

A SOCIAL AND BIOLOGICAL EVALUATION OF NEW ZEALAND MUDSNAIL
INVASION IN MICHIGAN RIVERS

By

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ABSTRACT

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New Zealand mudsnails (*Potamopyrgus antipodarum*; NZMS) are a small gastropod native to New Zealand and a documented worldwide invader. Michigan rivers are in the early phase of invasion, as NZMS were first detected in 2015 in the Pere Marquette River. In the early invasion process, information on likely vectors and the distribution of the species are critical to developing a well-informed management plan. My primary goals for this thesis were to address these informational needs. In order to document the distribution of NZMS, I developed a sampling methodology and evaluated its effectiveness, as standard methods for lotic sampling NZMS have not been developed in the literature. I conducted a total of 227 surveys in 12 Michigan rivers between 2015 and 2018. Survey data were analyzed using an occupancy model, resulting in a per survey detectability exceeding 96%. NZMS were detected in 5 Michigan rivers: the Pere Marquette, Boardman, Manistee, Au Sable, and Pine rivers. I estimate that more than 65 river kilometers within the Pere Marquette, Boardman, and Pine rivers are infested. The distribution of NZMS suggests discrete transport events, and that the introduction into Michigan likely occurred well before the first reported detection of this species. The literature indicates that the wading gear of anglers is the main vector of transport within and between rivers. I surveyed 308 anglers fishing the Pere Marquette River in 2016-2017. Even though 52% of anglers were aware of NZMS, there was not a significant difference in wader cleaning behaviors between anglers aware or unaware of NZMS, indicating that outreach efforts need to focus beyond the awareness stage. As no in-stream method of treatment is presently available, angler engagement should be a focus of future management efforts.

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I'd like to end with a note to my son and future children. Let this be proof that your age and gender don't define you. You can do anything you set your mind to with hard work and kindness. Don't let others tell you what you're capable of and instead find that out for yourself. Education is important but so are life's experiences. I hope the work I have done to better myself will also ensure you have the life you deserve and every opportunity your heart desires.

TABLE OF CONTENTS

LIST OF TABLES	ix
LIST OF FIGURES	x
CHAPTER 1: EVALUATION OF A QUALITATIVE SURVEY FOR EARLY DETECTION MONITORING OF NEW ZEALAND MUDSNAIL	
INTRODUCTION	1
METHODS	4
Study Sites	4
Qualitative Survey Protocol	7
Analysis	9
RESULTS	12
Detection Probability	12
Time to First Detection	15
Survey Modifications	17
Decision Support Tool	19
DISCUSSION	20
Survey Design Considerations	23
Decision Support Tool	25
Survey Modifications	27
Future Research	28
APPENDICES	30
APPENDIX A: Qualitative survey data sheet	31
APPENDIX B: R code for the occupancy models	32
WORKS CITED	33
CHAPTER 2: DISTRIBUTION OF NEW ZEALAND MUDSNAILS (POTAMOPYRGUS ANTIPODARUM) IN MICHIGAN RIVERS FROM 2015 TO 2018	
INTRODUCTION	37
METHODS	40
Study Sites	40
Qualitative Survey Implementation	41
Analysis	42
RESULTS	42
New Zealand Mudsnail Distribution Across Michigan	42
New Zealand Mudsnail Distribution within the Pere Marquette River	51
New Zealand Mudsnail Distribution within the Boardman River	52
New Zealand Mudsnail Distribution within the Manistee River	54
DISCUSSION	56
WORKS CITED	60

CHAPTER 3: THE RELATIONSHIP BETWEEN ANGLER AWARENESS OF NEW ZEALAND MUDSNAIL INVASION AND ACTION TAKEN TO PREVENT SPREAD	64
INTRODUCTION.....	64
METHODS.....	71
Study Sites	71
Angler Survey	72
Analysis	74
RESULTS.....	76
DISCUSSION	87
Conclusion	95
APPENDICES.....	97
APPENDIX A: IRB exempt form	98
APPENDIX B: Anger survey response sheet.	99
APPENDIX C: Decontamination effectiveness table	100
WORKS CITED.....	103

LIST OF TABLES

Table 1. Single searcher detection probabilities for New Zealand mudsnails calculated with the inclusion and exclusion (represented by an occupancy of 1) of non-detection sites for the three levels of relative abundance and the overall single search detection probability.....	13
Table 2. The time until first detection of New Zealand mudsnails summarized across three relative levels of abundance.....	16
Table 3. Detection probability for New Zealand mudsnails given different survey lengths of 5, 10, 15, and 20 minutes.....	18
Table 4. Decision support tool to assist balancing limiting resources across a gradient of detection probabilities. The probability of failing to detect at a system level is a function of site level detectability, true occupancy within a system, and the number of sites surveyed. For example, the probability of failing to detect New Zealand mudsnails within a defined system that has 5% true occupancy is 0.603 (approximately 60%) when 20 sites within a system are surveyed with a 0.5 site level detection probability.....	20
Table 5. Sampling intensity and frequency of detection of NZMS in a broad-scale survey of Michigan rivers, 2015 and 2018. Some access points were surveyed more than once during a single year, and as such the number of survey locations is not equal to the number of survey events.....	43
Table 6. Summary of river kilometers infested with NZMS in the Pere Marquette, Boardman, and Manistee rivers. Results from the Au Sable River were not included as sampling was much more limited and sampling was taken over by other investigators after 2016. Surveys kilometers represent the distance between the most upstream and downstream points of detection and assume a continuous distribution of NZMS between survey points. Total river kilometers infested is based on the maximum distance for each river.....	45
Table 7. Five wader types were determined prior to the survey; each angler surveyed was evaluated for wader type.....	74
Table 8. Qualitative survey responses were coded for quantitative analysis.....	75
Table 9. Decontamination methods used in the angler survey and their effectiveness at inducing NZMS mortality.....	100

LIST OF FIGURES

Figure 1. Locations and results of the NZMS qualitative surveys, 2017.....	6
Figure 2. Qualitative surveys involved two to four searchers independently surveying up to a 50-meter stretch of river without overlapping when possible.....	8
Figure 3. Single searcher detection probabilities of New Zealand mudsnails by relative abundance with the inclusion and exclusion of non-detection sites of NZMS including in each level of relative abundance.....	13
Figure 4. Single and paired searcher detection probability of New Zealand mudsnails by abundance comparing the inclusion and exclusion of non-detections sites. In low abundance sites, 1-10 NZMS were detected; medium abundance sites, 11-100 NZMS were detected; high abundance sites, >100 NZMS were detected by each searcher.....	15
Figure 5. The time until first detection of New Zealand mudsnails summarized across three relative levels of abundance and compared across all levels of abundance. In low abundance sites, 1-10 NZMS were detected; medium abundance sites, 11-100 NZMS were detected; high abundance sites, >100 NZMS were detected by each searcher.....	16
Figure 6. Cumulative proportion of the time until first detection of New Zealand mudsnails calculated at three levels of relative abundance.....	17
Figure 7. Overall detection probability for New Zealand mudsnails given different survey lengths of 5, 10, 15, and 20 minutes.....	18
Figure 8. Conceptual model of the invasion process (from Heger and Trepl 2003).....	39
Figure 9. Location and results of NZMS qualitative surveys, 2015.....	47
Figure 10. Location and results of NZMS qualitative surveys, 2016.....	48
Figure 11. Location and results of NZMS qualitative surveys, 2017.....	49
Figure 12. Location and results of NZMS qualitative surveys, 2018.....	50
Figure 13. New Zealand mudsnail distribution and qualitative abundance in the Pere Marquette River between 2015 and 2018. Qualitative density levels include none (0), low (1-10), medium (11-100), and high (100+) found during a 20-minute timed search. Based on surveys conducted by Michigan State University.....	52
Figure 14. NZMS distribution and qualitative abundance in the Boardman River between 2016 and 2018. Possible density levels include none (0), low (1-10), medium (11-100), and high (100+). Based on surveys conducted by Michigan State University.....	54
Figure 15. New Zealand mudsnail distribution and qualitative abundance in the Manistee River between 2016 and 2018. Qualitative density levels are none (0), low (1-10), medium (11-100), and high (100+).....	55

Figure 16. Distribution of the New Zealand Mudsail in North America. Credit: USGS 2018...	65
Figure 17. NZMS attached to trash pulled out of the Pere Marquette River in Michigan. Photo credit: William Keiper.....	66
Figure 18. NZMS have been detected in 5 Michigan rivers including the Pere Marquette, Au Sable, Boardman, Manistee, and Pine Rivers. Red dots indicated points of detection as found by a series of detection surveys conducted by Michigan State University (MSU) and the Michigan Department of Environmental Quality (MDEQ; now the Michigan Department of Environment, Great Lakes, and Energy). Locations of detection on the Pine River are not indicated on this map as they were not confirmed by MSU/MDEQ surveys.....	70
Figure 19. Map of the survey range on the Pere Marquette. Red indicates extent of survey locations.....	72
Figure 20. Home states of anglers surveyed, outside of Michigan. Of the 308 anglers surveyed, 240 were from Michigan. Numbers in parentheses indicate number of respondents from the given state.....	77
Figure 21. Distance traveled by anglers to reach the Pere Marquette from their home.....	78
Figure 22. Distribution of usage of different wader types among anglers surveyed on the Pere Marquette River.....	79
Figure 23. Rivers traveled within 2 days pre and post fishing in the Pere Marquette.....	80
Figure 24. Map of western states that surveyed anglers have fished at some point prior to fishing the Pere Marquette River. Anglers were asked if they had ever fished in the western United States. Of the nine western states anglers reported traveling to, eight have known established populations of NZMS.....	81
Figure 25. The source where anglers first learned of NZMS invasion in Michigan.....	82
Figure 26. Wader decontamination methods of anglers.....	83
Figure 27. No significant difference was found between the awareness of the NZMS infestation and action taken to decontaminate waders (Fisher's Exact Test, $P=0.30$).....	84
Figure 28. Anglers who are aware of the NZMS infestation in the Pere Marquette River exhibit behaviors with varying levels of risk. The green boxes are the least risky behavior and should result in the lowest likelihood of NZMS spread. The red boxes are the riskiest behaviors and are most likely to lead to NZMS spread. The yellow box represents a moderate level of risk.....	86
Figure 29. Anglers who are unaware of the NZMS infestation in the Pere Marquette River exhibit behaviors with varying levels of risk. The green boxes are the least risky behavior and should result in the lowest likelihood of NZMS spread. The red boxes are the riskiest behaviors and are most likely to lead to NZMS spread. The yellow boxes represent a moderate level of risk.....	87

Figure 30. There are multiple steps that must occur for awareness of an issue to turn into effective the prevention of new infestations. Engagement is required to inform initial awareness and action for.....89

CHAPTER 1: EVALUATION OF A QUALITATIVE SURVEY FOR EARLY DETECTION MONITORING OF NEW ZEALAND MUDSNAIL

INTRODUCTION

Expanding globalization has come with ecological risks. The threat of non-native species introduction has increased as a result of frequent travel of humans and manufactured goods, with new invasion pathways being continually created (Hulme 2009; Meyerson and Mooney 2007). The economic, environmental, and social costs of controlling invasive species, while difficult to calculate, are estimated at hundreds of billions of dollars annually (Lovell et al. 2006; Pimentel et al. 2005). Effective management tools are critical for reducing costs of fighting the ongoing worldwide battle against invasive species. Natural resource managers are finding they must dedicate more time than ever to the management of invasive species. Management efforts must be planned for decades or even hundreds of years as established invasive populations are rarely able to be eradicated with the time and resources available (Keller et al. 2007). Invasive species management is a dynamic, time sensitive, and complex task with many aspects of the invasion process (the steps that must occur between introduction of a potential invader through establishment and spread) that must be taken into consideration. Preventing the introduction of an invasive species is the most cost-effective approach for managing the threat of new invasions (Ruesink et al. 1995), but prevention is not always feasible, so early detection and effective control is recommended as a cost-effective strategy to reduce the likelihood of widespread establishment.

