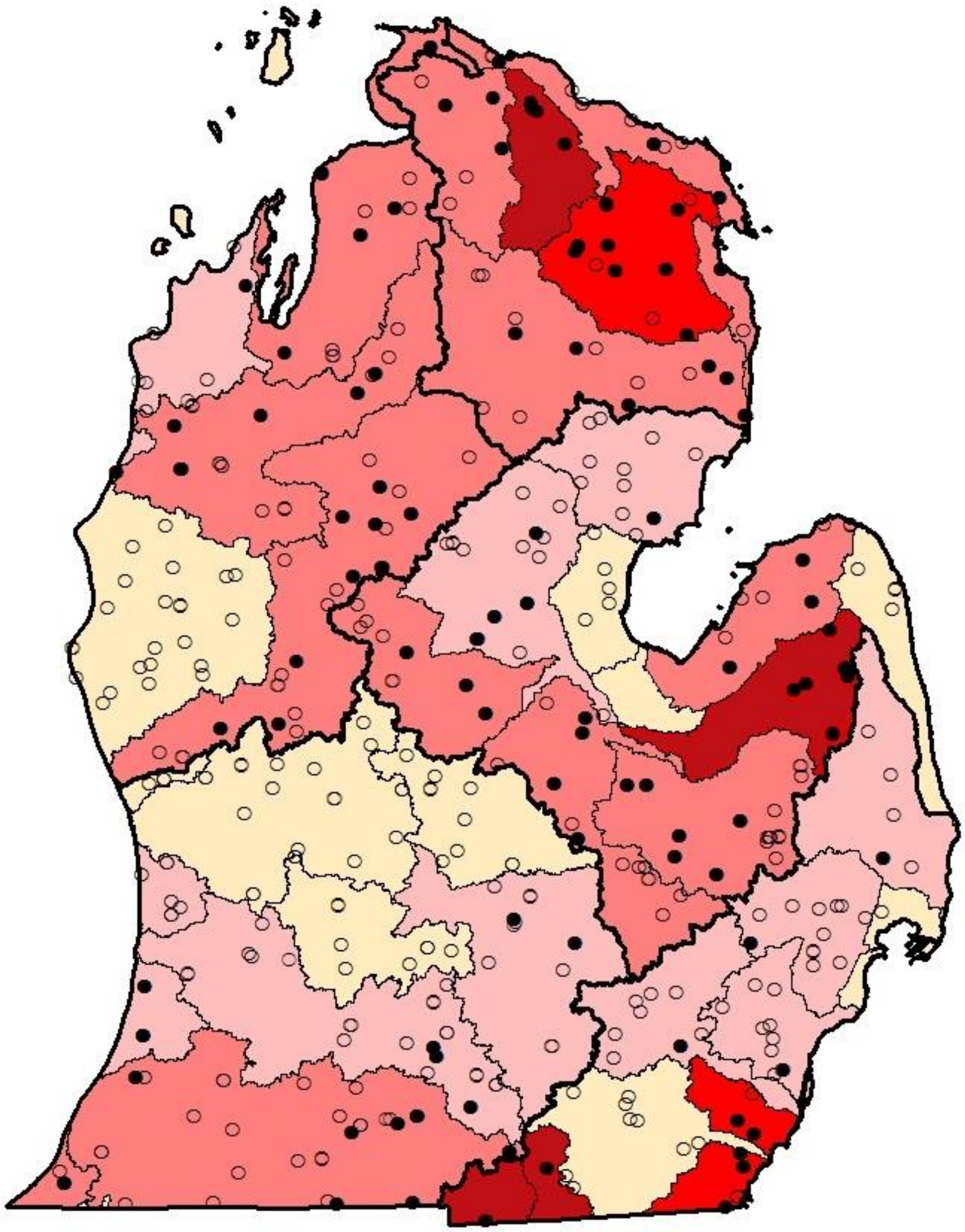


Figure 1.4: 1975 map of the "*diogenes* complex" detection. Black dots are sites where *diogenes complex* was detected, hollow dots are where the *diogenes complex* was not detected, but other crayfish were.

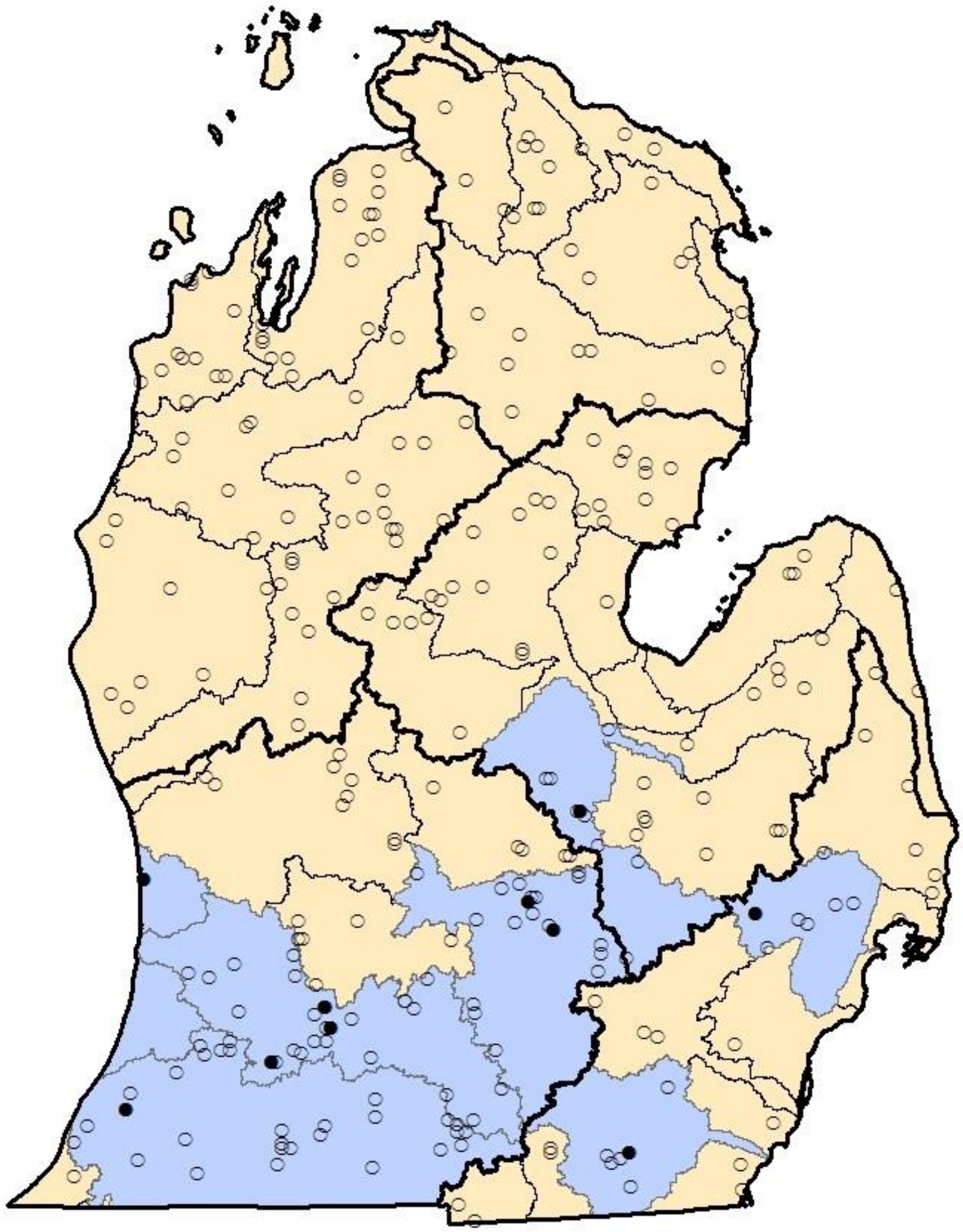


Figure 1.5: 2015 map of the "*diogenes* complex" detection. Black dots are sites a species was detected, hollow dots the *diogenes complex* was not detected. Shading represents percentage of sites in a watershed that contained the *diogenes complex* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%

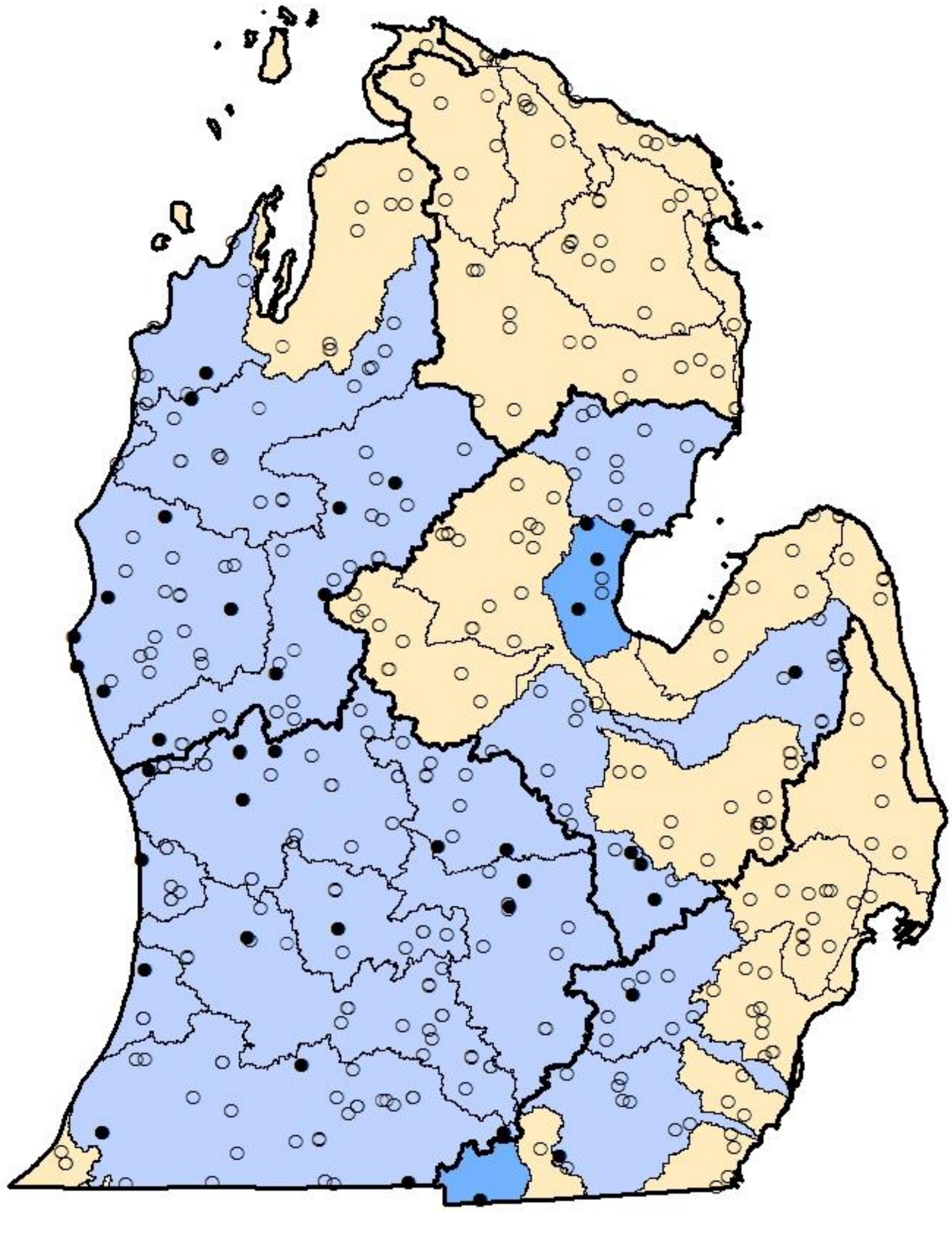


Figure 1.6: 1975 map of *Cambarus robustus* detection. Black dots are sites where *C. robustus* was detected, hollow dots are where the *C. robustus* was not detected, but other crayfish were.

