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*.

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

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 (Table 4). 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

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

	Crayfish Use						
County	No	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

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, Faxonius 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 5 km 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 5 km 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

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

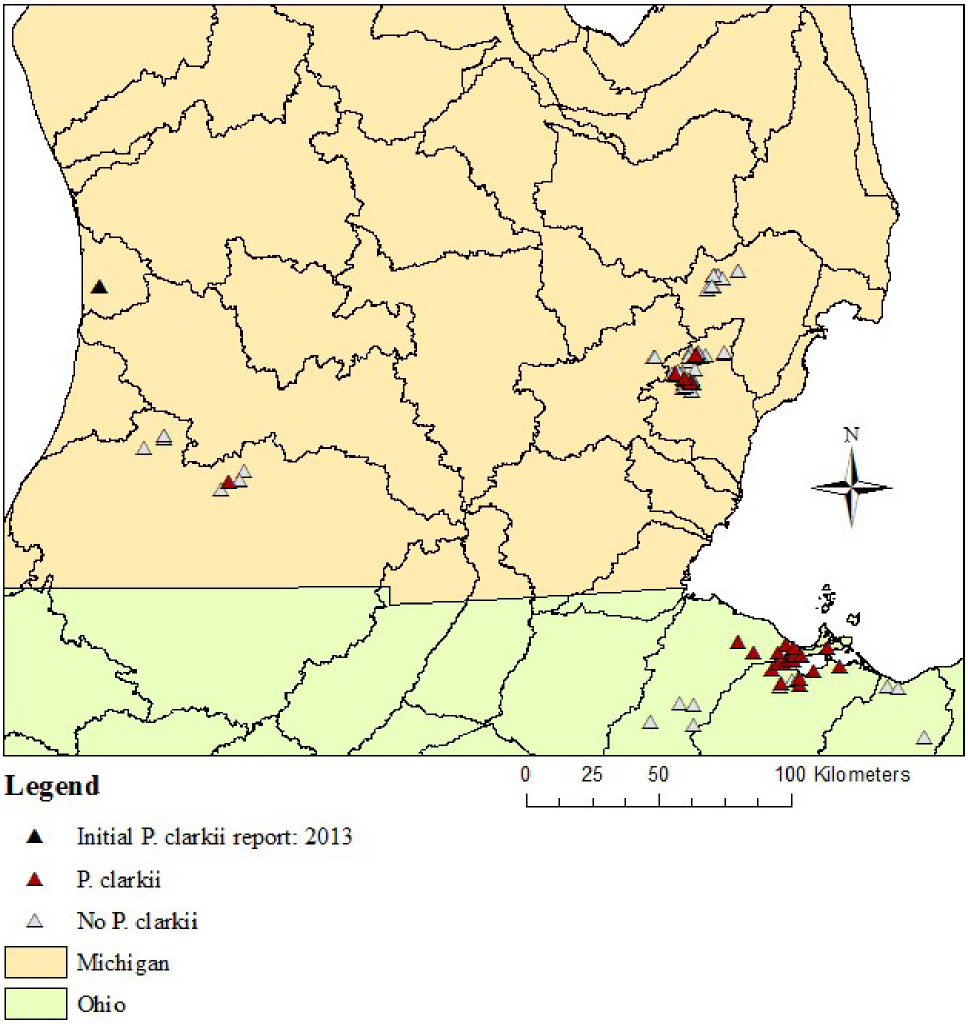

Figure 2. Field sampling sites within HUC-8 watersheds around Sandusky Bay, Ohio, Vicksburg, Michigan, and Novi/Farmington Hills, Michigan. Red symbols are sites where *P. clarkii* was detected, gray symbols are sampled areas where *P. clarkii* was not detected. The initial sighting of *P. clarkii* is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

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 2009). 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 2009; 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 proactive 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 ~ 30 km from Ontario, CAN, and ~ 40 km from Indiana, USA.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. The survey instrument for collecting data on crayfish use in Michigan classrooms.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2018/Supplements/MBI_2018_Smith_etal_Figure_S1.pdf