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Assessment of invasion risks for red swamp crayfish (*Procambarus clarkii*) in Michigan, USA

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Abstract: Non-native invasive crayfish continue to threaten ecosystems across the globe. However, factors that increase the risk of these introductions and subsequent establishment have yet to be fully elucidated. This study takes place in the US state of Michigan, where in 2013 several carcasses of red swamp crayfish (*Procambarus clarkii*) were discovered at popular fishing locations. Following this discovery, we explored possible modes of entry *P. clarkii* might use to invade Michigan by visiting various retailers that sold live crayfish and surveying classroom use of crayfish. We visited retail shops in 2014 and again in 2015 to determine if these stores continued selling live red swamp crayfish following a ban on possession of live red swamp crayfish enacted in late 2014. However, in 2017 we discovered established populations of *P. clarkii* in several ponds in southeast Michigan and a lake in the southwest portion of the state. These discoveries offered an opportunity to qualitatively compare our assessment of potential vectors with an ongoing invasion and to determine the effectiveness of the prohibition on live *P. clarkii* sales. Our assessment of potential vectors indicated that classrooms and live food markets are the most likely sources of the invasion, but none of the vectors we explored were risk free. In particular, we found that the number of retail shops selling live *P. clarkii* in 2014 actually increased following the prohibition, indicating the need to ensure the cooperation of industry and individuals in preventing the introduction and spread of non-native invasive crayfish. The results of this study can be used by natural resource managers to help identify vectors that move non-native invasive crayfish across political boundaries and illustrate the importance of restricting and prohibiting the movement of non-native invasive species across boundaries, or into new ecosystems.

Key words: Human mediated pathways, invasive crayfish, risk assessment

Introduction

Non-native 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). While many species currently pose an invasion risk to Michigan waters, this study focuses on the risk of red swamp crayfish (*Procambarus clarkii*) introduction. Red swamp crayfish are a species native to the Southcentral United States and Northeastern Mexico that prefers lentic waters and soft soils that permit the construction of shoreline burrows to escape desiccation (Huner and Lindqvist 1995; Taylor et al. 2015). *P. clarkii* are also capable of dispersing up to 1.6 km over dry land, allowing them to spread and become established in adjacent wetlands and waterbodies that are hydrologically disconnected (Banha and Anastácio 2014; Ramalho and Anastácio 2015).

Outside of its native range, *P. clarkii* has become invasive on every continent except Antarctica and Australia. Invaded habitats often include wetlands, lakes, and agricultural environments (Hobbs et al 1989). Red swamp crayfish have been particularly successful in areas lacking native crayfish, such as China and Africa. In China, introduced *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 Europe, *P. clarkii* is capable of surviving in environments different from the warm lentic systems with which it is often associated. In particular, Chucholl (2011) report the success of *P. clarkii* in a cold-water stream in Germany, indicating the species is able to persist in a wide thermal range of habitats. The species plasticity to environmental conditions is one life history trait that increases their invasiveness.

The risk of introduction and deleterious impacts of *P. clarkii* is elevated because of their aggressive behavior and ability to outcompete native species. In Oregon, for example, *P. clarkii* has been shown to compete with native signal crayfish (*Pacifastacus leniusculus*) for shelter (Hanshew and Garcia 2012; Pearl et al. 2013). In the Midwest and Southern U.S., *P. clarkii* has already been shown to outcompete native *Procambarus acutus* for shelter and even exclude *P. acutus* from uninhabited shelters (Grant and Figler 1996; Acquistapace et al. 2004).

Initial concern regarding the risk of red swamp crayfish invasion in Michigan arose when *P. clarkii* carcasses were observed in popular recreational fishing locations in 2013. The source of the carcasses was unknown, but resource managers speculated that live bait releases were likely the source of the detected specimens (MDNR 2013). The live bait trade is a documented pathway for introducing invasive species (Kilian et al. 2012; Lodge et al. 2012; Drake et al. 2015), but availability of *P. clarkii* as a live bait source was unanticipated because of state regulations that prohibit the use of nonnative crayfish for bait. However, this prohibition did not cover the possession of crayfish for other purposes such as aquarium or culinary ventures, or from other sources such as pet stores, or live food markets. This loophole inadvertently allowed anglers to access nonnative crayfish, such as *P. clarkii*, for bait from sources not regulated by the Michigan Department of Natural Resources (MDNR). The use of *P. clarkii* as bait, procured from unknown sources, coupled with the known invasiveness of *P. clarkii* in introduced habitats around the world (Huner and Barr 1983; Cruz and Rebelo 2007; Hanshew and Garcia 2012) prompted the MDNR to investigate the risk of potential introduction pathways in Michigan. The suspected pathways included incidental release from live food markets, bait bucket release, the pet trade; biological supply through classroom releases; and natural dispersal from invaded watersheds in Ohio (Norrocky 1983; Larson and Olden 2008; Peters and Lodge 2009).