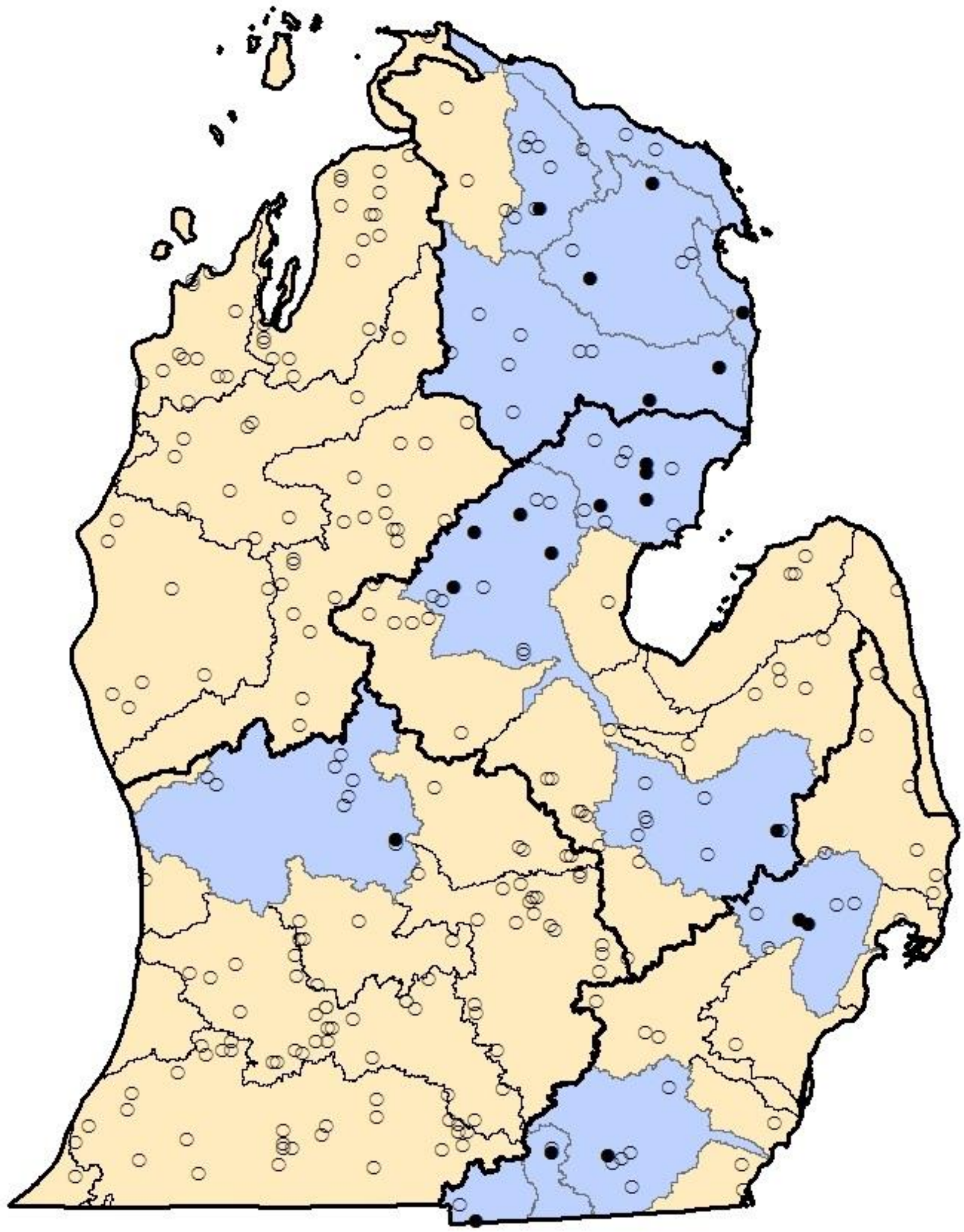


Figure 1.7: 2015 map of *Cambarus robustus* detection. Black dots are sites *C. robustus* was detected, hollow dots *C. robustus* was not detected. Shading represents percentage of sites in a watershed that contained *C. robustus* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%

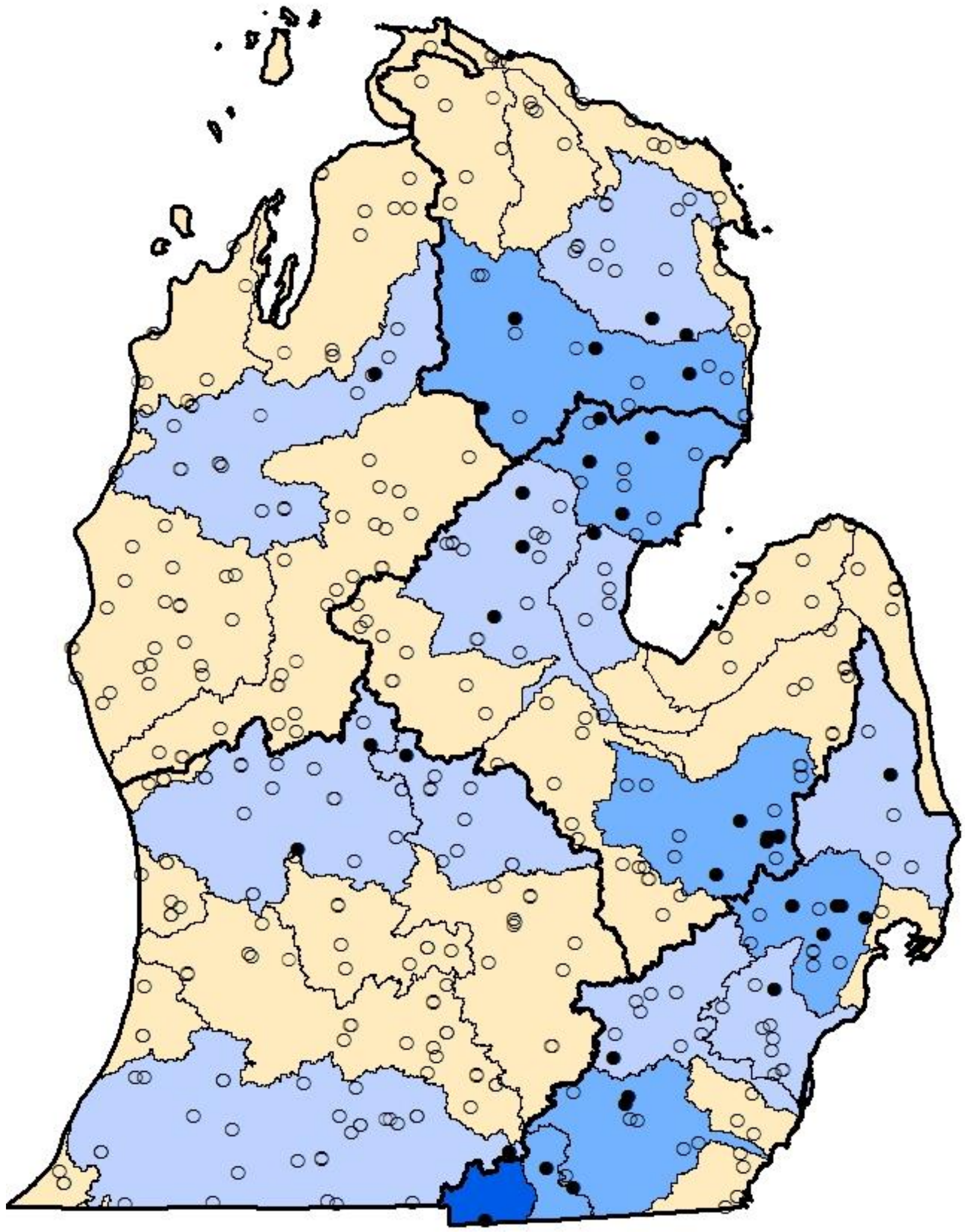


Figure 1.8: 1975 map of *Fallicambarus fodiens* detection. Black dots are sites where *F. fodiens* was detected, hollow dots are where the *F. fodiens* was not detected, but other crayfish were.