A critical first step in managing any potential invasion is identifying the organism's presence in a system (Mehta et al. 2007). However, a species is generally rare in the early stages of invasion and may only occur at a limited number of locations, making detection of the target

species challenging. As such, it is important to develop methods for early detection that are cost and time effective. In this study, characteristics of optimal early detection monitoring strategies were defined as yielding immediate or rapid detection results, high detection probability per sampling effort, cost conscious and efficient, and practical to implement. These characteristics should be considered in the development and analysis of targeted surveys.

Developing early detection surveys also requires knowledge of the target species' biology and invasion history. The New Zealand mudsnail (*Potamopyrgus antipodarum*; hereafter NZMS) is a gastropod native to New Zealand with established invasive populations found throughout the world (Bowler 1991). The NZMS is small (approximately 4-6 mm when fully mature in Michigan rivers) and cryptic in appearance, making it easily overlooked by the untrained eye. However, when observed, they are readily distinguished from most native snails that occupy overlapping habitats. NZMS tend to be patchy in their distribution and occupy a variety of substrates in shallow, low flow areas. The species is parthenogenic in many areas of their introduced range, with self-cloning females making up entire populations (Wallace 1992). The ability to reproduce asexually leads to large population increases, as females can reproduce spring through fall, brooding up to 70 young per reproductive event (McKenzie et al. 2012). Their ability to reproduce through parthenogenesis increases the likelihood of establishment after being introduced to novel environments because a single individual can result in the proliferation of a new infestation.

NZMS invasions have resulted in several negative impacts, including competition with native macroinvertebrates, alteration of algal assemblages, and adverse impacts on fish health (Kerans et al. 2005, Bennett et al. 2015, Vinson and Baker 2008). In the western United States, NZMS have reached densities upwards of 500,000/m² (Hall et al. 2006). At these levels, NZMS

have the ability to change the ecological structure and function of their invaded ecosystems (Hall et al. 2006). The adverse impacts and the continued spread of NZMS across the US are of concern to natural resource managers and anglers alike. Currently no large-scale treatments are available for open-water environments to effectively control NZMS populations. Instead, current work is focused on documenting distribution (see Chapter 2 of this thesis) and developing education/outreach campaigns (see Chapter 3 of this thesis) to prevent the introduction and spread to other waters.

New Zealand mudsnail was first detected and reported serendipitously in the Pere Marquette River in 2015 through an incidental observation by a recreationist (Sarah LeSage, Michigan Department of Environment Great Lakes and Energy, personal communication). However, NZMS was detected in 2013 in the Boardman River as part of ongoing macroinvertebrate sampling conducted by a university but was not reported to resource agencies until 2016 (Au Sable Institute, unpublished data). Since the initial reported detection in The Pere Marquette River, NZMS has been detected in five Michigan's rivers: Pere Marquette (detected 2015), Au Sable (detected 2016), Boardman (detected in 2013, but not reported until 2016), Manistee (detected 2017), and Pine Rivers (detected 2017). All five rivers are classified as coldwater streams and are popular destinations among trout and salmon anglers, supporting thousands of angler days per year (Michigan Department of Natural Resources, 2019). Angler movements among multiple rivers over short time periods (i.e., hours or days) poses a risk of secondary spread from a NZMS establishment as well as a mode of long-distance dispersal (i.e. cross-continental). Transport risk is high because NZMS have the ability to attach to waders or other angling equipment and survive nearly two days on a dry surface (Alonso and Castro-Díez 2012; Alonso and Castro-Díez 2008; Oregon State University 2010). NZMS natural dispersal is

relative slow within rivers because of their limited mobility and are likely dispersed the greatest distances within streams via drift and transport in the gastrointestinal (GI) tract of fish (Bruce et al., 2009) that consume NZMS, but don't have the ability to digest the snails so they are expelled alive.

No standard qualitative or quantitative survey methods for early detection and monitoring of NZMS have emerged from the scientific literature. Previous studies on NZMS have most commonly used quantitative techniques (with the goal of determining population densities) such as D-framed kick-nets, Hess and Surber samplers with various mesh sizes, standard sized viewing buckets, and ponar dredges (Moffitt et al. 2011, Bennett et al. 2015, Levri et al. 2008, Vinson 2004, and Schreiber et al. 2003). The protocols for previous studies have generally followed those most commonly used for traditional macroinvertebrate sampling, where effectiveness (i.e., detection probability) for NZMS specific sampling has not been specifically evaluated.

The purpose of this study was to develop and evaluate a visual qualitative survey technique for the efficient detection of NZMS. I evaluated this method by determining the consequences of duration of search time, number of surveyors, and the number of sites surveyed on the detectability of NZMS. In brief, I found that this method provides a cost effective way to detect NZMS in Michigan rivers or rivers with similar characteristics

METHODS

Study Sites

This study focused on Michigan rivers with established NZMS populations and rivers that were considered to be at high risk of invasion based on relatively high angler activity, using professional judgement of fisheries biologists and available estimates of angler effort from creel surveys, and proximity to known infested rivers. The rivers surveyed were all within the Lake

Michigan drainage basin and included the Pere Marquette, Manistee, Boardman, Baldwin, White, Rogue, Pine, Muskegon, Little Manistee, Platte, and Betsie Rivers and Slagle Creek (Figure 1). The Pere Marquette, Betsie, Boardman, Rogue, White, Pine, and part of the Manistee Rivers are designated as Michigan Natural Rivers, which provides additional protections against development and use. The Pere Marquette, Manistee, and Pine Rivers have an additional federal designation as Natural Wild and Scenic Rivers. All surveyed rivers are located on the western half of Michigan's Lower Peninsula and are considered coldwater fisheries. The rivers cover a collection of habitat types but are all generally low gradient, tree lined streams.

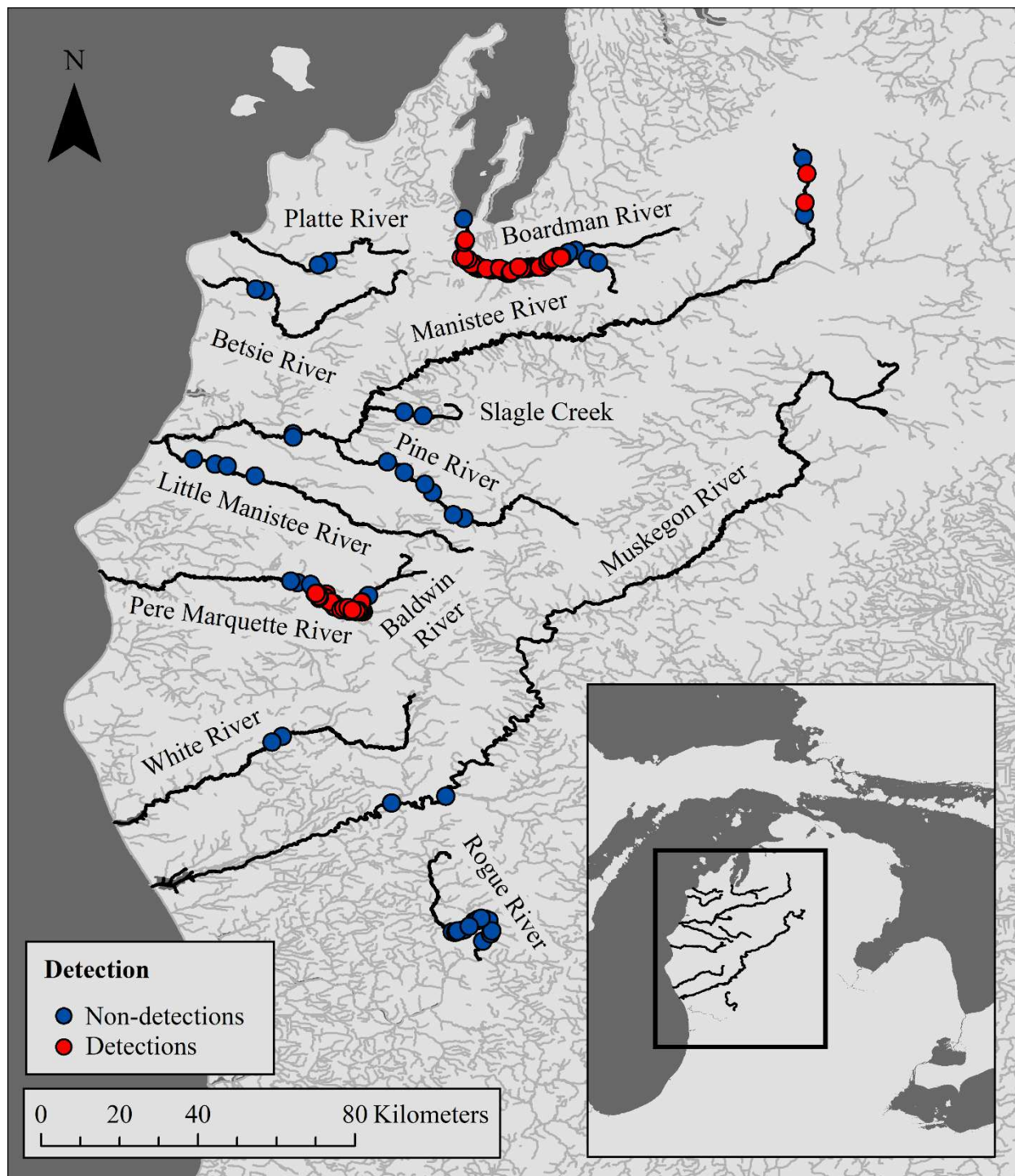


Figure 1. Locations and results of the NZMS qualitative surveys, 2017.

Qualitative Survey Protocol

Qualitative visual surveys were developed and implemented to detect NZMS. The survey methodology consisted of two to four individuals searching up to 50 meters of river (determined using a rangefinder) for 20 minutes (Figure 2). Each searcher would enter the river at a central access point and determine their approximate 50 meter search range. When two searchers were present, searchers would move in opposite directions from the central access point. When additional searchers were present, they would survey the opposite riverbank as the other two searchers. Each searcher would travel along the bank while wading in the river, examining all available substrates. This involved picking up vegetation, leaf packs, woody debris, etc. from the river's edge and visually inspecting for the presence of NZMS. Each searcher kept track of their own search time using a digital watch. The individual survey event ended when the 20-minute timer was up. In the event that the searcher reached the 50-meter extent of their search area prior the 20 minutes ending, they would move back towards the access point and continue searching the same area. This helped to standardize search effort between searchers.

To ensure searcher independence, communication regarding observed snails was prohibited between active searchers. The first 25 NZMS detected per searcher were collected and preserved in 95% ethanol, labeled, and stored to ensure correct and consistent identification among searchers. In a further attempt to standardize search effort, each searcher would pause their own 20-minute timer while collecting the first 25 NZMS observed, ensuring a total search time of 20 minutes was achieved. Surveys were generally completed in 30 minutes or less, allowing no more than 10 minutes to collect NZMS detected. The survey time when NZMS were first detected by each searcher was recorded along with a qualitative level of abundance (e.g., none [0 individuals detected], low abundance [1-10 detected], medium abundance [11-100 detected], high abundance [>100 detected]; Appendix A).

Surveys focused on stream margins where NZMS were most commonly detected in pilot qualitative surveys that occurred in 2015 and 2016. However, all observed available habitat types were surveyed to avoid any habitat type sampling bias, but particular attention was paid to woody debris and rooted submerged vegetation, as this was observed to be the preferred habitat of NZMS within the survey areas. Surveys were performed simultaneously with each searcher covering a different section of river without overlap. If overlap could not be avoided, searchers refrained from searching the same area at the same time.