Ecological risk assessment involves determining the likelihood that an undesirable environmental effect is going to result from some form of human activity. The evidence discussed above clearly documents the undesirable effects that can result from *P. clarkii* invasion. The United States Fish and Wildlife Service Ecological Risk Screening Summary found a high climate match for *P. clarkii* in Michigan (USFWS 2015). Here we sought to assess the likelihood of an invasion occurring, and particularly the most likely means by which such an invasion might occur. Previous studies have suggested that areas of high human use exhibit a high potential for crayfish introduction and spread (Puth and Allen 2005). Following previous studies on the risk of spread of aquatic invasive species (Drake and Mandrak 2014; Drake et al 2015) in the Great Lakes region, we used a variety of methods to conduct a semi-quantitative risk assessment to evaluate several potential invasion pathways. Qualitative methods were then applied to determine the relative likelihood that each of these entry routes could result in *P. clarkii* introduction to Michigan.

Subsequent to the completion of our risk assessment, the first detections of live *P. clarkii* were reported and confirmed in Michigan. The infestations allowed us to evaluate the credibility of our survey methods and further determine the consequences of crayfish usage and sale in Michigan.

Methods

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'. While traveling between identified stores, any additional stores encountered that fit the categories of a potential crayfish vendor were visited opportunistically. When inquiring about the availability of live crayfish, we attempted to give the impression that we were anglers potentially interested in crayfish for bait, food, or pets, depending on the shop.

After leaving a location, we 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, we 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. Store surveys took place between May 30th and June 13th of 2014 and between May 13th and May 20th of 2015.

On November 7 2014, Aquatic Invasive Species Order No. 1 of 2014 took effect (MDNR 2014), prohibiting the possession of live *P. clarkii*, and detailing a penalty where the owner would stand before a judge and face a potential fine of $10,000 and a felony charge (Natural Resources and Environmental Protection Act 451 of 1994; Amended 2014). This Order was communicated to the public through a statewide press release on November 10, 2014. The various industries of concern were additionally notified by a mailing campaign conducted by the Michigan Department of Natural Resources Fisheries Division and in person during MDNR shop inspections. In 2015 we re-visited 60 of the 85 shops that had been visited in 2014. Our resampling of shops was intended to assess compliance habits of businesses that sold live crayfish, or that might have begun selling live crayfish. Stores that were re-visited in 2015 were surveyed in the same manner as in 2014.

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 (IRB #: x16-328e). Surveys were distributed during the Michigan Science Teachers Association (MSTA) Conference in Lansing, MI, on March 4, 2016, in a Department of Natural Resources sponsored room at the conference titled 'DNR at MSTA'. This room was chosen because of its emphasis on biology, natural resources, and outdoor education. We 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 were collected at the only door to the conference room, ensuring that all surveys that were distributed were returned. 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 S1). 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). In accordance with our IRB permit; data for teacher surveys was reported at the county level to gain regionally relevant information while ensuring the anonymity of the teachers and school districts being surveyed.

Natural dispersal from a neighboring watershed

To assess the risk of natural dispersal we 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 (WPSC) in Ottawa and Sandusky Counties, Ohio. We 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 in expanding distances from WPSC between and beyond historical sampling sites where crayfish burrows were visible and in support of ongoing studies in Ohio (Thoma; unpublished data).

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 non-native species were preserved in 90% ethanol. At each sampling location, GPS coordinates were recorded in association with crayfish identifications.

Introduced Range

When *P. clarkii* were reported in several locations in the summer of 2017 we responded to all reports to confirm whether or not the report was valid. Upon identifying several areas that harbored *P. clarkii*, trapping efforts were conducted to determine the range of *P. clarkii* within the state. Trapping efforts were focused within a 5 km radius around initial *P. clarkii* observations. Authors used a combination of the Michigan Imagery Public, USGS NHD, Base Feature Hydro Lines, and USA Wetlands layers from ArcGIS, provided by the MDNR, and noted waterbodies not on the layer while traveling between locations to help identify potential sampling areas. After a waterbody was identified, efforts were then made to gain access to any private waterbodies. When permission to sample the location was granted, two minnow traps were baited with mesh bags filled with approximately 100g of dog food and deployed for at least 72 hours in each location, and were checked every 24 hours. Minnow traps were modified by enlarging the entrances to 65mm to allow for larger crayfish to enter. If no *P. clarkii* were detected in 72 hours of trapping, then traps were removed to be used at other locations.