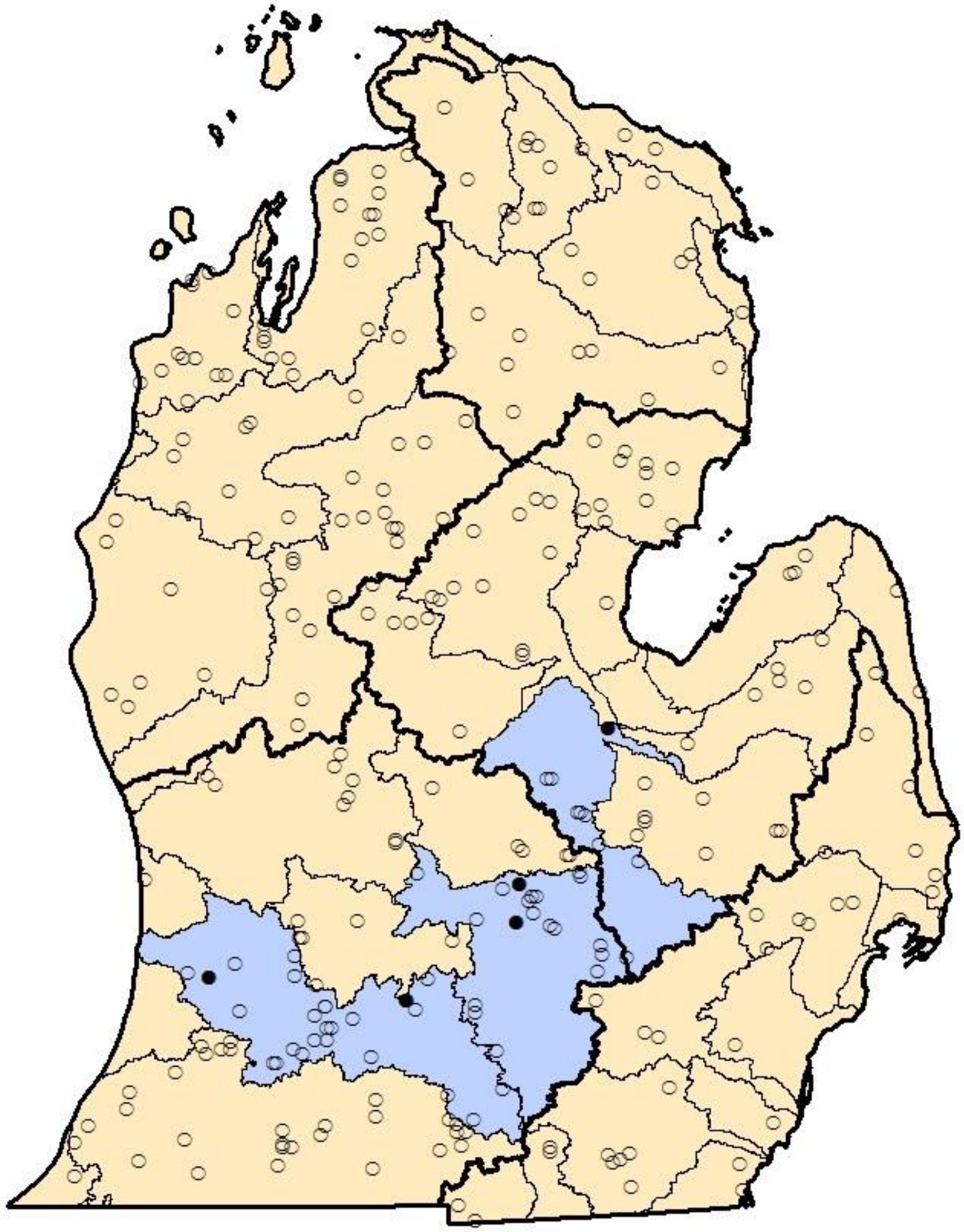


Figure 1.9: 2015 map of the *F. fodiens* detection. Black dots are sites *F. fodiens* was detected, hollow dots *F. fodiens* was not detected. Shading represents percentage of sites in a watershed that contained *F. fodiens* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%

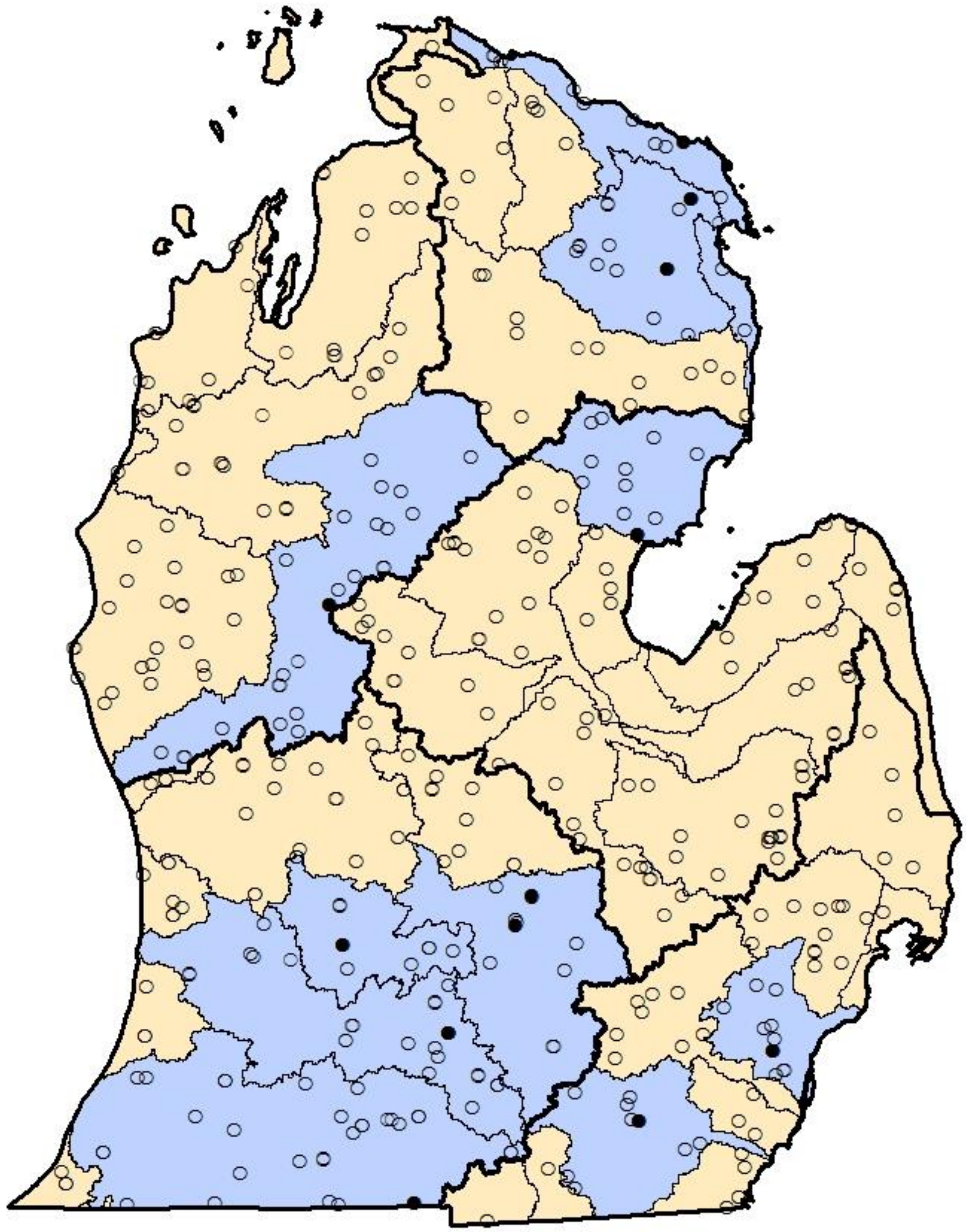


Figure 1.10: 1975 map of *Orconectes immunis* detection. Black dots are sites where *O. immunis* was detected, hollow dots are where the *O. immunis* was not detected, but other crayfish were.

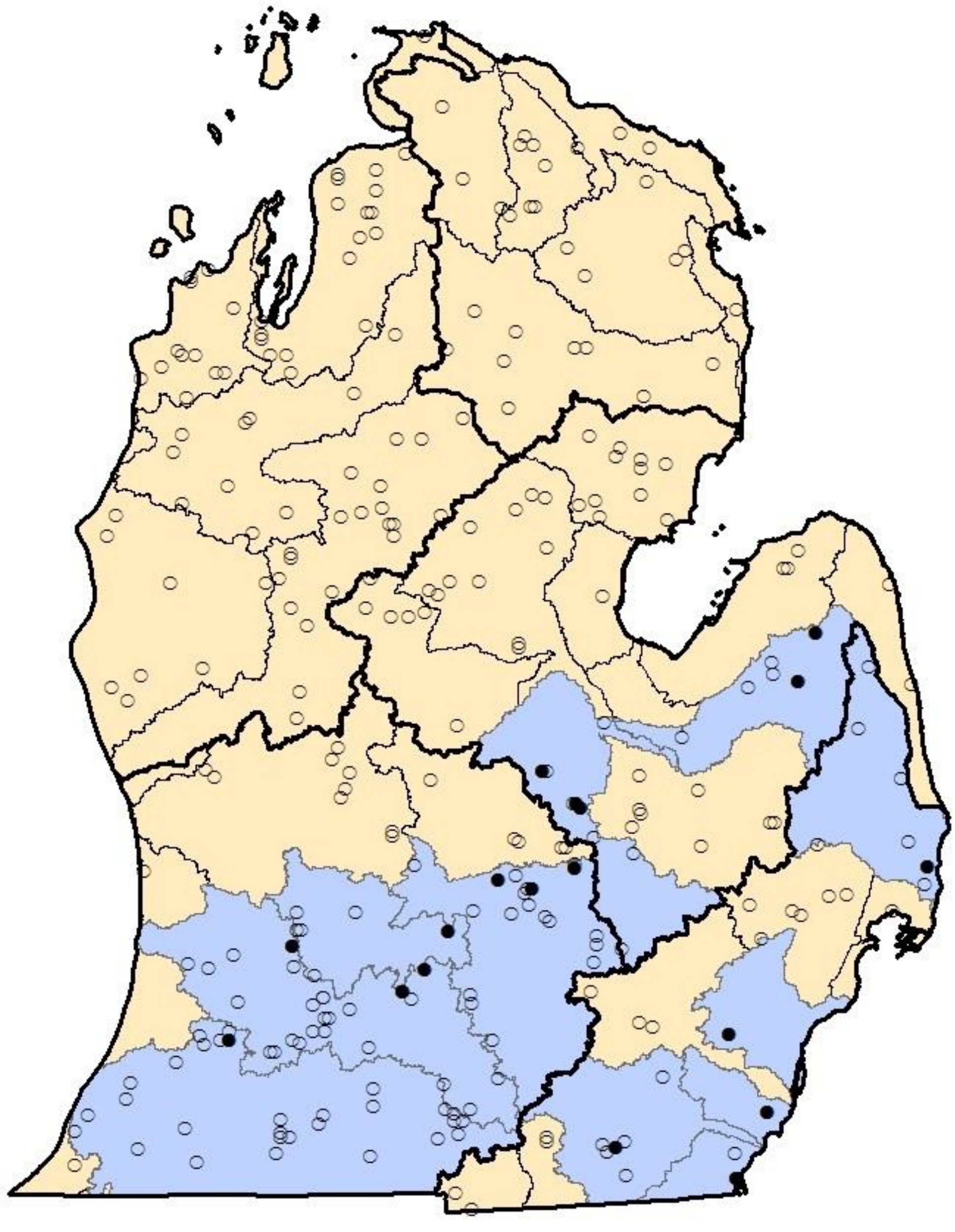


Figure 1.11: 2015 map of *Orconectes immunis* detection. Black dots are sites *O. immunis* was detected, hollow dots *O. immunis* was not detected. Shading represents percentage of sites in a watershed that contained *O. immunis* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%