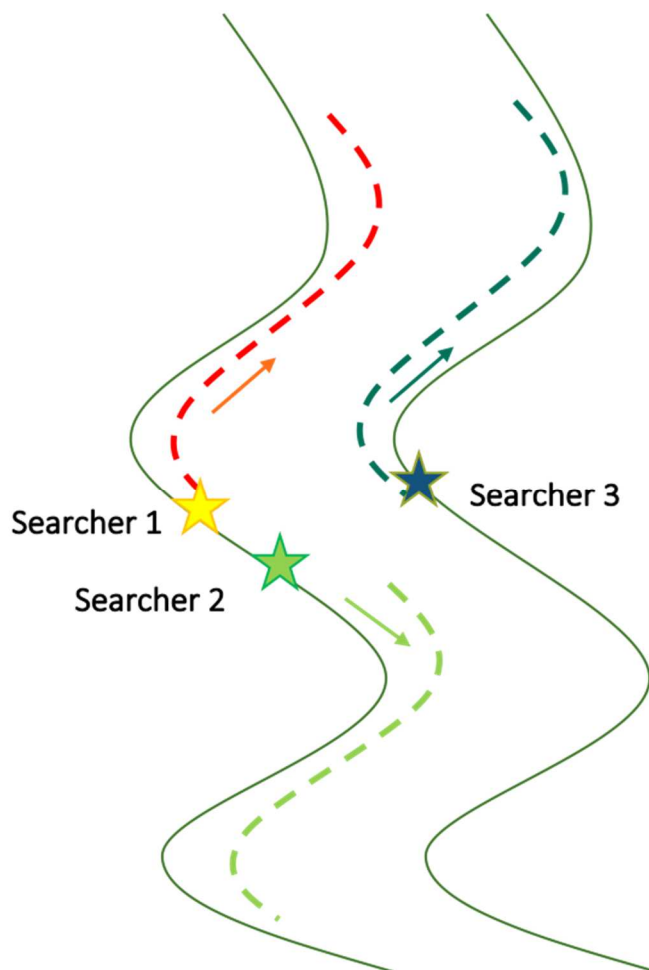


Figure 2. Qualitative surveys involved two to four searchers independently surveying up to a 50-meter stretch of river without overlapping when possible.

Analysis

I analyzed my summary data using an occupancy framework (e.g., MacKenzie et al. 2006), treating each observer as an independent sampling event. The following occupancy model allowed me to obtain estimates of site occupancy and detection probability (MacKenzie et al. 2002, 2006):

$$L(\psi, p) = \left(\psi^{n \cdot \Pi} p_t^{n_t} (1 - p_t)^{n - n_t} \right) \times (\psi \Pi (1 - p_t) + (1 - \psi))^{N - n}.$$

where t is the number of searchers at a site, N is the total number of sites surveyed, n is the number of sites where at least one detection occurred, ψ is the probability of occupancy, p is the detection probability for a single searcher, and n_t is the number of detections on t^{th} survey. In the analysis, the independent surveyors served as analogs to repeated samples over time in traditional occupancy analyses. I implemented this occupancy model and obtained estimates via the unmarked package in R (R Core Team 2018; Appendix B).

To implement this model, assumptions consistent with other applications of occupancy analysis were used (e.g., MacKenzie et al. 2006). The first assumption was that if NZMS were present, the organism was present through the entire search area at a given site. This assumption was necessary for the occupancy model and implies that if one searcher detected NZMS at a site and their paired searcher did not detect NZMS at the same site, the site was actually occupied, and the detection was imperfect. The second assumption was that the site was closed to NZMS; meaning no immigration or emigration during the survey. The third assumption was that the probability of detection was consistent across all sites within a river system. Finally, it was assumed that NZMS were identified accurately.

As in other typical applications of occupancy analysis, I assumed that detection probability was constant across sites, or was a function of observable covariates (e.g., water depth and turbidity; Bailey et al 2014). I was interested in how detection probability might vary

with the relative abundance, which is not a covariate that can be observed independently of the detection process. As such, I used a posteriori designation of relative abundance to approximate detection probability across categorical levels of abundance (i.e., low, medium, and high). At each site, I used the maximum level of relative abundance detected at a site as the true site abundance. Due to lack of guidance in the literature, I ran the occupancy model two ways: including and excluding sites where no NZMS were detected. One model included all of the non-detect sites, with the assumption that some of the non-detect sites were actually occupied at each of the various levels of abundance. The second model excluding the non-detect sites, treated the non-detects as uninformative relative to the question of how detection probability differs with relative abundance.

Additional iterations of the occupancy model were used to determine how the survey parameters, specifically total survey time, influenced detectability. Three additional model runs occurred to evaluate the influence of survey time. Those included, 1) with any detection made >15 minutes excluded, 2) with detections made >10 minutes excluded, and 3) with any detections made >5 minutes treated as non-detections,

Detectability was estimated with the occupancy model for each searcher working independently. However, the qualitative timed search protocols called for at least 2 searchers per site working independently. I estimated detectability for two searchers using the following equation:

$$p_d = 1 - (1 - p_s)^2$$

where, p_d is the probability of detection with two searchers and p_s is the probability of detection for a single searcher determined from the occupancy analysis.

I created a decision support table that allowed for adjustments of three model inputs (e.g., number of survey sites, probability of true occupancy [percentage of surveyed sites that are actually occupied], and detection probability). The following binomial distribution formula was used to build the decision support tool:

$$P(x) = \frac{k!}{x!(k-x)!} (\psi p)^x q^{(k-x)}$$

where k is the number of sites sampled, ψ is the probability of occupancy, p is the detection probability for a single searcher, q is the probability of failure (failing to detect, taking into account both the probability of detection and probability of occupancy), and x is the number of successes (detections). If we are calculating zero detects, then the formula becomes:

$$P(x) = \frac{k!}{0!(k-0)!} (\psi p)^0 q^k$$

which reduces to q^k OR $(1 - \psi p)^k$

Thus, to determine the required sample size (number of survey sites) for a given probability of detection $P(x)$, we get:

$$k = \frac{\ln(P(x))}{\ln(q)}$$

RESULTS

Detection Probability

In 2017, I conducted 227 surveys in 12 rivers with two to four searchers at each survey event for a total of 504 individual searcher observations. NZMS were detected in three of the rivers surveyed: Pere Marquette, Boardman, and Manistee rivers. There were 226 individual searcher detections from the three rivers totaling 180 unique sites where NZMS were detected. Some sites were sampled multiple times throughout the summer, particularly on the Pere Marquette as it was the focus of my other NZMS studies (see chapters 2 and 3 of this Thesis).

Using the full data set, the estimated detectability per independent searcher was 0.816 ± 0.027 , and estimated occupancy at 0.57 ± 0.034 . Or more simply stated, each searcher was estimated to detect NZMS 81% of the time when the species was present. This estimate indicates that approximately 57% of surveyed sites were likely occupied by NZMS. Based on this result, I estimated that the detectability for two searchers working independently was 0.966 using the above described equation: $p_d = 1 - (1 - p_s)^2$. Estimates of detectability increased as the a posteriori designation of relative abundance increased (Table 1; Figure 3).

Table 1. Single searcher detection probabilities for New Zealand mudsnails calculated with the inclusion and exclusion (represented by an occupancy of 1) of non-detection sites for the three levels of relative abundance and the overall single search detection probability.

Abundance	N	Occupancy	Detectability	
			Single Searcher $\pm$ SE	Two Searchers
Overall	512	0.57 ± 0.034	0.816 ± 0.0267	0.966
Low	306	0.404 ± 0.094	0.342 ± 0.0844	0.567
Low	74	1	0.554 ± 0.0578	0.801
Medium	281	0.19 ± 0.039	0.679 ± 0.0809	0.897
Medium	49	1	0.0735 ± 0.0631	0.930
High	389	0.429 ± 0.037	0.967 ± 0.0145	>0.999
High	157	1	0.968 ± 0.014	>0.999

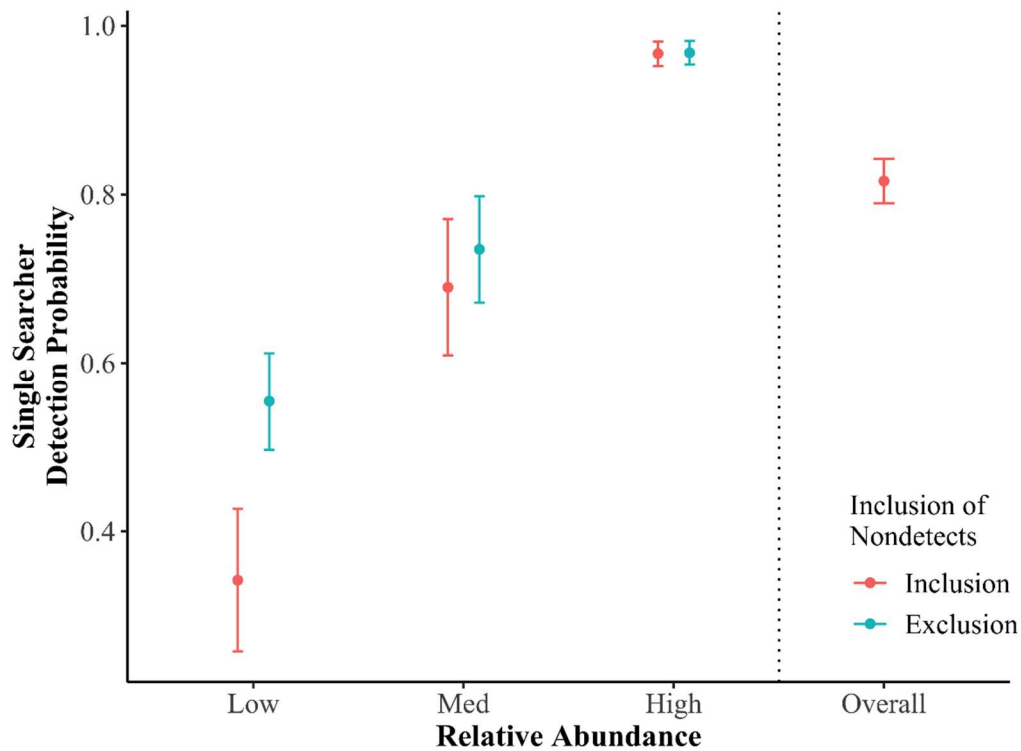


Figure 3. Single searcher detection probabilities of New Zealand mudsnails by relative abundance with the inclusion and exclusion of non-detection sites of NZMS including in each level of relative abundance.

Detection probability estimates were influenced by whether sites with no NZMS detections were included or excluded from the analysis, with the discrepancy decreasing as

relative abundance increased. When sites where NZMS were not detected by any searcher were included in the occupancy model, occupancy for sites with a low qualitative abundance (NZMS < 11; n=306) was estimated to be 0.404 ± 0.094 and detectability for one independent searcher was 0.342 ± 0.084 (Figure 3). In contrast, when I excluded sites with no detections (n=57 retained) meaning the true occupancy equaled 1 (i.e., 100% of sites were known to be occupied under model assumptions), the probability of detection for one searcher was 0.554 ± 0.058 with low qualitative abundance. When non-detect sites were included in the occupancy model for a medium qualitative level of abundance ($11 < \text{NZMS} < 100$; n=281), estimated occupancy was 0.19 ± 0.039 and the detectability for one independent searcher was 0.679 ± 0.081 . When only sites occupied at a medium abundance (n=36) were included, the probability of detection increased to 0.735 ± 0.063 . For sites with a high qualitative abundance of NZMS ($\text{NZMS} > 100$; n=389) and with sites with non-detection included in the model, occupancy and detectability were estimated at approximately 0.429 ± 0.037 and 0.967 ± 0.015 respectively. Finally, when only sites where NZMS were detected at high abundance were included in the occupancy model (n=133), the estimated detectability per searcher was 0.968 ± 0.014 .