Results

Retail stores

During the course of the 2014 and 2015 field season, we visited a total of 125 shops. These shops consisted of 80 food markets, 25 pet stores, and 20 tackle shops. Of the 80 food markets, all eight (10%) that carried any live crayfish included *P. clarkii* in their inventory, and three (3.75%) additional stores indicated a willingness to order live crayfish (Table 1, Figure 1). 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 positively identified while in tanks. Three (15%) of the 20 tackle shops sold live crayfish, all of which were native *Faxonius immunis.* When we asked tackle shop clerks about the source of their crayfish they generally indicated that they were 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 60 shops that were re-visited in 2015, 43 (69%) were food markets, 10 (17%) were pet stores, and 7 (12%) were tackle shops. We found that of the four (9%) food markets selling live *P. clarkii* in 2014, all of them were still selling live *P. clarkii,* in 2015. Additionally, three (7%) food markets that were not selling crayfish in 2014 had begun selling *P. clarkii,* in 2015. The remaining 36 (84%) food markets never sold crayfish during either visit.

Of the seven (64%) pet stores that were selling crayfish in 2014, six (55%) were still selling crayfish and one shop that had sold crayfish 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 never sold crayfish in either year. We could not identify the species of crayfish in the aquaria, although they appeared to be in the genus *Procambarus.*

Of the five (63%) tackle shops 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 did not sell crayfish either year. All tackle shops sold native *F. 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 indicated in 2014 that they caught their own (Table 2).

Classroom use

A total of 157 surveys were returned during the course of the conference. All but two of the respondents taught in the Lower Peninsula, representing 45 counties (Table 3, Figure 1). Of the 157 respondents, 18 (11.4%) reported using live crayfish in their classes. 'Risky' acquisition was reported on ten (6.4%) occasions and 'risky' disposal was reported on five (3.2%) occasions. Teachers that reported crayfish use in their classroom were from 11 counties; six of the 18 teachers reporting use of live crayfish were from Wayne County (Detroit region) a densely populated area with an abundance of artificial retention ponds connected by drain systems.

Natural dispersal from a neighboring watershed

A total of 31 locations in northwestern Ohio were visited in 2015-2016, 12 were dipnetted due to standing lentic water, and 19 were sampled by burrow excavation (Figure 2). Red swamp crayfish were found in 17 of these locations and were the dominant species at ten sites. In six sites, *P. clarkii* was the only species observed, possibly having extirpated native species (Thoma unpublished data). Of the 124 crayfish observed, 87 (70%) were *P. clarkii*. The following six species were found co-occurring with *P. clarkii* during the surveys: *Cambarus polychromatus, Cambarus thomai, Creaserinus fodiens, F. immunis, Faxonius propinquus,* and *Faxonius rusticus*.

Introduced Range

The initial sites of confirmation were a private pond in Farmington Hills, Michigan, a retention pond in Novi, Michigan, and Sunset Lake in Vicksburg, Michigan. The survey was focused on the Novi and Farmington Hills populations in order to better focus resources. A total of 67 locations were trapped between the Novi and Farmington Hills epicenters. All of these sites, whether they were streams or retention ponds, could be described as lentic systems at the time of sampling. There were 11 locations within 5km of the Novi epicenter where we confirmed *P. clarkii*. Of these 11 locations, *P. clarkii* was the only species of crayfish captured at five sites. Within the Novi region, the two furthest sites were 7.09 km from one another. Two of the sites where *P. clarkii* was detected were ponds that shared a culvert system within a private neighborhood and were located 3.5 km away from the next nearest site where *P. clarkii* was detected. *P. clarkii* was not detected in other immediately adjacent waterbodies to this neighborhood between the next nearest detection. Another five of the sites where *P. clarkii* was detected were all retention ponds that shared a drainage system and were located 2.42 km away from the remaining four sites which all were located on the same golf course in separate ponds. Of the four sites on the golf course, two were found north of a stream, and two south of the same stream, although no *P. clarkii* were detected within the stream, only native *F. virilis*.

Within 5km of the Farmington Hills epicenter there were four locations where we confirmed *P. clarkii*. All of these sites shared a contiguous intermittent wetland system, and the furthest two sites were within 0.25 km of one another. *P. clarkii* was the sole crayfish species observed within this area.

Trapping was conducted at additional waterbodies outside of the Novi, Farmington Hill, and Sunset Lake areas in response to public reports of *P. clarkii*. No *P. clarkii* were observed at these additional locations and it was apparent that the reported crayfish were native species upon further conversation with residents and investigation of the sites (Figure 2).