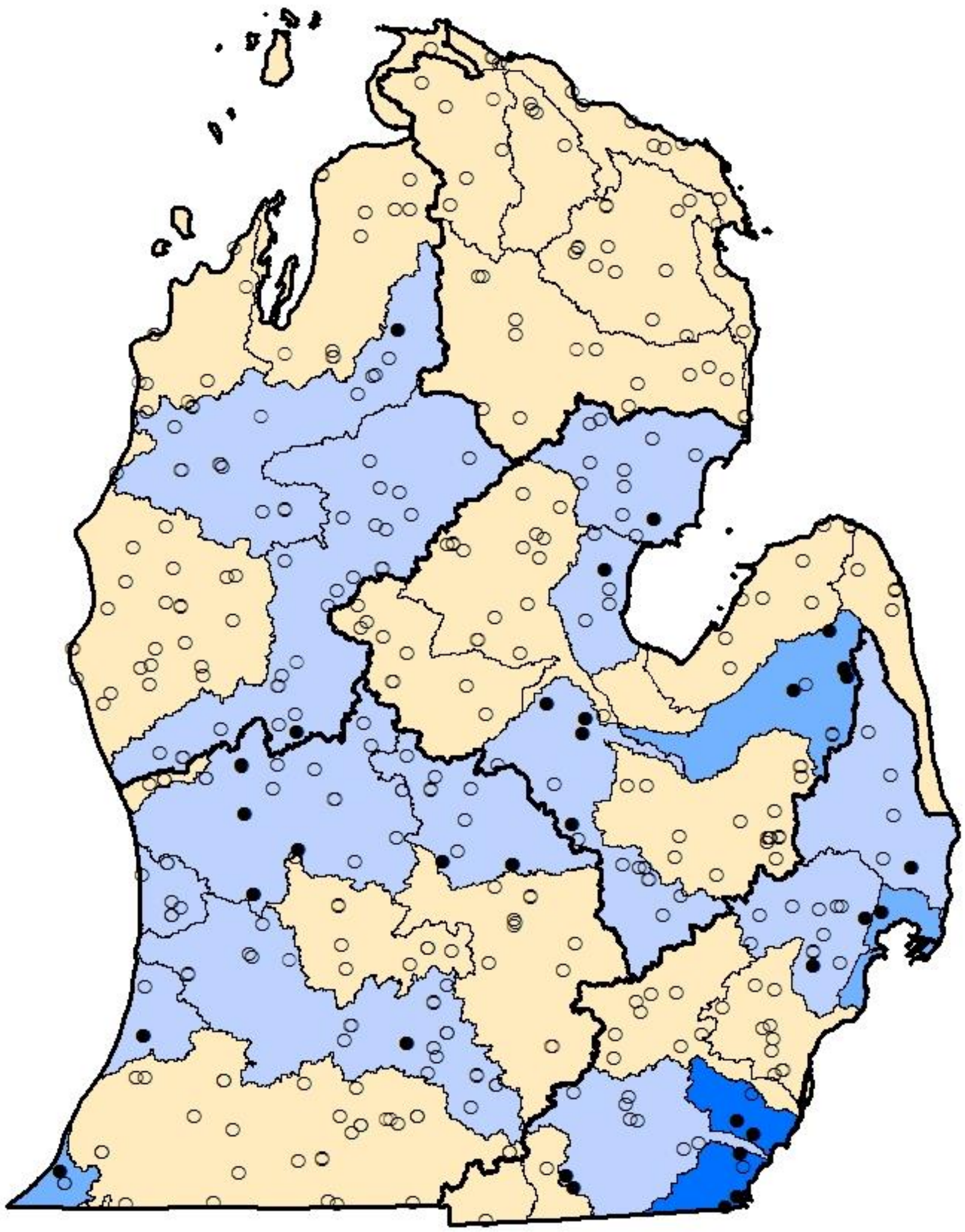


Figure 1.12: 1975 map of *Orconectes propinquus* detection. Black dots are sites where *O. propinquus* was detected, hollow dots are where the *O. propinquus* was not detected, but other crayfish were.

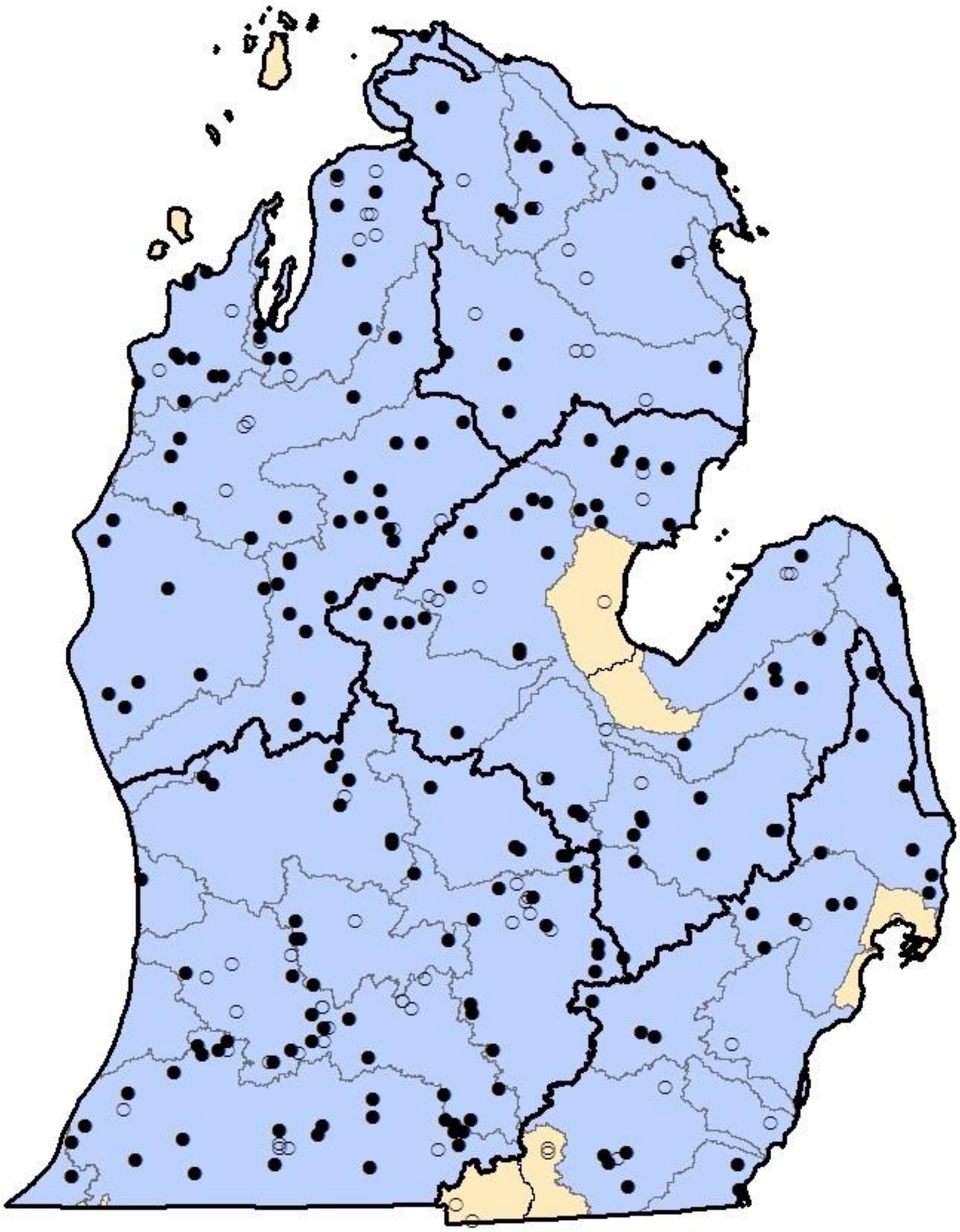


Figure 1.13: 2015 map of *Orconectes propinquus* detection. Black dots are sites *O. propinquus* was detected, hollow dots *O. propinquus* was not detected. Shading represents percentage of sites in a watershed that contained *O. propinquus* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%

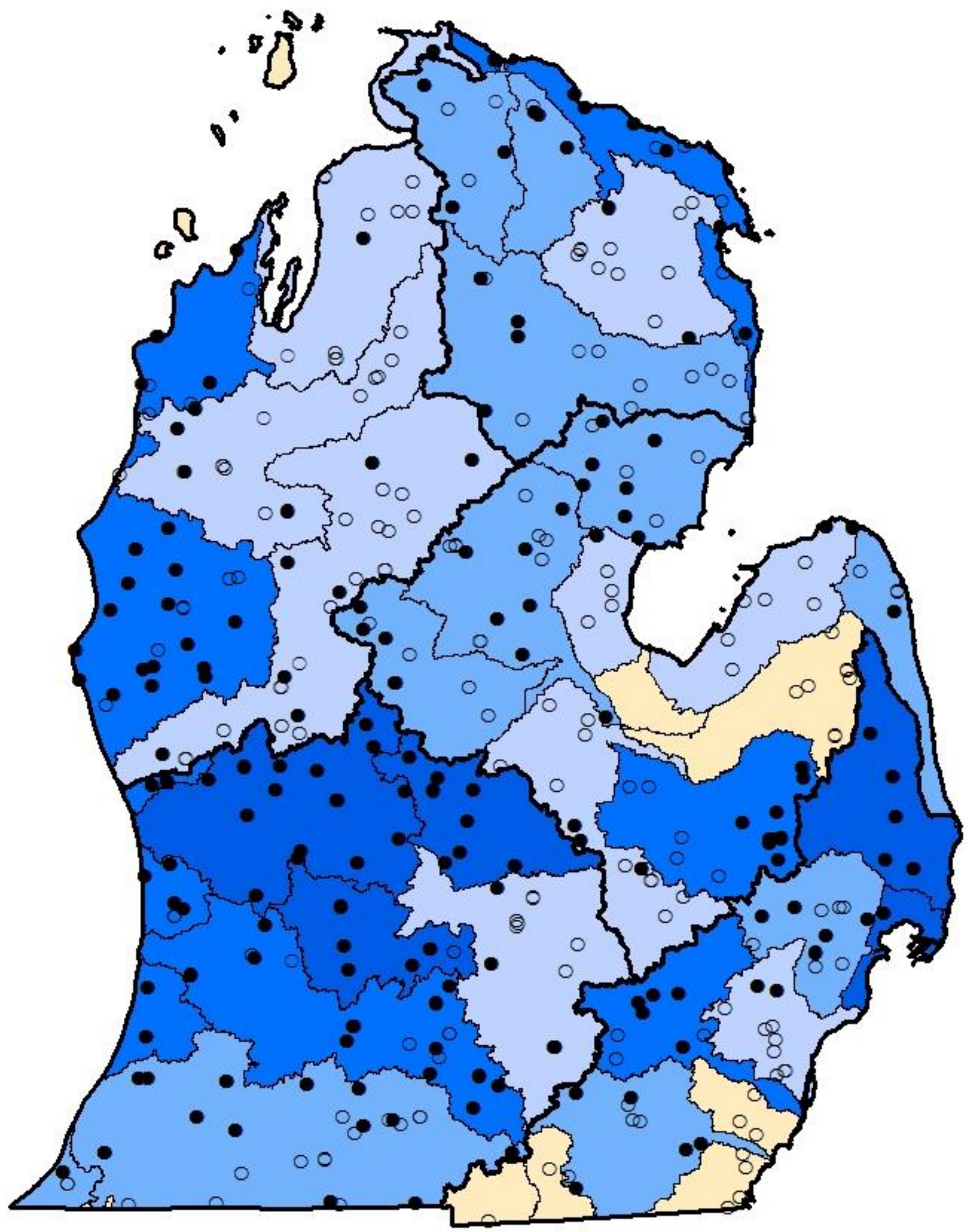


Figure 1.14: 1975 map of *Orconectes virilis* detection. Black dots are sites where *O. virilis* was detected, hollow dots are where the *O. virilis* was not detected, but other crayfish were.