Detection probability of two searchers working independently gives a detection probability of 0.966 when two individuals search independently at a site (Figure 4). Similarly, the inclusion of a second searcher always led to higher overall survey detectability with diminishing returns as you move from low to high relative abundance of NZMS, regardless of the inclusion or exclusion of non-detect survey data.

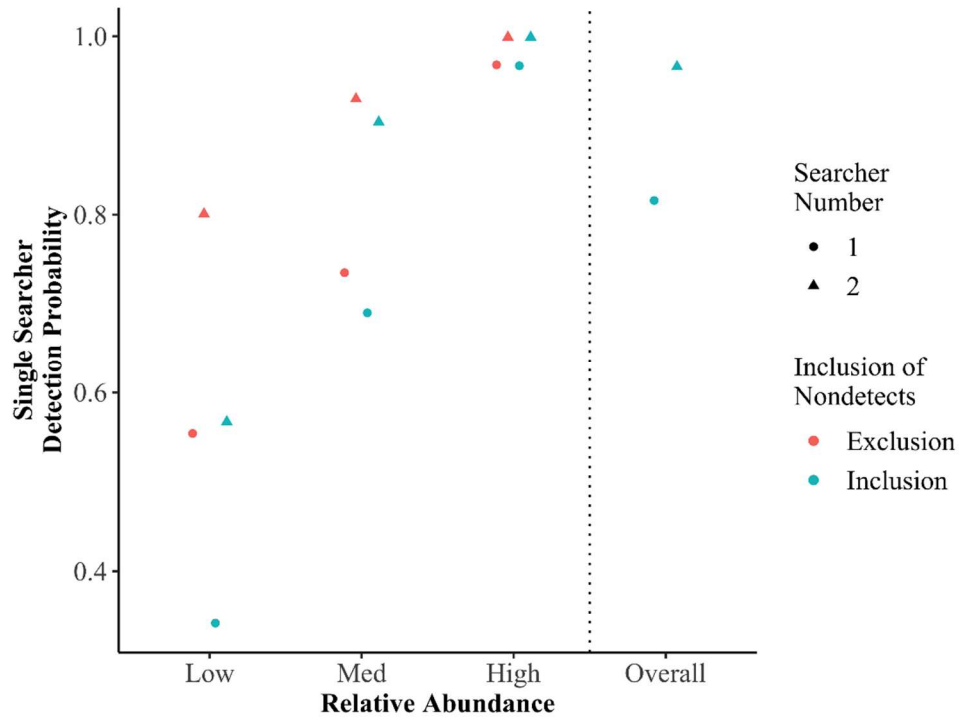


Figure 4. Single and paired searcher detection probability of New Zealand mudsnails by abundance comparing the inclusion and exclusion of non-detections sites. In low abundance sites, 1-10 NZMS were detected; medium abundance sites, 11-100 NZMS were detected; high abundance sites, >100 NZMS were detected by each searcher.

Time to First Detection

In the event that NZMS were detected during a timed search, the time until first detection of NZMS for each searcher ranged from 1 second to 19 minutes 43 seconds. The average time until first detection followed a general pattern of increased time with decreased abundance. The average time until first detection with high NZMS abundance was approximately 47 seconds ($0.787 \text{ minutes} \pm 0.153$; Figure 5; Table 2), followed by approximately 4 minutes and 11 seconds ($4.125 \text{ minutes} \pm 0.829$) for medium abundance, and 7 minutes and 44 seconds ($7.739 \text{ minutes} \pm 0.781$) for low abundance. At a high level of abundance, all detections were made within 11 minutes and 10 seconds from the start of the sampling; medium abundance detections made within 18 minutes; and low abundance detections made within 19 minutes and 43 seconds (Figure 6).

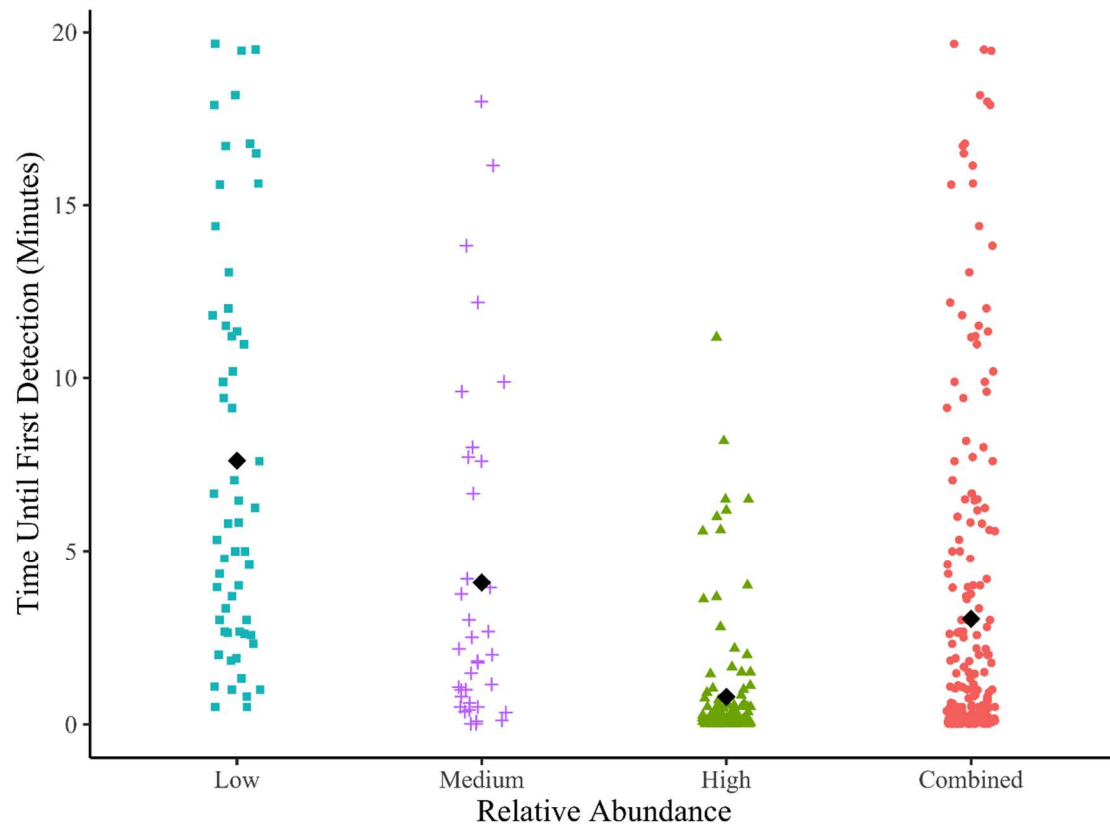


Figure 5. The time until first detection of New Zealand mudsnails summarized across three relative levels of abundance and compared across all levels of abundance. In low abundance sites, 1-10 NZMS were detected; medium abundance sites, 11-100 NZMS were detected; high abundance sites, >100 NZMS were detected by each searcher.

Table 2. The time until first detection of New Zealand mudsnails summarized across three relative levels of abundance.

Density	n	Time until First Detection of NZMS					
		Mean	Median	Variance	Standard Error	Minimum	Maximum
High	133	0.78	0.15	3.11	0.15	0.016	11.16
Medium	36	4.19	2	24.02	0.82	0.016	18
Low	57	7.73	5.81	34.14	0.78	0.5	19.66

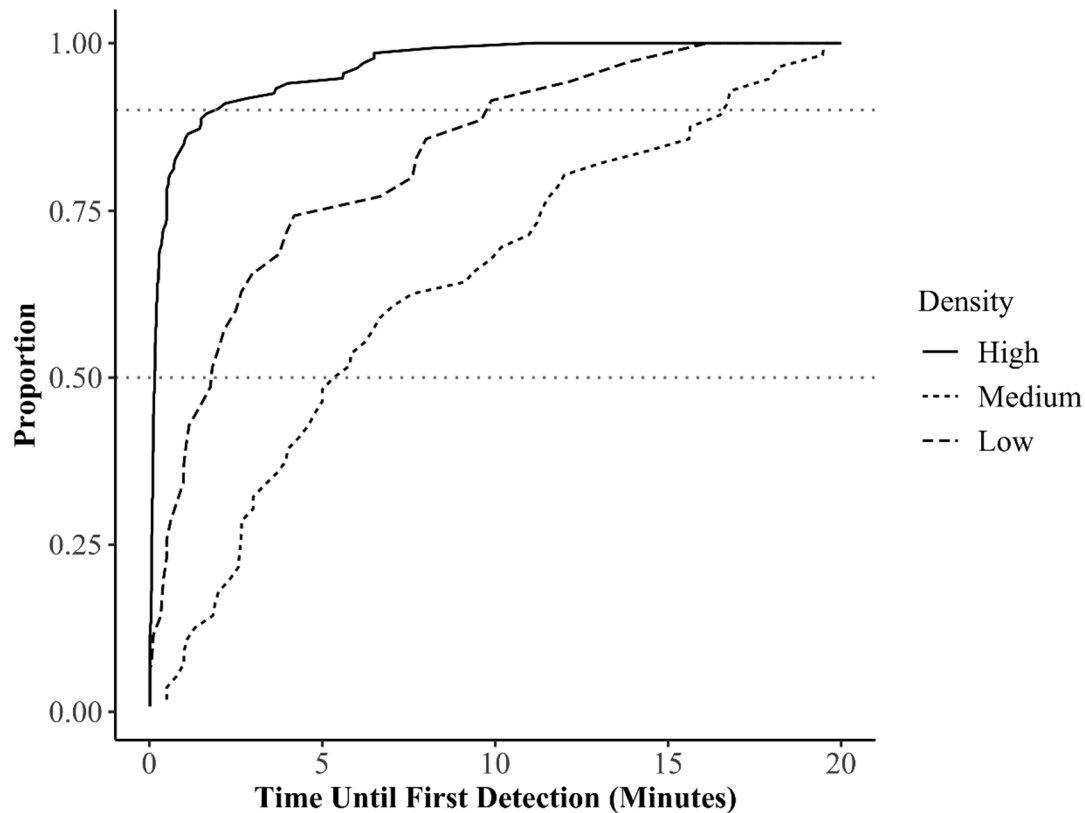


Figure 6. Cumulative proportion of the time until first detection of New Zealand mudsnails calculated at three levels of relative abundance.

Survey Modifications

The overall detection probability (not taking NZMS abundance into consideration) was reduced to 0.789 ± 0.028 , if the survey length was reduced from 20 minutes to 15 minutes. This resulted in the loss of approximately 1% (0.014) of detections (Table 3). If the survey time was further reduced to 10 minutes, the overall detection probability subsequently reduced to 0.791 ± 0.028 , resulting in a loss of approximately 2% (0.021) of NZMS detections. When survey time was reduced to 5 total minutes of search time, then the overall detection probability was reduced to 0.772 ± 0.033 , causing 4% (0.04) of NZMS detections to be missed. These calculations are based on the overall detection probability and would likely be different when various levels of NZMS abundance were taken into account. The overall detection probability changed with different survey durations. Reducing the survey time to 15, 10, or 5 minutes yields an overall

detectability of 0.798, 0.791, or 0.772 respectively (Figure 7; Table 3). Seventy-nine percent of detections occurred within the first 10 minutes of the surveys.

Table 3. Detection probability for New Zealand mudsnails given different survey lengths of 5, 10, 15, and 20 minutes.