Discussion

Our findings suggest there are non-trivial risks of *P. clarkii* introduction associated with each entry vector surveyed. Current state regulations that prohibit nonnative crayfish as bait seem to be effective at reducing the presence of *P. clarkii* in bait shops. However, despite the absence of *P. clarkii* in bait shops it appears anglers are purchasing *P. clarkii* from live food markets for use as bait. Anglers that purchase crayfish at live food markets instead of bait shops seem to receive an economic advantage, which is a likely reason anglers are using non-traditional shops for sources of bait. For example, crayfish sold in bait shops were $5 to $6 per dozen, whereas crayfish cost $4 to $6 per pound in food markets, which might include two dozen or more crayfish. Further, store clerks at several live food markets asked if we planned on fishing with the crayfish after purchase, which indicates there might be a culture of buying crayfish from food markets with the intention of using them for bait. 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 possible that *P. clarkii* purchased for the purpose of angling will be released into Michigan waterways. Anglers that purchase crayfish in food markets could easily transport them to other locations. Anglers in Ontario traveled a median of 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 outside of the Great Lakes Basin.

All crayfish observed in bait shops were native *F. 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, which is located within a watershed known to be invaded by *P. clarkii.* The proximity of the distributer to known *P. clarkii* populations increases the risk of this farm also being infested with *P. clarkii.* The potential risk increases when considering the potential for species misidentification. Lodge et al. (2000) and Peters and Lodge (2009) describe the difficulty of identifying crayfish species by natural resource managers and conservation officers and it is reasonable to think that crayfish farm staff may have similar difficulties, especially after considering the large volume they are required to check or sort during regular operations. A few misidentified crayfish could result in a high risk activity if *P. clarkii* were misidentified and accidentally mixed in with *F. immunis* bait shipments.

Pet stores and classroom settings also represent a potential vector of *P. clarkii* introductions in addition to other non-native crayfish species. Biological supply companies are known to ship *P. clarkii* to schools as part of science education kits (Larson and Olden 2008; Peters and Lodge 2011). Published and unpublished surveys from around the United States indicate that teachers routinely use crayfish acquired from biological supply companies, and that these crayfish are often sent home with students or released following use (Larson and Olden 2008; Larson, *unpublished data*). Our results in part concur with these assessments, that teachers in Michigan do exhibit risky behavior regarding the acquisition and disposal of crayfish. Despite the survey's inability to cover a representative data set for the entire state, the results indicate that communication with teachers regarding relevant regulations and best practices of disposal and euthanasia of live animals could be improved. This data set should be built upon with more surveys of teachers' behaviors related to acquisition and disposal of crayfish, but in the meantime can serve as an initial guide in the allocation of management outreach efforts. We also do not know the level of compliance/noncompliance in biological supply companies that provide crayfish to schools. We attempted to contact known biological supply companies to inquire about crayfish use and distribution, but no company responded. Even if biological supply companies comply with requests to cease shipments of *P. clarkii* to the state, and substitute a native species such as *Faxonius virilis, F. immunis* or *P. acutus,* there still exists a risk related to the accidental mixing of species in shipments if facilities are not properly managed. Although this study did not investigate the likelihood of pet crayfish release into the wild, the release of non-native invasive crayfish by hobbyists has been documented as a vector for introduction in other studies (Lodge et al. 2000; Peters and Lodge 2011; Chucholl 2013; Loureiro et al. 2015; DiStefeno et al. 2016). Regardless of the actual likelihood of introduction through classroom releases, *P. clarkii* females have been observed carrying as many as 701 eggs in recently discovered Michigan populations (Smith, personal observation, unpublished data). Their high fecundity means that only a few individuals or one gravid female could initiate an invasion in a wetland or waterbody. Further, proper disposal is key; crayfish flushed down toilets or disposed in the trash can potentially survive in the sewer and spread from there (Indiana Biological Survey 2008). If someone does possess live *P. clarkii*, we recommend that specimens are humanely euthanized before disposing of them in order to prevent further introductions.