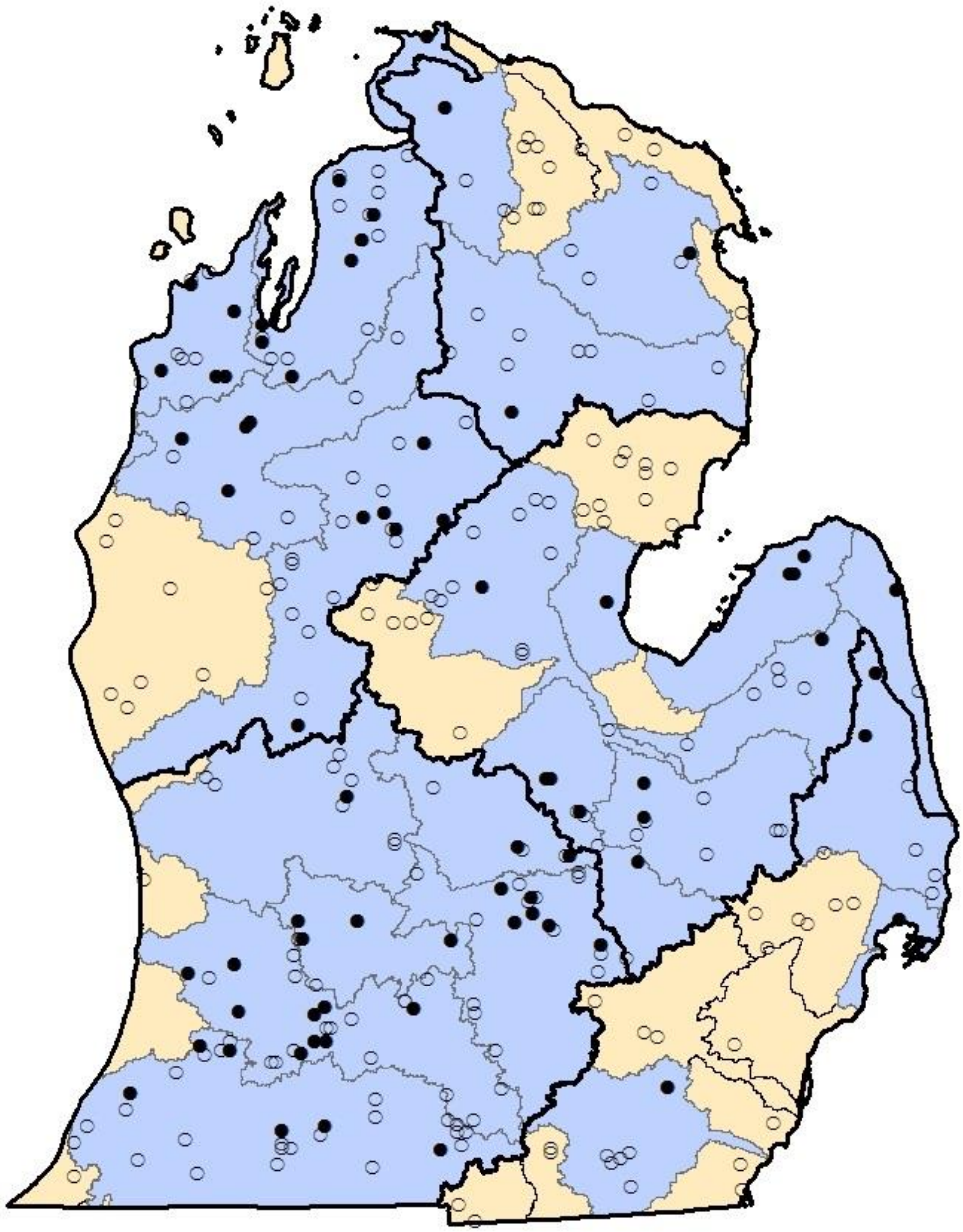
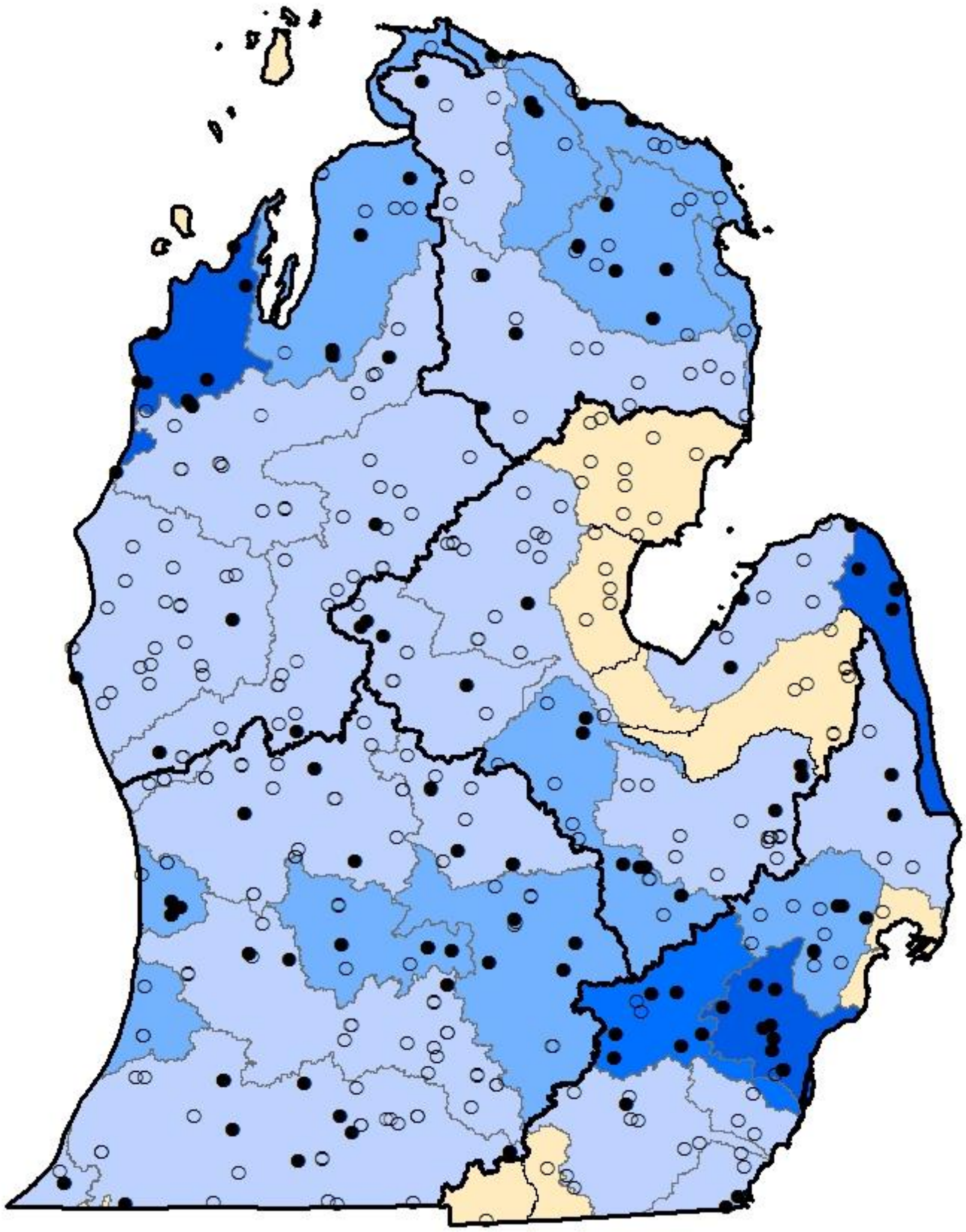
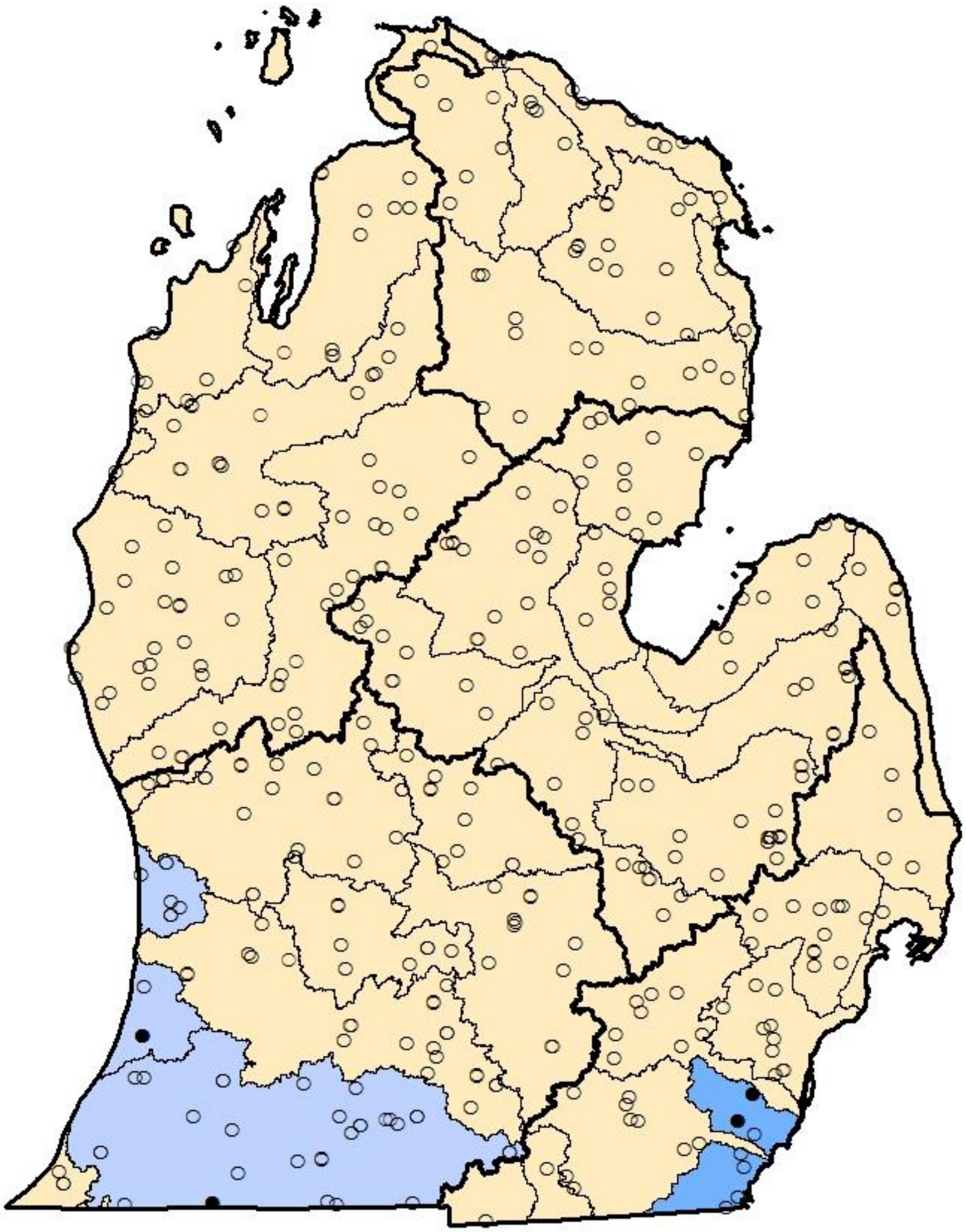


Figure 1.15: 2015 map of *Orconectes virilis* detection. Black dots are sites *O. virilis* was detected, hollow dots *O. virilis* was not detected. Shading represents percentage of sites in a watershed that contained *O. virilis* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%



1.16: 2015 map of *Procambarus acutus* detection. . Black dots are sites *O. virilis* was detected, hollow dots *O. virilis* was not detected. Shading represents percentage of sites in a watershed that contained *O. virilis* and goes from light to darkest: 1-25%, 26-50%, 51-75%, 76-100%. *P. acutus* was not detected in 1975.