Survey Length (Minutes)	Detection Probability $\pm$ SE
20	0.812 ± 0.027
15	0.798 ± 0.028
10	0.791 ± 0.028
5	0.772 ± 0.033

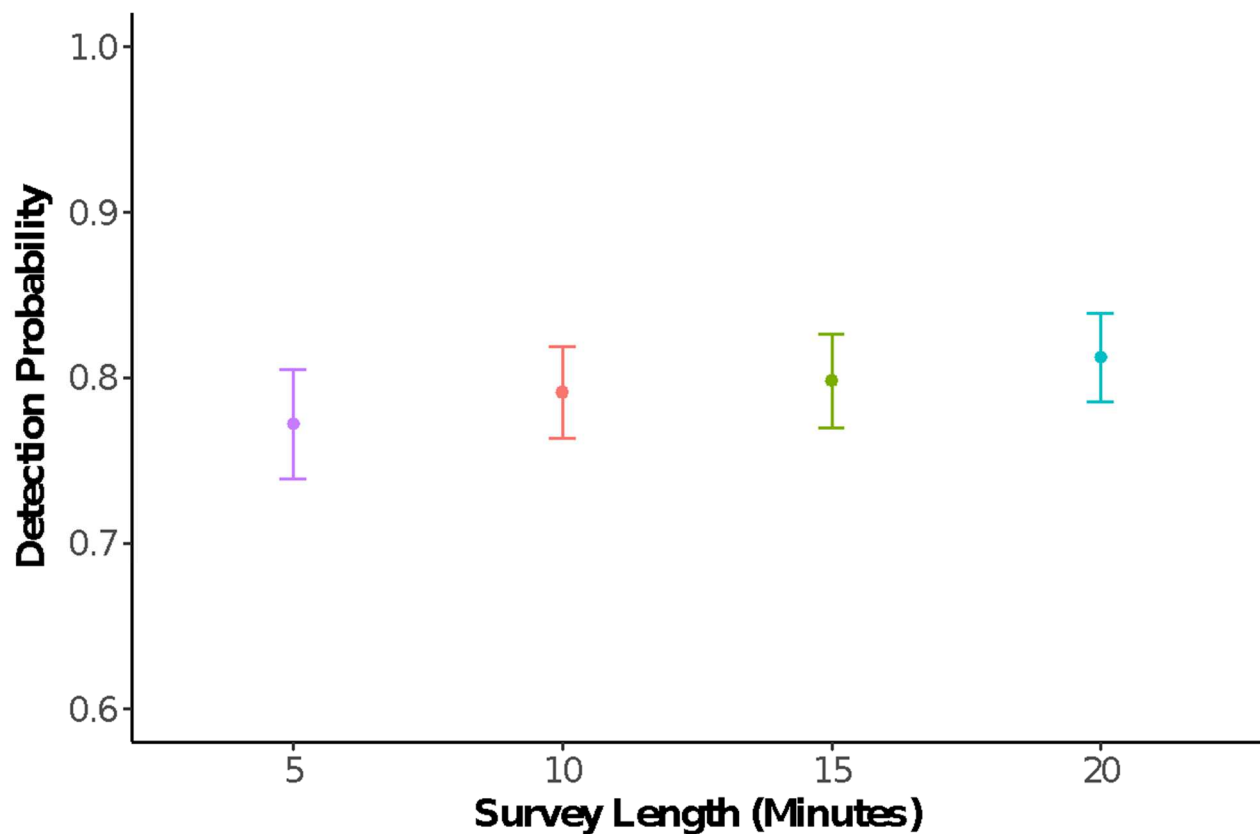


Figure 7. Overall detection probability for New Zealand mudsnails given different survey lengths of 5, 10, 15, and 20 minutes.

Decision Support Tool

A monitoring decision support tool was built using the above described binomial distribution formula (Table 4). As the number of sites surveyed increases, the probability of failing to detect NZMS decreases (i.e., the probability of detection increases). Similarly, an increase in the site level detection probability (the probability of detecting NZMS at each site when they are present), results in a decreased probability of failing to detect present NZMS. When site occupancy (the percentage of sites surveyed with NZMS) increases, the probability of failing to detect NZMS when they are indeed present, falls. For example, when 100 sites are surveyed with a unique sampling event detection probability of 0.8 (80%) and the true occupancy of all sites surveyed is 0.01 (1/100 sites are occupied), the probability of failing to detect NZMS is 0.448 (i.e., the overall detection probability is approximately 63%).

Table 4. Decision support tool to assist balancing limiting resources across a gradient of detection probabilities. The probability of failing to detect at a system level is a function of site level detectability, true occupancy within a system, and the number of sites surveyed. For example, the probability of failing to detect New Zealand mudsnails within a defined system that has 5% true occupancy is 0.603 (approximately 60%) when 20 sites within a system are surveyed with a 0.5 site level detection probability.

n Sites	Occupancy	Site Level Detection Probability							
		0.5	0.6	0.7	0.8	0.9	0.95	0.99	1
10	0.01	0.951	0.942	0.932	0.923	0.914	0.909	0.905	0.904
10	0.05	0.776	0.737	0.700	0.665	0.631	0.615	0.602	0.599
10	0.1	0.599	0.539	0.484	0.434	0.389	0.369	0.353	0.349
20	0.01	0.905	0.887	0.869	0.852	0.835	0.826	0.820	0.818
20	0.05	0.603	0.544	0.490	0.442	0.398	0.378	0.362	0.358
20	0.1	0.358	0.290	0.234	0.189	0.152	0.136	0.124	0.122
50	0.01	0.778	0.740	0.704	0.669	0.636	0.620	0.608	0.605
50	0.05	0.282	0.218	0.168	0.130	0.100	0.088	0.079	0.077
50	0.1	0.077	0.045	0.027	0.015	0.009	0.007	0.005	0.005
75	0.01	0.687	0.637	0.590	0.547	0.508	0.489	0.474	0.471
75	0.05	0.150	0.102	0.069	0.047	0.032	0.026	0.022	0.021
75	0.1	0.021	0.010	0.004	0.002	0.001	0.001	0.000	0.000
100	0.01	0.606	0.548	0.495	0.448	0.405	0.385	0.370	0.366
100	0.05	0.080	0.048	0.028	0.017	0.010	0.008	0.006	0.006
100	0.1	0.006	0.002	0.001	0.000	0.000	0.000	0.000	0.000
200	0.01	0.367	0.300	0.245	0.201	0.164	0.148	0.137	0.134
200	0.05	0.006	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
200	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

DISCUSSION

Detection of an invasive species early in the invasion process can strengthen management efforts by improving the likelihood of implementing effective response actions. Newly infested areas are more quickly identified and appropriate steps can be taken to eradicate or control the invasion. Any lost time can result in further spread and establishment of an

ecologically harmful organism. The consequences of delays in the initial detection of invasive species can be often measured in millions of dollars and years of effort (Anderson 2005).

The results show that the protocol I developed and implemented is highly effective at monitoring for NZMS in lotic habitats. The qualitative timed searches require only minimal equipment, time, and training. I found approximately 6-10 sites could be surveyed per day (depending on the distance between sites) following this protocol. Due to the minimal costs and training required, the timed searches are a good option when implementing citizen scientist monitoring. Further, when comparing to other detection techniques such as the use of environmental DNA (eDNA), detection and species confirmation results of these visual surveys are available immediately upon completion as they do not require the additional laboratory work that is necessary for an eDNA confirmation.

The analysis required me to make several assumptions, most of which were well justified and went unviolated. Given the limited mobility of NZMS (Richards 2002 as cited in Proctor et al. 2007), the assumption that snails do not move in or out of the designated 50-meter search area within the search time is virtually guaranteed. Also, confidence in proper identification for this species was high based on the use of voucher specimens which allowed me to re-examine snails to confirm the species when identification was in question. However, the assumption that the occupancy status is the same at all locations within a survey site was likely violated. NZMS distribution was patchy within areas of infestation (sometimes high abundance patches were observed a few meters away from seemingly uninfested areas). The consequence of violating this assumption is that some observations of a searcher not detecting NZMS when paired with a second searcher who did detect NZMS within the same survey event would bias the detection probability low. In comparison, the model would assume that NZMS were missed by the second

searcher if they were observed by the first searchers within the site, this violation would bias estimates of the detection probability low. Thus, true detectability is likely somewhat higher than estimated, and as such, the estimated detection probabilities were likely conservative. A study by Hayes and Monfils (2015) that utilized occupancy modeling to bird point counts also dealt with patchy target organisms, however the birds were patchy in time and not in space as the NZMS are in this study. Hayes and Monfils similarly found that estimates of detectability were not constant through time, violating the occupancy model's assumptions.

Detection probabilities differed as relative abundances of NZMS changed. Lower abundances of NZMS were associated with lower detection probabilities. By running two occupancy models for each level of abundance (with and without the non-detection sites), I was able to capture two possible detection probabilities. The occupancy model run that included the sites where no NZMS were found was the more conservative of the two. Assuming that some but not all of the sites where NZMS were not detected were occupied by a low level of NZMS abundance, the true detectability for NZMS at low abundances is likely between 0.342 – 0.567 (i.e., between 34% - 56% probability of detecting present NZMS). Similarly, true detectability when qualitative abundance of NZMS is medium is likely between 0.679 – 0.735. The two models did not vary substantially when NZMS abundance is high, so the true detectability is likely around 0.968. As such, to achieve similar detection probabilities when species are rare, additional effort (e.g., increased survey sites or searcher numbers) is required. These findings are similar to those from Hoffman et al. (2016), who evaluated aquatic invasive species (AIS) surveys and found that additional effort was required to detect rare AIS than abundant native species.

As I expected, the time to first detection increased as the qualitative level of abundance decreased. On average, it took more time to detect NZMS when they were present in low abundances than it did to detect them when they were present in medium or high abundance. The average time until first detection for all three levels of NZMS abundance (low, medium, and high) were under 8 minutes (7.74 minutes), and 75% of all observations were made in less than 4 minutes (3.95 minutes; Figures 5 & 6, Table 2). When looking at the cumulative distribution figure (Figure 6), it can be seen that the rate at which initial detections of NZMS accumulates is highest when relative abundance of NZMS at a site is high, i.e. higher NZMS abundance leads to quicker detection. This suggests that a shorter search time may be appropriate. To consider shortening the survey length, I reran the occupancy model an additional 3 times; once with all sites where NZMS were detected after 15 minutes recorded as non-detections, then again for 10 and 5 minutes. The ideal search time depends on tradeoffs between available resources and an acceptable detection probability. If surveys were reduced to 5 minutes, approximately 4% of detections would be lost, however the reduced cost of a shorter survey length may lead to the opportunity to bring on additional searchers or add additional survey sites.

Survey Design Considerations

The goal of early detection monitoring systems is to maximize the probability of detecting new invasions shortly after they occur (Mehta et al. 2007 and Brooks and Klinger, 2009). To achieve this goal within the context of this study, there are several survey design considerations, some of which are under the investigator's control, and some of which are not. In the context of our survey protocol, the number of searchers deployed, the duration of the survey, and the number of sites surveyed each has an influence on the probability of detecting NZMS within a river system. Increasing each of these three survey characteristics increases the probability of detecting NZMS within the system.

Our overall estimate of detectability was 0.816 or approximately an 81% chance of one searcher detecting NZMS when they are present at a site when following the previously described survey protocols. The addition of a second searcher drops the probability of missing NZMS when they are indeed present down 15% (Figure 4). We can also look at what this increase in detectability would mean when we look at high, medium, and low levels of abundance. Using our lower end of the detectability estimates for each qualitative abundance level (including the non-detection sites in our occupancy model) we note an increase in detectability in all three levels with the addition of the second searcher. Surveys conducted where low NZMS abundances were recorded increase in detectability from 0.342 to 0.567 when a second searcher is added. Surveys conducted where medium NZMS abundances were recorded increase in detectability from 0.679 to 0.897 when a second searcher is added. Finally, surveys conducted where high NZMS abundances were recorded increase in detectability from 0.967 to 0.999 when a second searcher is added. Detectability estimates can be calculated for greater than 2 searchers with a modification to the previously described equation and may be beneficial if site occupancy and abundance are low.