Although this study focused on *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, Faxonius limosus, F. rusticus,* and *F. virilis*. The pet trade leaves room for any number of the world's 669 crayfish species to become a threat to Michigan's waters (Crandall and De Grave 2017). It would be reasonable to assume, however, that *P. clarkii* is the most likely crayfish to become invasive in Michigan based on the large quantities observed in the food trade within Michigan's urban centers and the ongoing invasion in the Novi, Farmington Hills, and Sunset Lake areas. It remains unclear how the *P. clarkii* discovered in southern Michigan in 2017 arrived in the state. The lack of connection between several of the invaded systems suggests that there were multiple introduction events, potentially from unique sources. Genetic analyses are planned to assess relatedness of the new populations in Michigan and populations from potential sources to aid in determining the sources of the 2017 invasions. Every known population of *P. clarkii* in Michigan has been found well within the expected distances traveled by anglers with live bait, or within the same county as aquarium shops and schools reporting the use of crayfish. These uses support the assumptions about how a species might spread (Drake and Mandrak 2010; Drake et al. 2015). It is unlikely that *P. clarkii* invaded from established populations in Ohio given non-detects in recent intensive and extensive stream surveys between Sandusky Bay and the invasion centers (Smith 2016). We note that *P. clarkii* has shown westward expansion into the adjacent Portage watershed, outside of Sandusky Bay. This shows that *P. clarkii* is capable of expanding its range across watersheds, however, Smith (2016) did not detect *P. clarkii* between the currently infested areas of Southeast Michigan and the known range in Ohio. Methods used by Smith (2016) reported a 67% probability of detection for *P. acutus*, a native species with similar life history to that of *P. clarkii,* when dipnetting. The survey of the Sandusky Bay region also shows that where *P. clarkii* have been detected historically they have remained in abundance, and may be displacing other species.

In order to prevent potential damage to Michigan's wetland and aquatic ecosystems we suggest prohibition on the importation and possession of all crayfish in order to curtail any further potentially invasive species entering the state. Although the MDNR's memorandum made the possession of live *P. clarkii* illegal, there were still live food and pet markets that sold live *P. clarkii,* including several new shops. Studies have concluded that increased education and outreach, organized by and framed in terms relevant to key stakeholder groups, can be an effective strategy for increasing compliance and awareness of non-native invasive species (Diaz et al. 2012; Olden and Tamayo 2014; Oele et al. 2015; Seekamp et al. 2016). A directed effort is required to enforce existing laws regarding the sale and possession of *P. clarkii* in the introduction pathways we evaluated, especially for food markets and biological supply companies. Prevention efforts targeted at increasing awareness opportunities for the public and policy makers in ways that engage those involved with organisms in trade pathways (e.g., live food markets, pet store, bait shops), using language that appeals to their concerns, can be effective (Larson et al. 2011). Considering the effects that *P. clarkii* have had on crayfish populations and ecosystem health in other regions, we recommend a thorough investigation and implementation of management strategies to prevent the spread or potentially eradicate existing populations of *P. clarkii* in the state.

Despite this study's focus on the Lower Peninsula of Michigan, the information and suggestions from this study are applicable to other states, nations, and regions. Our findings suggest the invasion of *P. clarkii* into Michigan could have resulted from several pathways of introduction. Each of these pathways present in other areas, and have acted as initial gateways for invasion for other species in other regions of the globe (Hobbs et al 1989; Peters and Lodge 2009; Lodge et al 2012; Chucholl 2013). Peters and Lodge (2009) pointed to weak links and loopholes within policy between nations and states/provinces as a means by which non-native invasive species can find themselves far away from their native habitats. Experience in Michigan points to the need for pro-active and inclusive legislation and outreach to effectively manage vectors of introduction before a crisis point is reached. In Michigan the state regulator was unable to manage vectors of introduction other than the bait trade until there was evidence that *P. clarkii* was already being introduced to the state. We encourage agencies to proactively create policy that would restrict or prohibit the introduction of potentially invasive species, and to build better programs that communicate the risks of non-native invasive species to its citizens. These policy and communication efforts should stress that moving species to habitats where they are not native can pose significant ecological threats to native species. Neighboring management bodies should also be made aware of any ongoing ecological invasions that are occurring, as to be properly informed about potential risks and make proactive management decisions in preparation for potential invasion. We note that the closest populations of *P. clarkii* relative to political boundaries outside Michigan are ~30km from Ontario, CAN, and ~40km from Indiana, USA.

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Table 1. Summary table detailing crayfish availability by shop type during covert inspections, and whether crayfish available in each store included *P. clarkii*.

Shop Type	No Crayfish	Sold Crayfish	Sold *P. clarkii*	Total (%)	
Live Food	72	8	8	80	(64)
Pet	12	13	13	25	(20)
Tackle	17	3	0	20	(16)
				125	(100)

Table 2. Changes in behavior associated with the sale of crayfish detailed by shop type between 2014 and after prohibition of live possession in April of 2015.

Shop Type	Sold both years	Stopped selling in 2015	Began selling in 2015	Never sold	Total
Live Food	4	0	3	36	43
Pet	6	0	1	3	10
Tackle	4	0	1	2	7
					60

Table 3. Michigan teacher survey of crayfish use; response by county.