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Chapter 2:

Assessment of Potential Pathways of Entry for Red Swamp Crayfish in Michigan's Lower Peninsula

Introduction:

Invasive species have threatened Michigan's native flora and fauna since European colonizers began introducing plants and animals from Europe to help them acclimatize to the continent (Phillips 1928, Dunlap 1997). During the 19th and 20th centuries, the Great Lakes saw invasions, such as sea lamprey (*Petromyzon marinus*) and alewife (*Alosa pseudoharengus*), that are largely attributed to the construction of the Welland and Erie canals (Applegate 1950, Smith 1970). Other species arrived as purposeful introductions to serve commercial or sporting desires, such as rainbow smelt (*Osmerus mordax*) which were stocked as forage for sport fishes (Van Oosten 1937). Global trade has also increased the risk of species arriving in the Great Lakes from across the globe via ballast water, as was the case with several Ponto-Caspian species including the round goby (*Neogobius melanostomus*), Dreissenid mussels, and at least one amphipod (*Echinogammarus ischnus*) (Witt et al. 1997, Benson 2016a,b,c, Fuller et al. 2016). Currently there is considerable concern about the artificial connection of the Mississippi and Great Lakes watersheds via the Chicago Area Waterway System (CAWS); a system that harbors invasive species, such as the bighead carp (*Hypothalmichthys nobilis*) and silver carp (*H. molitrix),* that present risks to the Great Lakes and its tributaries (Cooke and Hill 2010, Cuddington et al 2014).

Large scale operations are not the only introduction vectors for invasive species; many invasions can be attributed to the unknowing actions of individuals, such as the release of pets or study organisms, incidental escape from small scale aquaculture, or the dumping of bait buckets into waterbodies (Capelli and Magnuson 1983, Lodge et al. 2000, Larson and Olden 2008, Chang et al 2009, Drake and Mandrak 2014). While many species currently threaten to invade Michigan waters, this study will focus on the risk of red swamp crayfish (*Procambarus clarkii*) introduction, a species native to the Southcentral United States and Northeastern Mexico.

The red swamp crayfish is a popular aquaculture species primarily raised for food, and accounts for roughly 80% of the total farmed crayfish in the world, with the U.S. producing up to 60,000 tons a year (Hobbs et al. 1989, Huner and Lindqvist 1995). In its home range, *P. clarkii* prefers lentic waters and soft soils that permit the construction of shoreline burrows to escape desiccation (Huner and Lindqvist 1995, Taylor et al. 2015). They are also capable of dispersing up to 1.6 km over dry land, allowing them to invade adjacent waterbodies that are not connected by stream flow (Banha and Anastácio 2014, Ramalho and Anastácio 2015).

Outside of its home range, *P. clarkii* has become invasive on every continent except Antarctica and Australia, often invading wetlands, lakes, and agricultural environments (Hobbs et al 1989). They have been particularly successful in areas lacking native crayfish such as China and Africa. In China, studies show that *P. clarkii* has damaged native vegetation and macroinvertebrate communities, and the burrowing activity has destroyed rice fields and irrigation systems (Li and Xie 2002, Li et al. 2005). In Africa, *P. clarkii* were first introduced in Uganda and Kenya to serve as a food source, but also as a predator of snails carrying schistosomiasis, in hopes of curtailing the effects of this disease in the human population (Mkoji et al. 1999, Foster and Harper 2007). Since their introduction into Africa, *P. clarkii* has spread outside of the initial aquaculture and introduction sites into neighboring systems, disrupting native food webs by destroying macrophyte beds and competing with native crabs (Foster and Harper 2007).

In Europe, *P. clarkii* success can be attributed to its resilience to *Aphanomyces astaci*, a fungal pathogen that has proven lethal to Astacid crayfish outside of North America and the Parastacid crayfish of the Southern Hemisphere (Huner and Lindqvist 1995). This resilience to disease has allowed Cambarid crayfish such as *P. clarkii* and North American Astacid crayfish like the signal crayfish (*Pacifastacus leniusculus*) to invade European streams with little biotic resistance from native crayfish (Aquiloni et al. 2011, Chucholl and Schrimpf 2016). European studies have also shown that *P. clarkii* is capable of living in environments different from the warm lentic systems with which it is often associated. In particular, a study from Germany reports the success of *P. clarkii* in a cold water stream, suggesting it is able to persist in a range of habitats (Chucholl 2011).

Red swamp crayfish affect native food webs by shifting trophic structures, altering macrophyte and macroinvertebrate communities, and decreasing biodiversity (Ilheu et al. 2007, Klose and Cooper 2012, Carreira et al. 2014). In Portugal, *P. clarkii* have been observed to selectively feed on macrophytes, removing species in a sequential order and resulting in a shift of dominant vegetative cover and loss of species richness (Carreira et al. 2014). Foraging and burrowing habits in loose substrates have also been shown to negatively affect macrophyte communities by increasing turbidity (Anastácio and Marques 1997, Angeler et al. 2001). The foraging selectivity of *P. clarkii* on periphyton has also been related to decreases on macroinvertebrate species richness (Klose and Cooper 2012). When preferred vegetative food sources are scarce, adult *P. clarkii* have been shown to adapt by becoming more predatory, focusing on macroinvertebrates, with evidence that their preferences can alter macroinvertebrate populations and species richness (Correia 2002). Young *P. clarkii* have also been shown to decrease macroinvertebrate diversity in wetland systems due to their predatory behavior (Correia and Anastácio 2008).

Studies examining vertebrate species' relationships with *P. clarkii* show further negative implications of introductions. Amphibian populations have been shown to be at particularly high risk of *P. clarkii* predation due to their resistance to toxins found in the protective layer of amphibian eggs (Gamradt and Kats 1996, Cruz et al. 2008). Though few studies have covered the topic, predation of fish eggs has also been observed during a study looking at potential egg predators of the endangered razorback sucker (*Xyrauchen texanus*) (Mueller et al. 2006).

Studies focused on rusty crayfish (*Orconectes rusticus)* invasions in Wisconsin have shown that invasive crayfish can have detrimental effects on food webs and biodiversity in areas that already have native congeneric crayfish (Lodge and Lorman 1987, Lodge et al. 1994, Dorn and Mittelbach 2004). Despite native congeners, *O. rusticus* has become a successful invader in the Great Lakes by aggressively outcompeting native species for important resources such as shelter from predation and by attaining a size refuge from predation, allowing them to become more influential in food web dynamics than native congeneric crayfish (Capelli and Munjal 1982, DiDonato and Lodge 1993). These traits of successful invasion tactics are likely to be exhibited by *P. clarkii* when competing in appropriate habitats with other burrowing and lentic bound or congeneric species in Michigan. In Oregon, *P. clarkii* has been shown to compete with native signal crayfish (*Pacifastacus leniusculus*) for shelter (Hanshew and Garcia 2012, Peal et al. 2013). In the Midwest and Southern U.S., *P. clarkii* has already been shown to outcompete native *P. acutus* for shelter and even exclude *P. acutus* from uninhabited shelters (Grant and Figler 1996, Acquistapace et al. 2004). Furthermore, populations of *P. clarkii* found around the Sandusky Bay of Northern Ohio are beginning to become numerically dominant in wetlands once dominated by native species such as *C. diogenes, C. polychromatus,* and *P. acutus* (Thoma unpublished data, Thoma and Smith personal observation 2015).

Although no live specimens of *P. clarkii* have been found in Michigan, carcasses were observed in locations with high fishing pressure, raising concerns that anglers might be using *P. clarkii* as fishing bait in Michigan waters; these actions have the potential to result in the release of live specimens (MDNR 2013). The presence of carcasses, coupled with accounts describing *P. clarkii* as an ecologically plastic decapod with the ability to survive in a wide range of temperatures and flow regimes and adapt to a wide array of competitors and predators, prompted the MDNR to investigate potential entry points that *P. clarkii* could use to enter Michigan's ecosystems (Huner and Barr 1983, Cruz and Rebelo 2007, Hanshew et al. 2012). Prior studies suggest that *P. clarkii* could enter the state from a variety of sources including incidental release from live food markets, bait buckets, the pet trade, classrooms that use crayfish supplied by biological supply companies, and natural dispersal from invaded watersheds in Ohio (Norrocky 1983, Larson and Olden 2008, Peters and Lodge 2011).

Because the prior literature suggests that *P. clarkii* could potentially cause ecological and economic issues in Michigan, this study sought to formally assess the potential pathways *P. clarkii* could use to enter Michigan. Using a combination of survey methods, this study looked at potential entry routes including the pet, bait, and food trades, use in classrooms, and natural dispersion from Ohio as likely modes of introduction. Qualitative methods were then applied to determine the relative likelihood that each of these entry routes could result in *P. clarkii* introduction to Michigan. Discussion was then structured around the likelihood these pathways could result in a successful invasion. This study's findings will hopefully help shape management decisions to prevent the introduction of *P. clarkii* and other non-native crayfish into Michigan, and mitigate the spread of any future introductions.

Methods:

Multiple methods were used to survey potential entry vectors of *P. clarkii* into Michigan. This study focused on food, bait, and pet shops, educational classroom use, and natural dispersal from a known population existing in the Sandusky Bay area of Ohio, all of which have been considered potential sources of invasion risk (Norrocky 1983, Larson and Olden 2008, Peters and Lodge 2011).

Retail Stores

Retail stores were surveyed during the summers of 2014 and 2015 to identify where individuals might buy live *P. clarkii* for personal use. Store surveys focused on commonly known store genres that sell live crayfish including pet stores, bait shops, and food markets. Store surveys focused on major population centers in Michigan's southern Lower Peninsula including Battle Creek, Bay City, Detroit Metropolitan area, Grand Rapids metropolitan area, Lansing, Kalamazoo, and Saginaw. Initially stores were selected by conducting an internet search with the following terms in each city; 'bait shop', 'bait store', 'fish market', 'live food market', 'pet shop', 'pet store', 'seafood market', and 'tackle shop'. Stores were then visited haphazardly, allowing for additional stores to be discovered and visited while traveling between identified locations. When inquiring about the availability of live crayfish, I attempted to give the impression that I was an angler potentially interested in crayfish for bait.