As noted, the detection probability increases as the number of searchers increases in a non-linear manner. However, the decision to increase the number of searchers must be weighed against the increased costs incurred by employing additional personnel. I noted diminishing returns for the effort and costs associated with an additional searcher above two. A 3-searcher party yields a detection probability of approximately 0.994 (94%) and a 4-searcher party yields a detection probability of 0.999 (nearly 100%) to the overall detection probability of NZMS. Given that each additional searcher would add only a small increase in detectability, I would recommend considering a 2-searcher party for future implementation of these protocols.

When evaluating the survey, I also evaluated if a 20-minute search time is long enough. Figure 5 shows the time until NZMS were first detected against the three levels of abundance. We are much less likely to fail to detect NZMS for sites with high abundance than we would be to miss them at sites occupied with medium or low abundance given a 20-minute search time. From this, we can see that 89% of all mudsnail detections occurred within the first 10 minutes, considering all levels of abundances (Figure 6). Since the average time until first detection was below 8 minutes for all levels of abundance (high, medium, and low), a longer search time provides minimal extra detection capacity. The relatively quick time until first detection in infested sites is likely due to the implementation of the survey as a targeted search for NZMS. Survey efforts were focused in near shore, low flow areas containing vegetation or woody debris where NZMS are more often found when abundances are low. Time, resources, and goals (e.g., the highest acceptable risk of failing to detect NZMS when present) should be evaluated to determine the most appropriate survey duration. Finally, the incorporation of a spatially adaptive cluster sampling design into the current occupancy framework has been shown to reduce model bias and can increase detectability, especially when working with rare, patchy species (Pacifi et al. 2016).

Decision Support Tool

The true occupancy of NZMS is unknown, but it is reasonable to assume it would initially be low (e.g., 1%) during the early phase of introduction and establishment into an area. Thus, when planning a survey, one needs to decide what level of occupancy is the goal for detection as well as the presumed detection probability for a single observer. Once these considerations are made, choices and tradeoffs between the number of searchers per site and number of sites visited can be evaluated. As such, decisions have to be made about acceptable detection probabilities. Detection probability is highest when site abundance is high, survey

length is maximized, and searcher numbers are increased beyond one. The combined influence of these considerations can be estimated via application of the binomial distribution shown in table 4.

The higher the detection probability, number of searchers, and number of sites visited, the lower the probability of failing to detect NZMS when they are present within a system. However, even when the number of sites surveyed is increased, when true occupancy is low (e.g., 0.01), the chance of failing to detect NZMS remains high. Occupancy will be low when NZMS, or other invasive species, are first introduced into a system. Thus, maximizing confidence in NZMS detection is critical.

Similar decision support tables have been made for determining the number of survey replicates per site necessary when designing a single-season occupancy study (MacKenzie and Royle 2005; Guillerá-Arroita, et al. 2010). However, the utility of these tables is their use for understanding occupancy of a given organism in a system by manipulating the target variance within the model (instead of the probability of failing to detect utilizing a binominal distribution as was done in this study). The approach I am proposing is novel to the best of our knowledge. Bailey et al. (2004) applied a variation of the likelihood-based occupancy model proposed by MacKenzie et al. (2002) when determining site occupancy and detectability for terrestrial salamanders. Other methods of estimating detectability have been implemented in studies including the capture-recapture methods analyzed in the CAPTURE program developed by Otis et al. (1978) used by Kery and Plattner (2007) in a butterfly monitoring program. While these methods could potentially be used for studies of the detection probability for NZMS or other invasive species, I found that application of the standard occupancy model fit our situation well without the need for additional effort, as a capture-recapture study would entail. Further, the use

of capture-recapture methods for an invasive species would be counterproductive to any control efforts.

Future adaptations of the decision support tool (Table 4) may consider incorporating cost estimates of various aspects of invasion management (e.g., early detection and rapid response costs). A survey by Hauser and McCarthy (2009) developed a simple detection and management model for a low-density invasive species in Australia, orange hawkweed (*Hieracium aurantiacum*). The model aimed to reduce costs associated with surveillance and management upon detection. Including treatment costs into my model were not ideal since management techniques of NZMS are currently limited to education/ outreach of potential vectors of spread.

Survey Modifications

Although the analysis shows that the survey is a highly effective early detection tool, it may be modified to better reflect the needs of other monitoring or research objectives. For example, it may not be necessary to capture even a qualitative level of abundance of NZMS when trying to document the spread of their distribution. If this is the case, the survey could be stopped as soon as the first searcher detects the presence of NZMS. This adaptation would allow for more surveys to be conducted in a stretch where NZMS are potentially established.

A possible shortcoming of this analysis is the uneven number of relative NZMS recorded. Through the implementation of our qualitative surveys, I found 133 sites to be at a high level of abundance, 36 sites with medium abundance, and 57 sites with low abundance (Table 3). These results likely skewed our overall detection estimates to reflect high abundance detection estimates. It is possible, and even likely, that NZMS were present at more survey sites than we observed (e.g., since NZMS are less likely to be detected at lower abundances, we likely more often failed to detect their presence at sites where they existed in lower abundances than when they were present in high abundance).

Future Research

Habitat and landscape features have the potential to impact the detectability and estimated occupancy of a species. Specifically, for NZMS additional site characteristics such as substrate, discharge, water depth, temperature, and season may be considered as covariates when estimating occupancy and detectability in lotic environments. This would require habitat mapping alongside qualitative survey implementation and would yield a more complete picture of how occupancy and the probability of detection changes given certain site characteristics. Performing such habitat surveys would be very time consuming, and the additional insight gained into detection probabilities may not provide much additional power for the overall sampling effort.

As the invasion process progresses, additional information needs to be collected to assess damage done and guide future management actions. In this study, a qualitative level of abundance was adequate to determine NZMS distribution within rivers. However, future studies may choose to evaluate quantitative abundance of NZMS. The literature provides mixed recommendations on quantitative NZMS techniques for lotic environments, however most require higher water velocities to be effective. As I was most regularly finding NZMS in areas of low flow, many of these techniques (e.g., Surber and Hess samplers) may not be ideal for all infested Michigan rivers. A study by Nett et al. (2012) ran occupancy analysis to do a comparative evaluation of sampling methods of round goby within the Great Lakes, and results helped guide the most effective sampling methods for round goby within the lakes. A similar effort could be made to evaluate quantitative survey methods for NZMS.

The early detection evaluation method presented in this chapter would benefit from further application to additional rare species (i.e., other early state invaders or rare, threatened and endangered species) to demonstrate its utility for other species of unknown occurrence and

distribution. Different species-specific protocols could be evaluated using a similar process and decisions made by implementing the binomial distribution decision support tool. The recent red swamp crayfish (*Procambarus clarkii*) invasion in Michigan may be an ideal candidate for application of this method. Future studies may consider comparing additional methods for early detection such as the use of environmental DNA (eDNA). Survey evaluation as demonstrated above may have utility when implemented for other invasive species monitoring producers and warrants further investigation.

APPENDICES

APPENDIX A: Qualitative survey data sheet.

New Zealand Mudsail Qualitative Data Sheet v1.0

Search for 20 minutes, preserving a sample of the first 25 snails collected from all searchers in 95% ethanol, or any snails you are uncertain are New Zealand mudsnails. Each person in the team should conduct their search independently, but you can work together to identify any animals found. We recommend a maximum of 4 people searching any single site.

Sampling Crew (full names): _____

Year _____ Month _____ Day _____

River Name: _____

Site_ID (added later by MSU/DNR/DEQ): _____

Latitude (decimal degrees): _____ Longitude (decimal degrees): _____

Location Description: _____

Circle the number of New Zealand mudsnails found.

Time until first detect:

Searcher 1:	NONE	LOW (1-10)	MEDIUM (11-100)	HIGH (>100)	_____
Searcher 2:	NONE	LOW (1-10)	MEDIUM (11-100)	HIGH (>100)	_____
Searcher 3:	NONE	LOW (1-10)	MEDIUM (11-100)	HIGH (>100)	_____
Searcher 4:	NONE	LOW (1-10)	MEDIUM (11-100)	HIGH (>100)	_____

Check habitats where mudsnails were found:

Gravel ☐ Silt ☐ Vegetation ☐ On wood ☐ Other ☐ _____

Available habitats surveyed:

Gravel ☐ Silt ☐ Vegetation ☐ On wood ☐ Other ☐ _____

Distance Surveyed (50 meters maximum) _____

Jar Label ID Number with snails found (or "none", if no snails found) _____

Map/Notes (Draw on back): _____

APPENDIX B: R code for the occupancy models.

```
library(unmarked)

# Import data file

nzms=read.csv(file="",head=TRUE,sep=",")

# put in site covariates if necessary
site.cov=read.csv(file="",head=FALSE,sep=",")

# put in observation covariates if necessary
obs.cov=read.csv(file="",head=FALSE,sep=",")

umf<-unmarkedFrameOccu(y=nzms, siteCovs=site.cov, obsCovs=
data.frame(obs.cov=obs.cov))

summary(umf)

detect.out<-occu(~1 ~1, umf)

backTransform(detect.out['det'])
backTransform(detect.out ['state'])
```

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WORKS CITED

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CHAPTER 2: DISTRIBUTION OF NEW ZEALAND MUDSNAILS (*POTAMOPYRGUS* *ANTIPODARUM*) IN MICHIGAN RIVERS FROM 2015 TO 2018

INTRODUCTION

The New Zealand mudsnail (*Potamopyrgus antipodarum*; hereafter NZMS) is a small aquatic gastropod native to New Zealand. It is considered to be a worldwide invader, with established populations in Australia (Loo et al. 2007), Asia (Hamada et al. 2013), Europe (Ponder 1988), and North America (Bowler 1991). The NZMS began its invasion of the United States in 1987, when first discovered in the Middle Snake River during a mollusk survey in Idaho (Bowler 1991). NZMS were first detected in the Laurentian Great Lakes in 1991 and in a Michigan river in 2015 through a non-target survey (Zaranko, et al. 1997; Michigan Department of Environmental Quality 2015). Since their time of detection, state management agencies have sought to document the early invasion dynamics of this species to protect Michigan's cold-water resources.

NZMS have been documented outcompeting native macroinvertebrates that make up the majority of food resources for sport fishes such as trout and salmon (Hall et al. 2006; Maret et al. 2008). NZMS are not themselves a substitute food source for sport fishes and have been known to survive digestion by trout (Bruce et al. 2009; Vinson and Baker 2008; McKenzie et al. 2012). State resource agencies and stakeholder groups are concerned because the rivers where NZMS were initially detected in Michigan support thousands of angler days annually, which translates into a significant source of income for the state.

Anglers and other outdoor recreationalists (e.g., sport paddlers) may unknowingly transport NZMS within and between waterbodies. NZMS are a small (ranging between 2 – 6 mm

in their invaded ranges), cryptic invader that are easily overlooked (McKenzie et al. 2012). They have the ability to survive out of water transport and readily attach to most any surface. Lengthy transport is possible due to the NZMS ability to seal its operculum, allowing it to survive nearly 2 days on a dry surface and up to several weeks with moisture (Alonso and Castro-Diez 2012). NZMS are parthenogenic in many areas of their invasion (Wallace 1992), and are highly fecund, producing up to 70 embryos each per breeding cycle (McKenzie et al. 2012; Richards 2004). These life history characteristics allow NZMS to quickly establish new populations once introduced.