County	Crayfish Use: No	Crayfish Use: Yes	Neither Risky Acquisition or Disposal	Risky Acquisition	Risky Disposal	Both Risky Acquisition and Disposal	Total Surveys
Allegan	2	0					2
Barry	1	0					1
Bay	6	0					6
Berrien	2	0					2
Branch	2	0					2
Calhoun	2	0					2
Charlevoix	1	0					1
Chippewa	1	0					1
Clinton	2	0					2
Eaton	1	1			1		2
Genesee	5	2		1		1	7
Gratiot	2	1		1			3
Hillsdale	1	0					1
Huron	4	0					4
Ingham	7	0					7
Ionia	2	0					2
Isabella	2	0					2
Jackson	6	0					6
Kalamazoo	7	0					7
Kalkaska	1	0					1
Kent	7	2		1	1		9
Lake	1	0					1
Lapeer	1	0					1
Lenawee	3	0					3
Livingston	1	1		1			2
Macomb	7	0					7
Manistee	1	0					1
Marquette	1	0					1
Mecosta	3	1			1		4
Midland	2	1	1				3
Monroe	2	0					2
Montcalm	1	0					1
Newaygo	2	0					2
Oakland	8	1	1				9
Oceana	1	0					1
Osceola	1	0					1
Oscoda	1	0					1
Ottawa	2	1	1				3
Saginaw	4	0					4
Shiawassee	3	0					3
Tuscola	1	0					1
Van Buren	3	0					3
Washtenaw	6	1	1				7
Wayne	19	6		4		1	25
Wexford	1	0					1
Total	139	18	4	8	3	2	157

Table 4. Number of responses concerning acquisition and disposal reported in the Michigan teachers survey of crayfish use. Some respondents reported multiple methods of acquisition and/or disposal. In the event that more than one response was listed, the most 'risky' response was considered for analysis

Acquisition Responses	Total
Biological Supply Company	5
Pet Store	3
Zoo, Nature Center, or Aquarium	0
Collected from the wild (by yourself or students)	8
Collected from the wild (by someone else)	2
Other	1
Disposal Responses	
They are returned to supplier	0
They are given away to students	1
They are given to another teacher	0
They are donated to a university, museum, or aquarium	1
They are kept in the classroom as pets until they die naturally	9
They are released into the wild	6
They are flushed down toilets	0
They are euthanized	2
They are disposed of in trash containers	1
They are eaten	2
Other	0