After leaving a location, I recorded the name, address, type of establishment (food market, pet store, or tackle shop), whether or not it carried live crayfish, species of any live crayfish, whether or not the establishment would be willing to order live crayfish, and any notes on the sale of other live organisms. In the event that a store did not sell live crayfish, I asked whether any nearby retailers might sell live crayfish. Any suggested shops were then visited and surveyed if they had not previously been surveyed that year.

Due to Aquatic Invasive Species Order No. 1 of 2014, which took effect on November 7 2014, prohibiting the possession of live *P. clarkii*, 62 shops that had been visited in 2014 were re-visited in 2015 (MDNR 2014). This resampling of shops was intended to assess compliance habits of businesses that sold live crayfish, or might have begun selling live crayfish. Stores that were re-visited were surveyed in the same manner as the prior year.

Classroom Use

Data on crayfish use in the classroom was collected through the distribution of anonymous surveys, approved by the Michigan State University Human Research Protection Program. Surveys were distributed during the Michigan Science Teachers Association (MSTA) Conference in Lansing, MI, on March 4, 2016. Surveys were distributed in a Department of Natural Resources sponsored room at the conference titled ‘DNR at MSTA’. This room was chosen because its emphasis on biology, natural resources, and outdoor education. I assumed that teachers that sought out lectures in this room were the most likely to use crayfish in their classrooms.

Upon entering the ‘DNR at MSTA’ lecture room, each teacher was handed a survey and asked to turn it in before leaving. Surveys consisted of one question regarding the county in which they taught and four multiple choice questions regarding grades taught, any crayfish use, means of crayfish acquisition, and means of crayfish disposal (Figure 2.1). Surveys were analyzed by assigning a value of ‘risky’ or ‘safe’ to the listed sources and disposal techniques. Sources regarded as ‘safe’ included collection from the wild or crayfish obtained from local nature centers. Sources regarded as 'risky' included biological supply companies, pet stores, or other written responses that suggested the possibility that the acquired crayfish were potentially a non-native species. For disposal techniques, 'safe' responses included anything that either ensured the crayfish were dead before disposal, involved release back to the site from which they were collected, or donation to a museum, university, or similar establishment. Disposal methods regarded as 'risky' included any method that created uncertainty about the fate of the crayfish, such as sending crayfish home with students, flushing live crayfish down toilets, throwing live crayfish in the trash, or releasing crayfish into the wild (if they had not been collected from the same site).

Natural dispersal from a neighboring watershed

To assess the risk of natural dispersal I assessed the presence and distribution of *P. clarkii* around the Sandusky Bay of Ohio, a region where their presence has already been documented (Norrocky 1983), and that is close to the southeastern border of Michigan. Survey sites were initially selected based on advice from a local expert (Thoma), who cited observations that a population of *P. clarkii* continued to persist in and around Winous Point Shooting Club in Ottawa and Sandusky Counties, Ohio. I sampled along ditch lines, and in creeks and wetlands where *P. clarkii* had been reported by Norrocky in the past (Norrocky 1983). Additional sites were sampled haphazardly between and beyond historical sampling sites where crayfish burrows were visible.

At each sampling site, standard dip netting techniques were used to sample crayfish where surface water was present (Olden et al. 2006). Standard burrow excavation methods were used in areas such as dried ditches and fields, in which burrows were excavated using a shovel and crayfish were extracted by hand (Ridge et al. 2008). After crayfish had been identified and sexed, native species were released and invasive species were preserved in 90% ethanol. At each sampling location, GPS coordinates were recorded in association with crayfish identifications.

Results:

Retail Stores

During the course of the 2014 and 2015 field season, 125 shops were visited; these shops consisted of 80 food markets, 25 pet stores, and 20 tackle shops. Of the 80 food markets, all 8 (10%) that carried any live crayfish included *P. clarkii* in their inventory, and 3 (3.75%) additional stores indicated a willingness to order live crayfish (Table 2.1a). Of the 25 pet stores, all of the 13 (52%) stores that sold live crayfish included in their supply either *P. clarkii* or other crayfish from the genus *Procambarus* that could not be identified while in tanks. Three (15%) of the 20 tackle shops sold live crayfish, all of which were native *Orconectes immunis.* When I asked tackle shop clerks about the source of their crayfish they generally indicated that they had been imported from Ohio. Four tackle shops did not have crayfish in stock at the time but three reported they would be buying crayfish from Ohio, while the remaining shop reported that they caught their own crayfish from a nearby waterway.

Of the 62 shops that were re-visited in 2015, 43 (69%) were food markets, 11 (18%) were pet stores, and 8 (13%) were tackle shops. I found that of the four (9%) food markets I revisited that were selling live *P. clarkii* in 2014, all of them were still selling live crayfish, including *P. clarkii*. Additionally three (7%) food markets that were not selling crayfish in 2014 had begun selling crayfish, including *P. clarkii*, in 2015. The remaining 36 (84%) food markets never sold crayfish during either visit.

Of the seven (64%) pet stores I revisited that were selling crayfish in 2014, six (55%) were still selling crayfish and one shop that had sold crayfish in 2014 had permanently closed by

2015. Additionally one pet shop that did not sell crayfish in 2014 had begun selling crayfish in 2015. The remaining three (27%) pet stores did not sell crayfish in either year.

Of the five (63%) tackle shops I revisited that sold crayfish in 2014, four (50%) continued to sell crayfish in 2015, and the tackle shop that reported they caught and sold their own crayfish in 2014 had permanently closed by 2015. One tackle shop that had not sold crayfish in 2014 had begun selling crayfish in 2015. Two tackle shops never sold crayfish either year. All tackle shops were selling native *O. immunis*, purchased from an Ohio bait dealer according to personal conversations with the store clerks in both 2014 and 2015, with the exception of the store that noted in 2014 that they caught their own. (Table 2.1b)

Classroom Use

A total of 157 surveys were returned during the course of the conference. All of the respondents, except for 2, taught in the Lower Peninsula, representing 45 counties (Figure 2.2). Of the 157 respondents, 17 (10.8%) reported using live crayfish in their classes. 'Risky' acquisition was reported on 10 (59% of crayfish users) occasions and 'risky' disposal was reported on 5 (29% of crayfish users) occasions. Teachers that reported crayfish use in their classroom were from 11 counties; 5 of the 17 teachers reporting use of live crayfish were from Wayne county (Detroit region).

Natural dispersal from a neighboring watershed

A total of 21 locations in northwestern Ohio were visited in 2015 (Figure 2.3). Red swamp crayfish were found in 13 of those locations and were the dominant species in 10. Of the 124 crayfish observed, 87 (70%) were *P. clarkii*. The following six species were found co-occurring with *P. clarkii* during the surveys: *C. polychromatus, Cambarus thomai, Fallicambarus fodiens, O. immunis, O. propinquus,* and *O. rusticus*.

Discussion:

My findings suggest there are currently non-trivial risks of *P. clarkii* introduction associated with each entry vector surveyed. Despite the absence of *P. clarkii* in bait shops I believe that some anglers are buying *P. clarkii* from food markets due to the lower prices; crayfish sold in bait shops were $5 to $6 for a dozen, whereas in food markets crayfish were $4 to $6 per pound, which might include 30 or more crayfish. At several food markets, store clerks asked if I planned on fishing with the crayfish I was thinking about purchasing. A recent study found 28% of Michigan anglers that use live bait release their bait into the water after fishing (Drake et al. 2015), so it is likely that if *P. clarkii* are purchased for the purpose of angling they will be released into Michigan waterways. As well, anglers in Ontario moved a median of roughly 290 km during fishing outings (Drake and Mandrak 2010). If Michigan anglers show similar mobility, they could potentially spread bait, including *P. clarkii,* a substantial distance across the state or even into other Great Lakes regions.

All crayfish found in bait shops were native *O. immunis*. However, bait shop clerks acknowledged that these crayfish were sourced from a distributer located in Ohio. The nearest crayfish farm to Michigan is located in Fremont, OH, and is within a watershed known to be invaded by *P. clarkii*, raising the possibility of this farm also being invaded by *P. clarkii* at some point. It is unlikely that workers at the crayfish farm would check or sort every crayfish going out for order, allowing for the possibility of *P. clarkii* being mixed into a shipment of *O. immunis*.

Pet stores and classroom settings also pose threats to the distribution of *P. clarkii*, as well as other non-native crayfish species. Although this study did not investigate the likelihood of pet crayfish release into the wild, it has already been documented as a vector for crayfish introduction in other studies (Lodge et al. 2000, Peters and Lodge 2011, Chucholl 2013, Loureiro et al. 2015). Even if the likelihood of pet release were to be low, *P. clarkii* females have been known to carry upwards to 400 young (Gherardi 2006). Their high fecundity means that only a few individuals or one gravid female could initiate an invasion. Even if pet owners and educators were to flush crayfish down a toilet, or dispose of them in the trash, any live specimens could potentially survive in the sewer or waste dumps and spread from there (Indiana Biological Survey 2008). If someone does possess live *P. clarkii* it is recommended that they are euthanized and any potential young are also destroyed before disposing of them in order to prevent introduction.

Although many of the examples of successful *P. clarkii* invasion overseas can be attributed to their role as a vector of diseases killing off native crayfish species, and possibly expediting their spread by taking away potential competitors, this will likely not be a concern for Michigan if *P. clarkii* successfully invade. Michigan's crayfish species are of the Cambaridae family, a group of crayfish that show little to no effects when exposed to the diseases carried by *P. clarkii* (Huner and Lindqvist 1995). Instead *P. clarkii* would likely compete directly for resources with native species including *Cambarus diogenes*, *C. polychromatus*, *F. fodiens* and the congener *P. acutus* (Gherardi and Daniels 2004, Cooper and Armstrong 2007, Hanshew and Garcia 2012, Thoma pers. obs. 2015). Native crayfish could also face greater risk of predation compared to *P. clarkii.* Great Lakes fish have already been shown to prefer crayfish with smaller bodies and chelae (DiDonato and Lodge 1993). The potential for *P. clarkii* to reach larger comparative sizes and displace native species from shelter could result in higher rates of predation for native crayfish (Gherardi and Daniels 2004, Hanshew and Garcia 2012).

Reaching a large size might also put *P. clarkii* above the gape limitations of some native fish, allowing them to affect native fish assemblages via egg predation (DiDonato and Lodge 1993, Mueller et al. 2006). Fish communities could be further affected as a result of macrophyte destruction, an important cover type for valuable Michigan fish species and their prey, due to consumption or increased turbidity from foraging habits (Wilson et al. 2004, Carreira and Rebelo 2014).