There are multiple stages to the invasion process: introduction/arrival, establishment, integration/reproductive success, and spread (Vermeij 1996; Heger and Trepl 2003; Figure 8). It is widely acknowledged that the best way to manage potential invasive species is early identification and response through the development of a targeted management strategy, ideally prior to establishment and integration (Myers et al. 2000; Simpson et al. 2009; Westbrooks 2004). Determining a baseline of the organism's distribution, establishment, and potential for harm are critical first steps to developing a management plan.

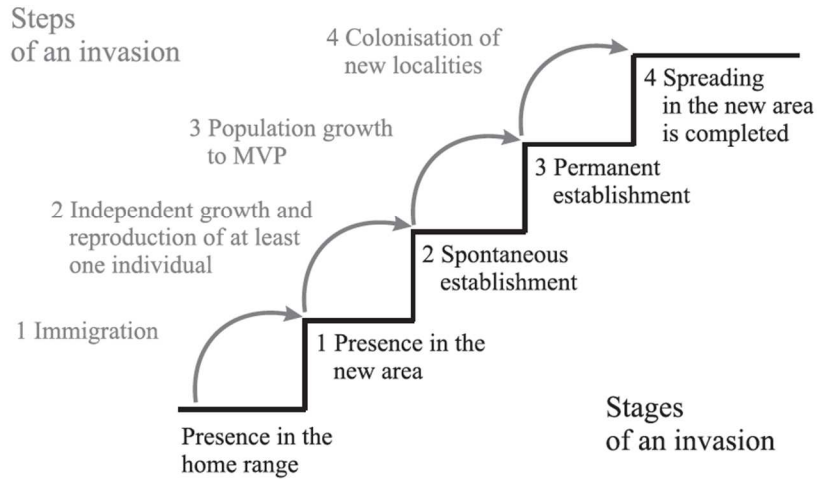


Figure 8. Conceptual model of the invasion process (from Heger and Trepl 2003).

The shape or pattern of an invaders distribution provides information on the processes leading to their spread. Disjoint colonies suggest multiple points of introduction (Wieferich et al. 2011), whereas a continuous distribution is more indicative of a diffusive, natural dispersal pattern (Suarez et al. 2001). For example, the special pattern observed for beach scale insects, an invasive pest on beech trees, has been documented to be the result of both human driven, large scale transportation as well as localized diffusive spread (Wieferich et al. 2011). Long-distance mediated transport of invasive species is thought to be a rare, yet significant means of aquatic invasive species spread (Suarez et al. 2001; Buchan & Padilla 1999). This information could thus help identify the vector for intra- and inter-river spread and colonization.

The goal of this study was to provide a snapshot of the broad-scale distribution of NZMS in Michigan rivers, focusing on rivers at perceived greatest risk. Further, I examined the distribution in the Pere Marquette and Boardman rivers in detail to document short-term

expansion of range. Results of this study inform targeted management efforts to areas that are infested or watersheds that are at risk of infestation because of proximity to infested sites.

METHODS

Study Sites

Twenty-one Michigan rivers were identified by the Michigan Department of Environmental Quality (MDEQ; now the Michigan Department of Environment, Great Lakes, and Energy) and the Michigan Department of Natural Resources (MDNR) across the state of Michigan as being at high risk of invasion based on their location and popularity as high-use angling locations. The Pere Marquette River is the primary river of interest for this study as it was where NZMS were first detected in the inland waters of Michigan. The area of the Pere Marquette where NZMS were initially observed is within Baldwin, MI (Lake County).

Broad surveys were conducted on the Slagle, Pokagon, Silver, and Little Beaver Creeks, and the Rouge, Bladwin, Muskegon, White, Baldwin, Little Manistee, Platte, Betsie, Rock, Upper Tahquamenon, Chocoday, Fox, and Paw Paw Rivers in 2016. Intensive surveys were conducted around the infested range of the Pere Marquette between 2015 - 2018. Similarly, sampling documented the infested range of the Boardman River in 2016 - 2018, where NZMS were detected in late 2016. NZMS were detected in the Manistee River in 2017 and surveys occurred between 2017 and 2018. NZMS were detected in late 2017 in the Pine River, as such sampling occurring only in 2018. NZMS were detected in the Au Sable River in 2016 and subsequent sampling occurred that same year. Other investigators focused on the Au Sable River starting in 2017, as such is outside the scope of my study.

Study sites were selected in 2015 based around where the initial detection of NZMS occurred. Additional rivers that spanned the upper and lower peninsula of Michigan and were thought to be at risk of NZMS invasion were surveyed in 2016. Both 2015 and 2016 surveys

were conducted by the MDEQ, with the exception of the Pere Marquette River, which was surveyed by Michigan State University personnel. Michigan State University took over survey implementation in 2017 and implemented an intensive evaluation of rivers with known NZMS infestation (the Pere Marquette, Boardman, and Manistee river) with additional limited evaluations of nearby rivers with high recreational use.

Qualitative Survey Implementation

Qualitative surveys were conducted in a standardized fashion and were found to be high effective in detecting the presence of NZMS (see Chapter 1 of this thesis). Each survey event was independently conducted by two to four surveyors and had a 20-minute search time with up to 50-meter search area (determined with a rangefinder) to standardize effort across surveyors. To ensure surveyor independence, communication regarding snails found was prohibited between surveyors. In a further attempt to standardize search effort, each surveyor would pause their own 20-minute timer while collecting a sample of found NZMS to ensure a full 20-minute search time. The independent surveys were limited to a total time of 30 minutes or less, allowing no more than 10 minutes to determine each surveyors' area and collect found NZMS.

The survey focused on near shore areas where NZMS were most commonly detected. Particular attention was paid to woody debris and rooted submerged vegetation, as this was observed to be the preferred (most commonly utilized) habitat of NZMS when detected in relatively low densities. Surveyors performed surveys simultaneously with each surveyor covering a different section of river without overlap. If overlap could not be avoided, then surveyors would refrain from searching the same area at the same time. The first 25 NZMS found were collected and preserved in 95% ethanol, labeled, and stored. A qualitative level of abundance was documented by each surveyor (none, low (1-10), medium (11-100), high (100+)). Overall survey detectability for two surveyors working independently was estimated at 96% (see

Chapter 1). Based on this evaluation, I view classification of sampled points as “infested” or “not infested” to be highly accurate.

In 2015, an intensive monitoring effort was implemented on the Pere Marquette. Approximately 68-river kilometers were surveyed using a raft to navigate between most survey locations with additional downstream locations surveyed at public access points. A limited monitoring effort was implemented in 2016 where resources allowed only for the United States Forest Service access sites along the Pere Marquette River to be surveyed. In 2017, a raft was again used to conduct surveys on the Pere Marquette and Boardman rivers in order to determine NZMS spread and distribution within each river, with surveys covering approximately 35 and 51-river kilometers respectively. Qualitative surveys were performed in close proximity (one-quarter or one-half mile) to each other. Additional rivers were surveyed via public access points. In 2018, a reduced effort to look at rivers with known populations was implemented in an attempt to document NZMS spread outside of the current documented ranges of infestation.

Analysis

Survey results were imported into and mapped in ESRI ArcGIS 10.6.1. Summary statistics were analyzed in Microsoft Excel 16.9. The above survey methods were analyzed for effectiveness in Chapter 1 of this thesis.

RESULTS

New Zealand Mudsail Distribution Across Michigan

A total of 21 Michigan rivers were surveyed from 2015-2018. Established populations of NZMS were detected in four Michigan rivers: the Pere Marquette, Au Sable, Boardman, and Manistee (Table 5). Another confirmed detection of NZMS was reported from the Pine River in late 2017, however my subsequent sampling did not detect any in the 7 sites sampled.

Throughout Michigan, 65.6 total river kilometers have been documented to be infested by

NZMS (Table 6). NZMS were present in 49% of survey events conducted between 2015 and 2018.

Table 5. Sampling intensity and frequency of detection of NZMS in a broad-scale survey of Michigan rivers, 2015 and 2018. Some access points were surveyed more than once during a single year, and as such the number of survey locations is not equal to the number of survey events.

River	No. of survey events	No. of surveys that resulted in detections
Pere Marquette		
2015 ^a	61	53
2016	11	3
2017	118	66
2018	10	5
Au Sable^b		
2016 ^a	17	6
Boardman^c		
2016 ^a	2	2
2017	61	50
2018	9	3
Manistee		
2016 ^a	3	0
2017	14	2
2018	5	3
Pine		
2017	6	0
2018	1	0
Rogue		
2016 ^a	2	0
2017	17	0
2018	3	0
Betsie		
2016 ^a	2	0
2017	2	0
Baldwin		
2016 ^a	4	0
2017	4	0

Table 5 (cont'd)

Little Manistee		
2016 ^a	7	0
2017	4	0
Platte		
2016 ^a	3	0
2017	3	0
White		
2016 ^a	3	0
2017	2	0
Muskegon		
2016 ^a	3	0
2017	2	0
Slagle Creek		
2016 ^a	2	0
2017	2	0
Rock		
2016 ^a	1	0
Upper Tahquamenon		
2016 ^a	1	0
Little Beaver Creek		
2016 ^a	1	0
Chocolay		
2016 ^a	1	0
Fox ^d		
2016 ^a	1	0
Pokagon Creek		
2016 ^a	1	0
Silver Creek		
2016 ^a	1	0
Paw Paw ^d		
2016 ^a	1	0
Total	391	193

^aSurveys conducted by the Michigan Department of Environmental Quality (now the Michigan Department of Environment, Great Lakes, and Energy).

^bEast, south, and main branch of Au Sable were surveyed.

Table 5 (cont'd)

^cSouth and north branch of Boardman surveyed in 2017 and 2018; main branch surveyed each survey occurred.

^dEast branch surveyed.

Table 6. Summary of river kilometers infested with NZMS in the Pere Marquette, Boardman, and Manistee rivers. Results from the Au Sable River were not included as sampling was much more limited and sampling was taken over by other investigators after 2016. Surveys kilometers represent the distance between the most upstream and downstream points of detection and assume a continuous distribution of NZMS between survey points. Total river kilometers infested is based on the maximum distance for each river.

River	River kilometers surveyed	River kilometers infested
Pere Marquette		
2015 ^a	68.2	15.0
2016	45.8	9.6
2017	35.2	19.8
2018	35.6	13.1
Boardman ^b		
2016 ^a	22.5	22.5
2017	51.6	33.6
2018	51.6	6.5
Manistee		
2016 ^a	16.4	0.0
2017	16.6	8.9
2018	16.6	12.2
Total	360.0	65.6

^a Surveys conducted by the Michigan Department of Environmental Quality (now the Michigan Department of Environment, Great Lakes, and Energy).

^b South and north branch of Boardman surveyed in 2017 and 2018; main branch surveyed each survey occurred.

Sampling locations and intensity varied by year. In 2015, the Pere Marquette was the only river selected for surveying to confirm initial NZMS detections in a previous non-target survey. Approximately 68 river kilometers were surveyed (Figure 9), and the initial distribution

was largely continuous. In 2016, survey coverage was expanded to include all rivers perceived to be at high risk of invasion (Figure 10). From these surveys, NZMS were confirmed in three rivers: Pere Marquette, Au Sable, and Boardman rivers. In 2017, 13 rivers were surveyed, focusing on rivers adjacent to the Pere Marquette and Boardman rivers where NZSMS were documented to be present (Figure 11). In addition to reconfirming the presence of NZMS in the Pere Marquette and Boardman rivers, the Manistee River was confirmed to contain NZMS in 2017. In 2018, sampling focused on rivers with known NZMS infestations to determine range expansion within these rivers (Figure 12). In addition, the Rogue River was surveyed as a follow-up to results from a preliminary eDNA survey.