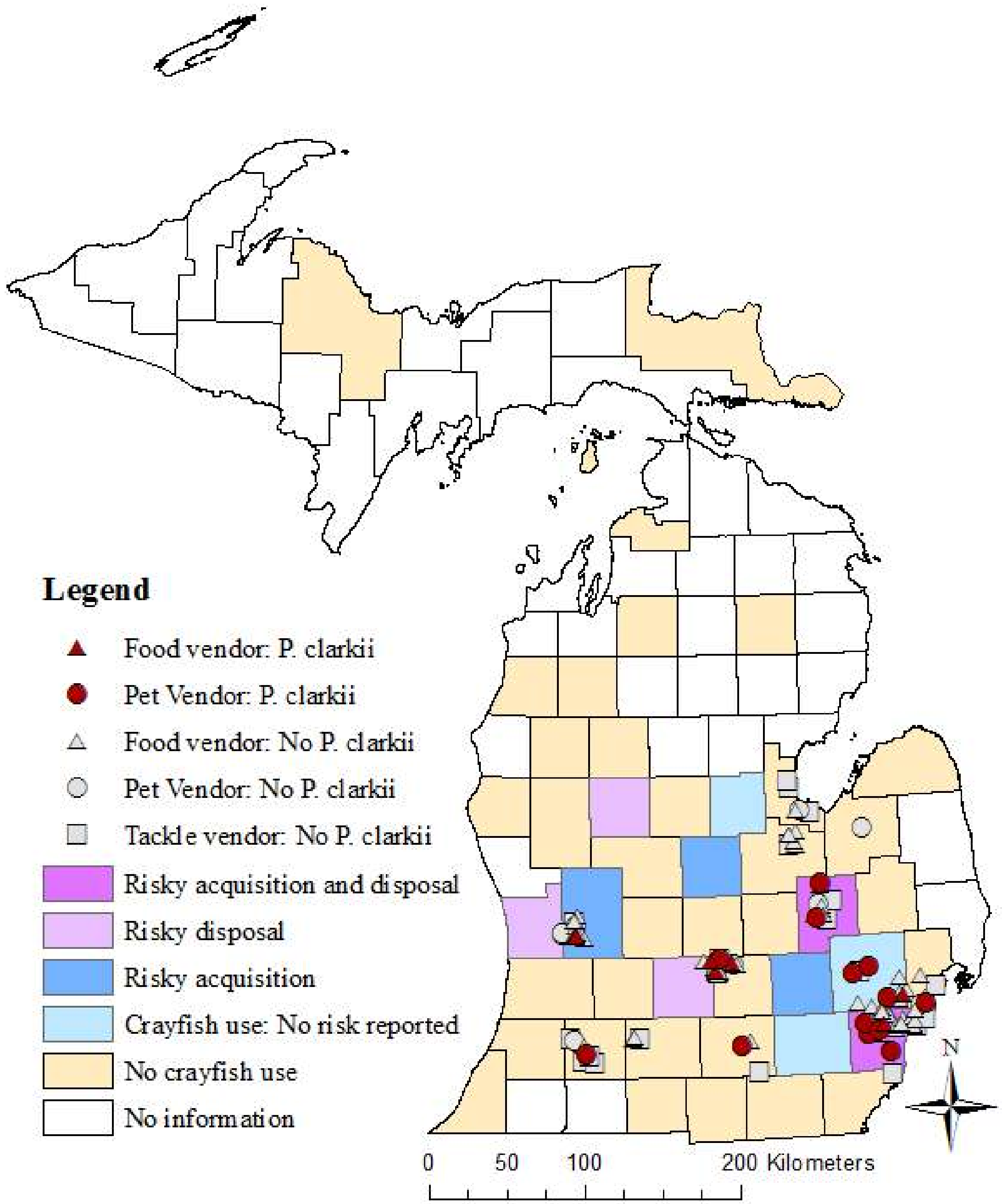


Figure 1. Map of counties surveyed for crayfish use in Michigan classrooms. The shading indicates the highest reported form of risk documented by a county. Red symbols indicate shops where live *P. clarkii* were sold, and gray symbols indicate surveyed shops that did not sell *P. clarkii*.