Ecological and economic impacts regarding *P. clarkii* burrowing habits are another concern. Despite the fact that five of Michigan's native species exhibit terrestrial burrowing behavior, they have not been shown to cause the significant destruction to dams and dykes that has been documented for *P. clarkii* (Correia and Ferreira 1995, Correia 2002, Klose and Cooper 2012). With Michigan's abundant managed wetlands and dams this could lead to other negative economic and ecological consequences of a *P. clarkii* invasion.

Although this study focused on the potential for *P. clarkii* invasion in Michigan, the concerns of introduction could be extended to other crayfish species. Hobbs et al. (1989) contains an extensive list of studies focused on the invasions of other crayfish including *P. leniusculus*, *Orconectes limosus*, *O. rusticus*, and *Orconectes virilis*. It would be reasonable to assume that *P. clarkii* are the most likely crayfish to be introduced based solely on the large quantities observed in the food trade within Michigan's urban centers. The pet trade however leaves room for any number of the world's 640 crayfish to become a threat to Michigan's waters (Crandall and Buhay 2008). Although none of Michigan's native crayfish species are in immediate danger of extinction there is evidence that several species could be slowly extirpated by invasive *O. rusticus*. In order to prevent the potential damage to Michigan's wetland and aquatic ecosystems it is suggested that tighter restrictions are placed in the importation and possession of non-native crayfish in order to stop any further potentially invasive species entering the state. Despite the MDNR's memorandum making the possession of live *P. clarkii* illegal, there were still shops continuing to sell live *P. clarkii*, including several new shops. If managers hope to prevent the introduction and spread of *P. clarkii* through food, pet, and bait trade then more effort must be made by the MDNR to enforce laws regarding *P. clarkii* sale and possession.

APPENDIX

Table 2.1a: Results of crayfish retailer visits

Shop Type	No Crayfish (%)		Sold Crayfish (%)		Sold *P. clarkii* (%)		Total (%)	
Food	72	0.90	8	0.10	8	0.10	80	0.64
Pet	12	0.48	13	0.52	13	0.52	25	0.20
Tackle	17	0.85	3	0.15	0	0.00	20	0.16
							125	1.00

Table 2.1b: Results of crayfish retailer shop re-visits

Shop Type	Sold both years (%)		Quit selling in 2015 (%)		Begun selling in 2015 (%)		Never sold (%)		Total (%)	
Food	4	0.09	0	0.00	3	0.07	36	0.84	43	0.69
Pet	6	0.50	0	0.00	1	0.10	3	0.30	10	0.18
Tackle	4	0.57	0	0.00	1	0.14	2	0.29	7	0.13
									60	1.00

Figure 2.1: The survey instrument for collecting data on crayfish use in Michigan classrooms.

Question 1:
What **Counties** do you teach?

Question 2:
What grades do you teach?
(Check all that apply)

- ☐ Grades1-5
- ☐ Grades 6-8
- ☐ Grades 9-12
- ☐ Other: ______________________________

Question 3:
Do you use **live crayfish** in your classroom?

- ☐ Yes
- ☐ No

Question4:
From **where** do you obtain your classroom crayfish?
(Check all that apply)

- ☐ Biological Supply Company
- ☐ Pet Store
- ☐ Zoo, Nature Center, or Aquarium
- ☐ Collected from the wild (by yourself or students)
- ☐ Collected from the wild (by someone else)

Other: ______________________________

Company Names: ________________________

Question 5:
How are crayfish typically disposed of in your classroom?
(Check all that apply)

- ☐ They are returned to supplier
- ☐ They are given away to students
- ☐ They are given to another teacher
- ☐ They are donated to a university, museum, or aquarium
- ☐ They are kept in the classroom as pets until they die naturally
- ☐ They are released into the wild
- ☐ They are flushed down toilets
- ☐ They are euthanized
- ☐ They are disposed of in trash containers
- ☐ They are eaten

Other: ______________________________

Figure 2.2: Map of counties surveyed for crayfish use in Michigan classrooms. Color coding indicates the highest reported form of risk from labeled counties.

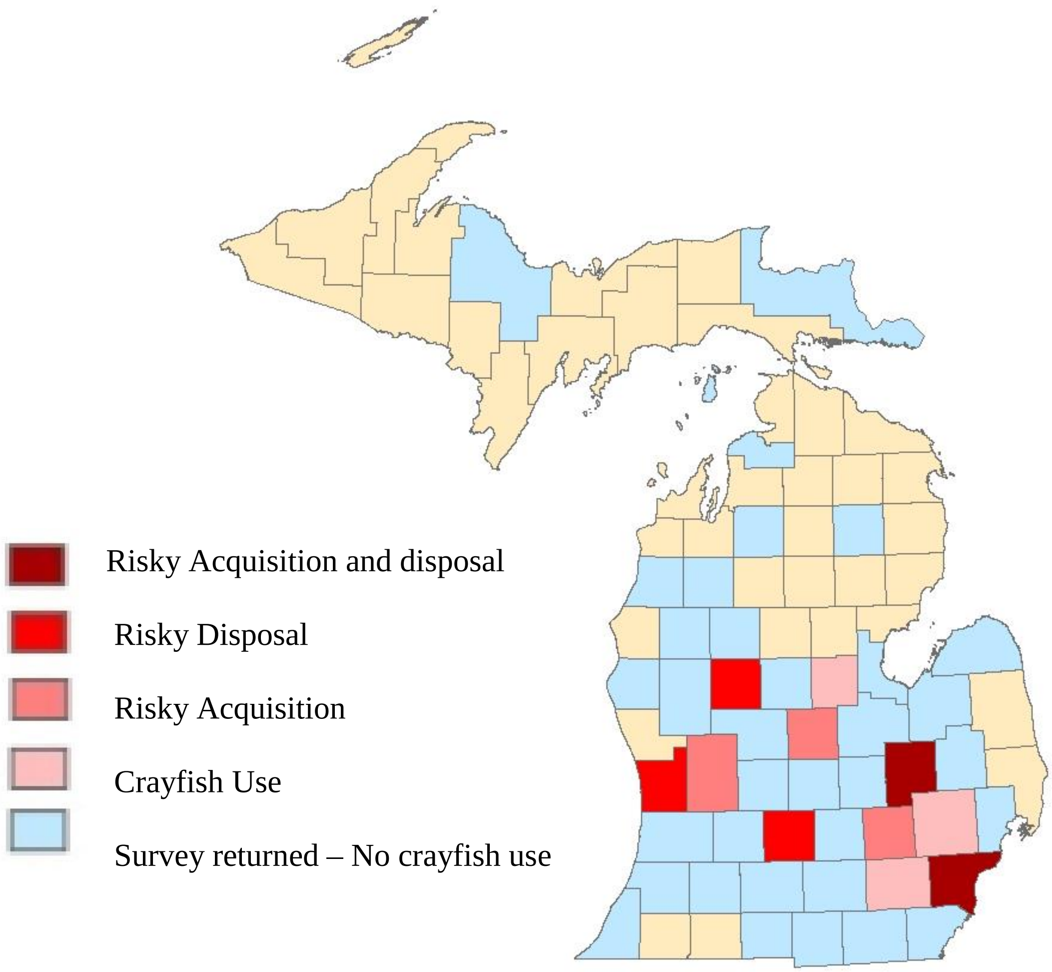
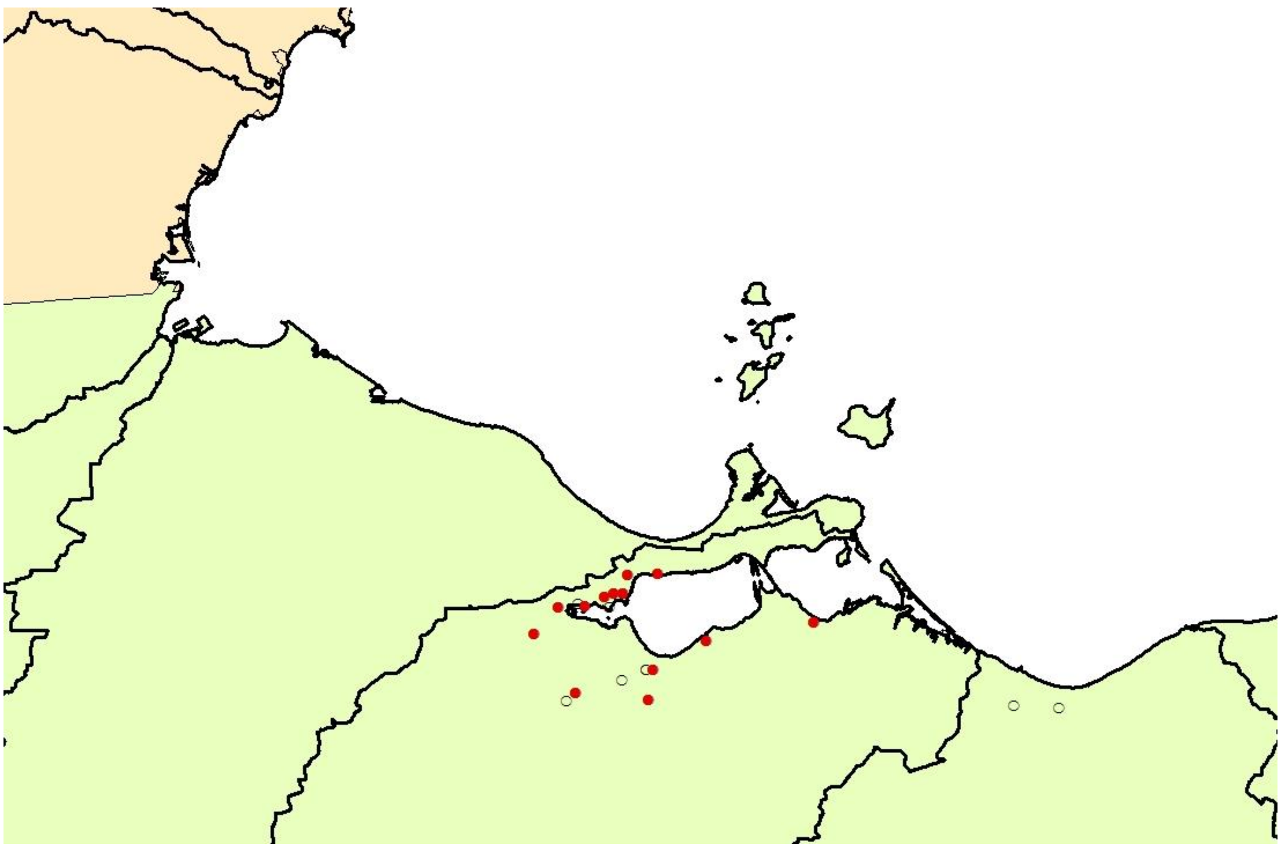


Figure 2.3: Survey sites around Sandusky Bay, Ohio. Filled dots are sites where *P. clarkii* was detected, hollow dots are where *P. clarkii* were not detected.



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