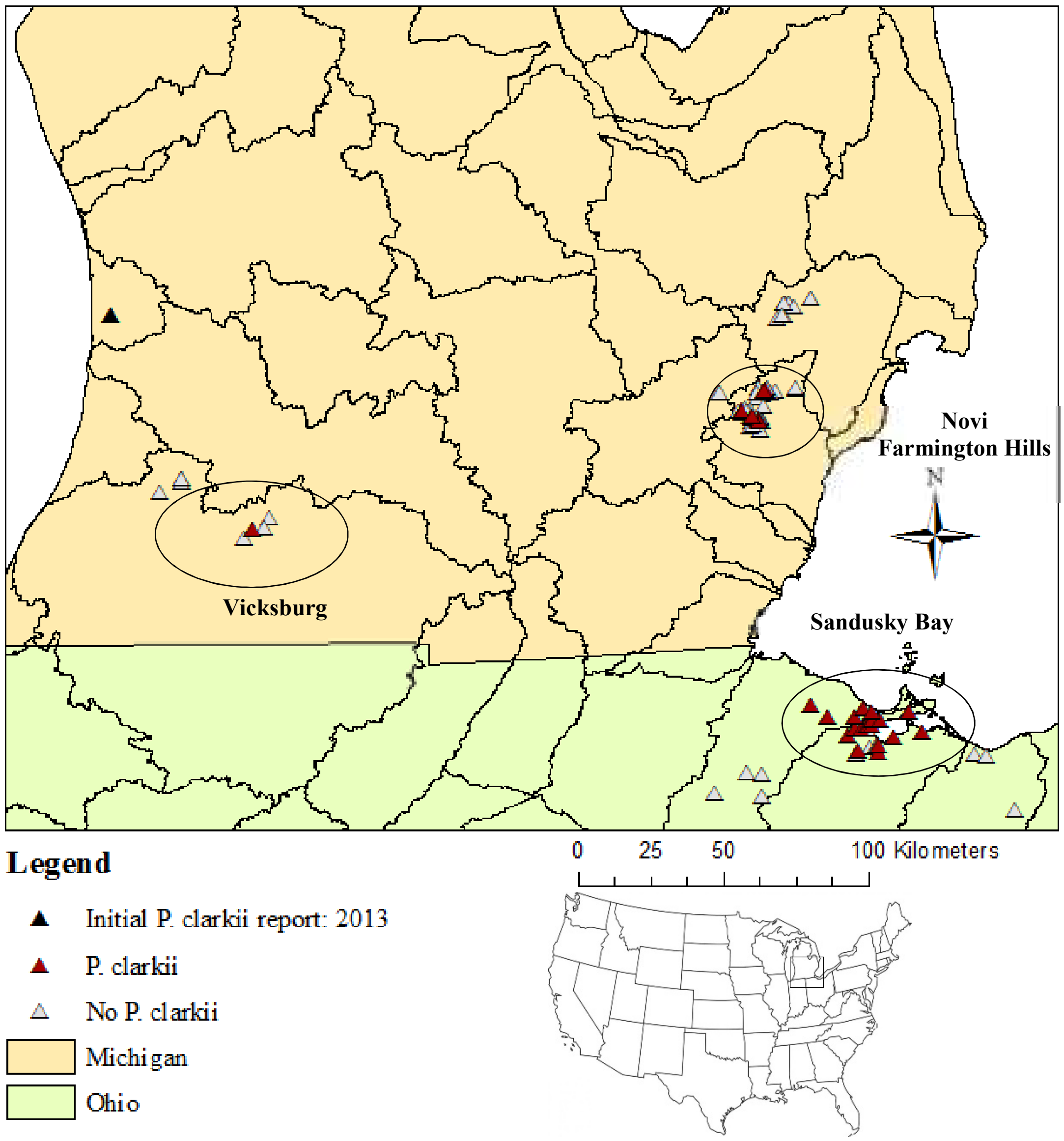
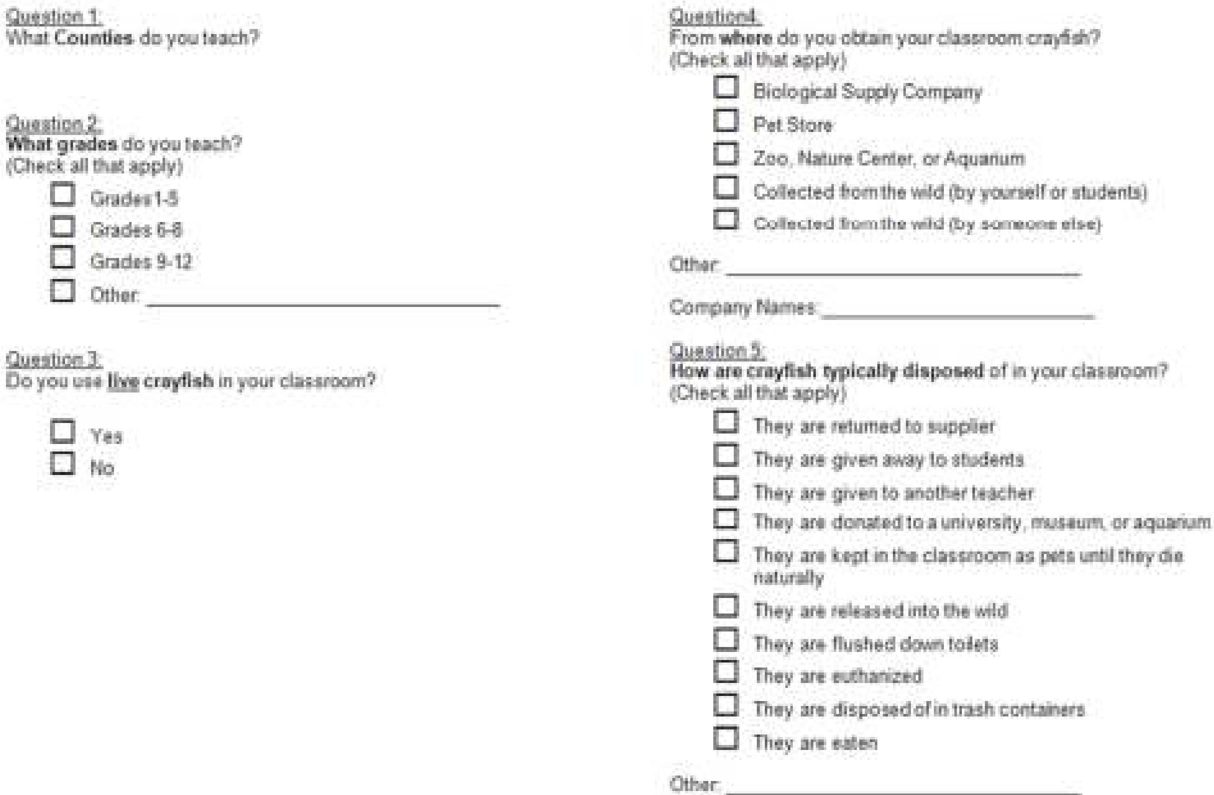


Figure 2. Field sampling sites within HUC-8 watersheds around Sandusky Bay, Ohio, Vicksburg, Michigan, and Novi/Farmington Hills, Michigan. Red symbols are sites where *P. clarkii* was detected, gray symbols are sampled areas where *P. clarkii* was not detected. The initial sighting of *P. clarkii* is marked with a black symbol; no live specimens were found there in subsequent visits and no further public reports have come in.

Appendix 1



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

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

- ☐ Grades 1-5
- ☐ 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 S1. The survey instrument for collecting data on crayfish use in Michigan classrooms.