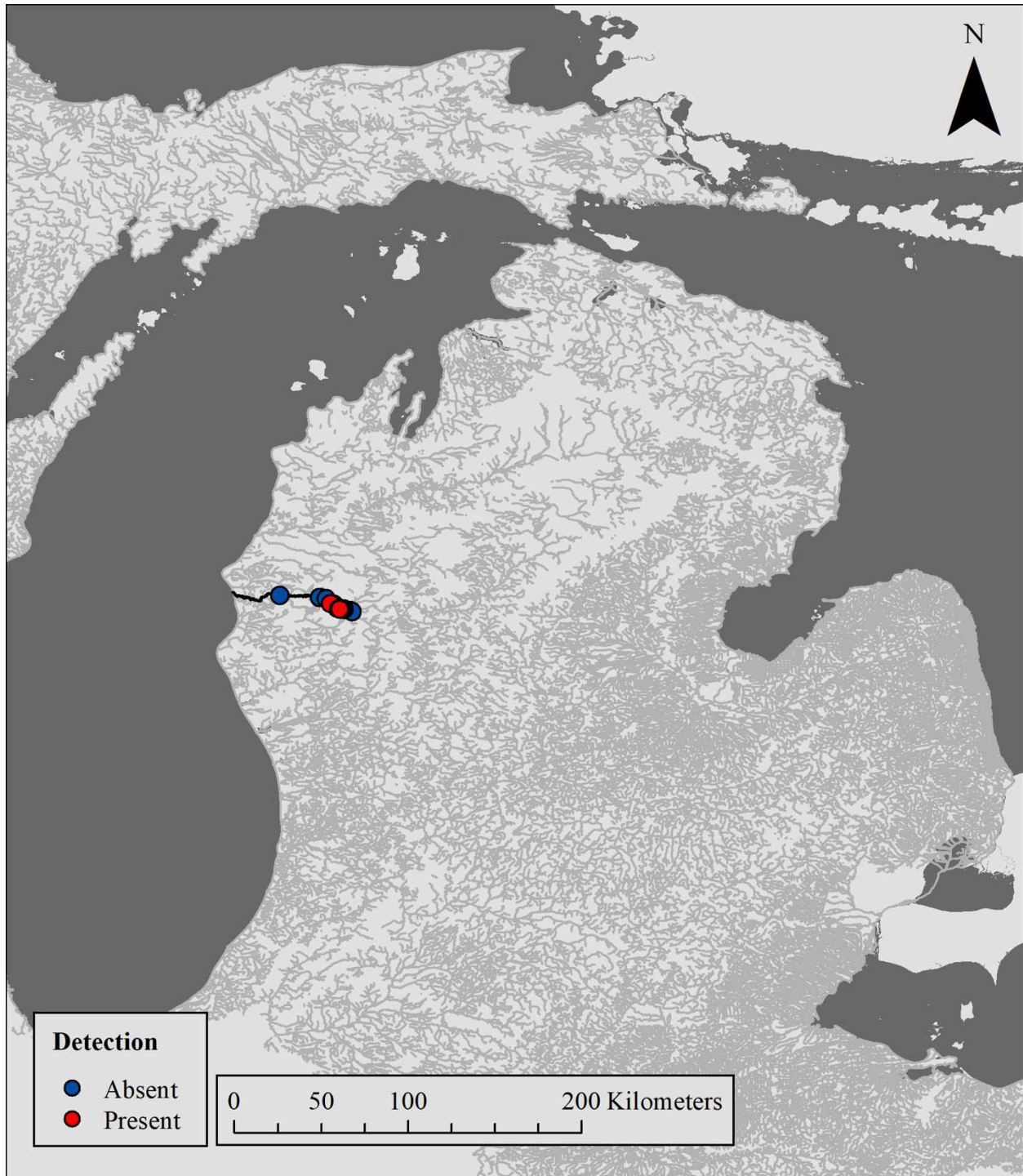


Figure 9. Location and results of NZMS qualitative surveys, 2015.

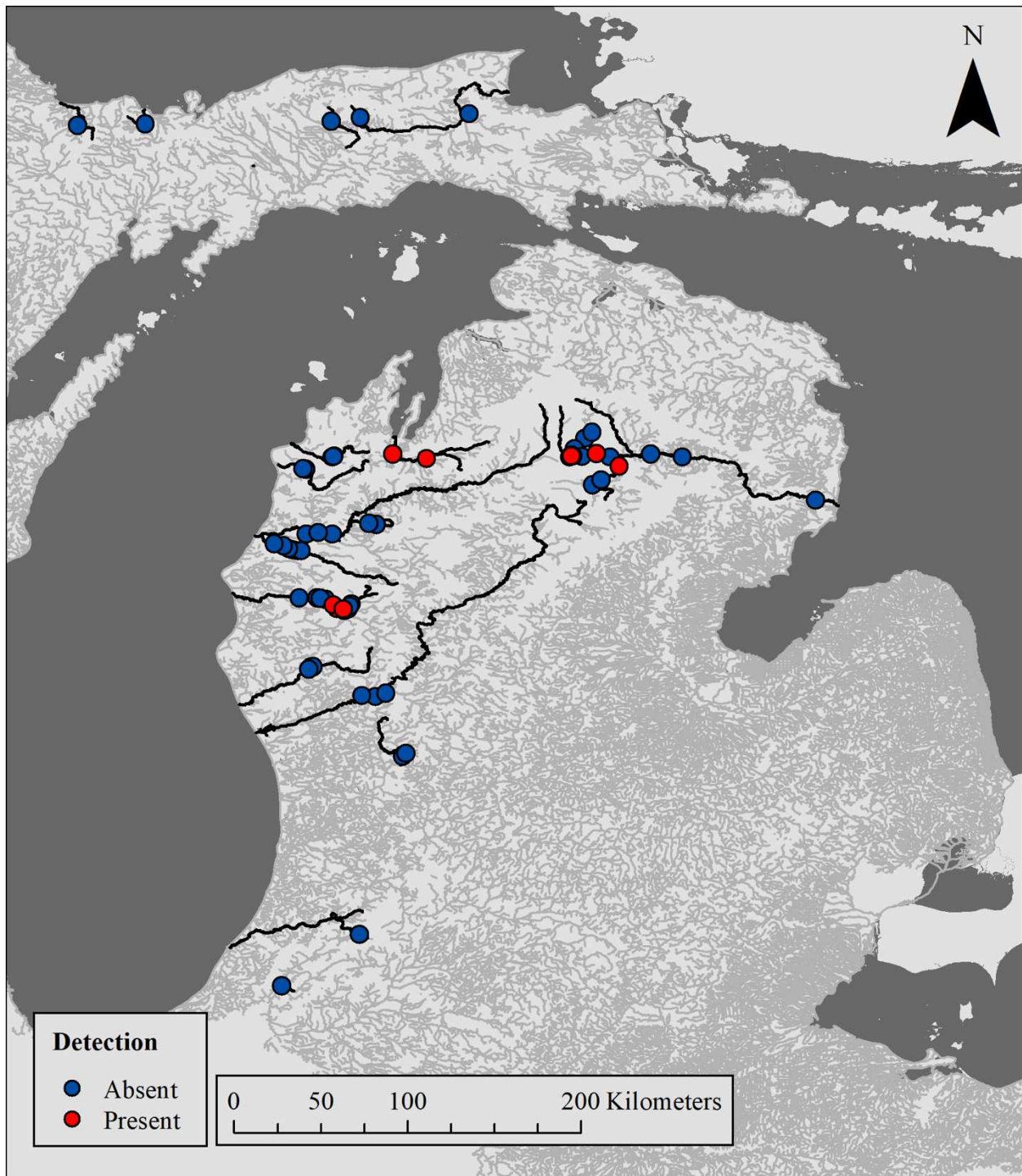


Figure 10. Location and results of NZMS qualitative surveys, 2016.

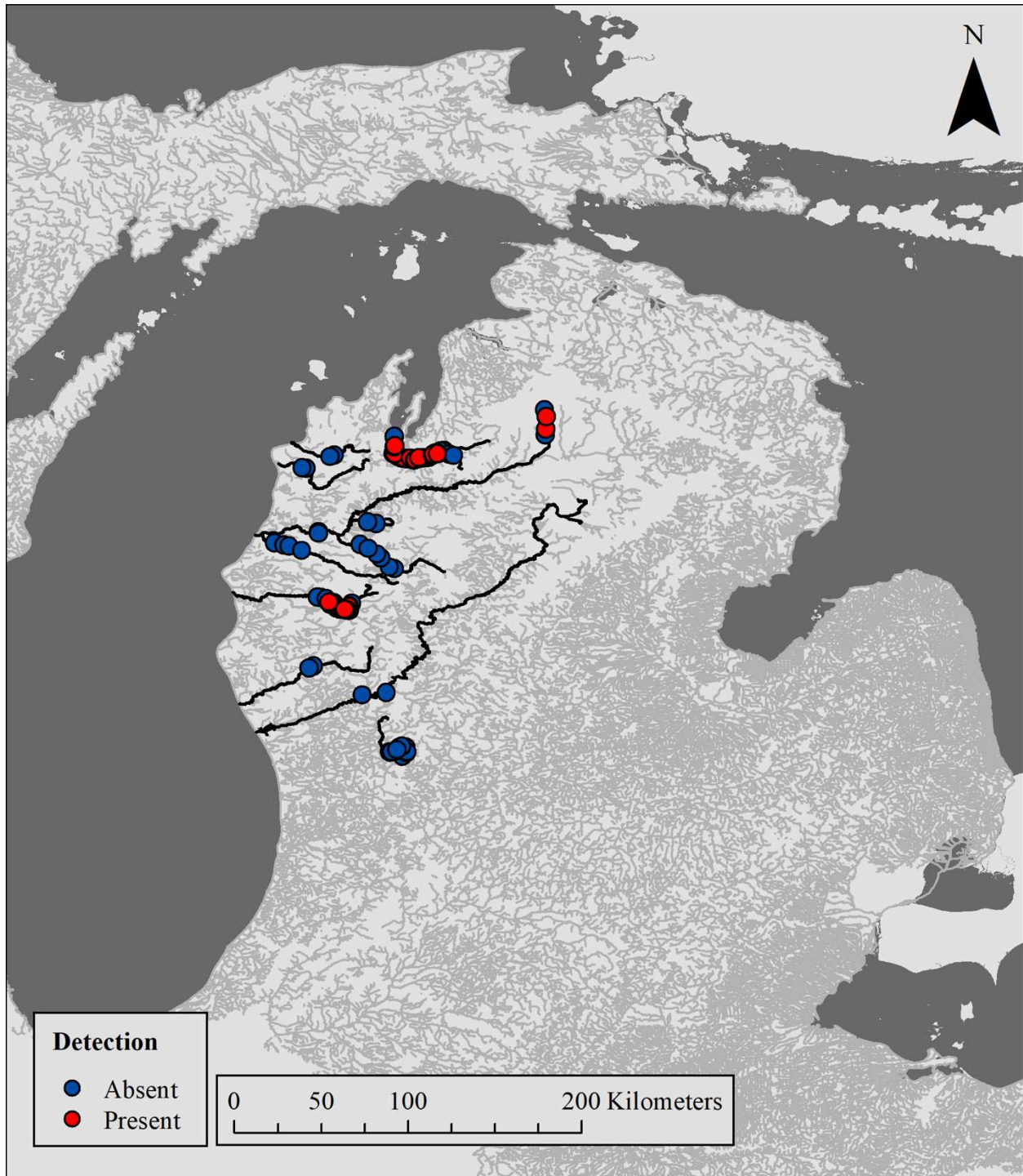


Figure 11. Location and results of NZMS qualitative surveys, 2017.

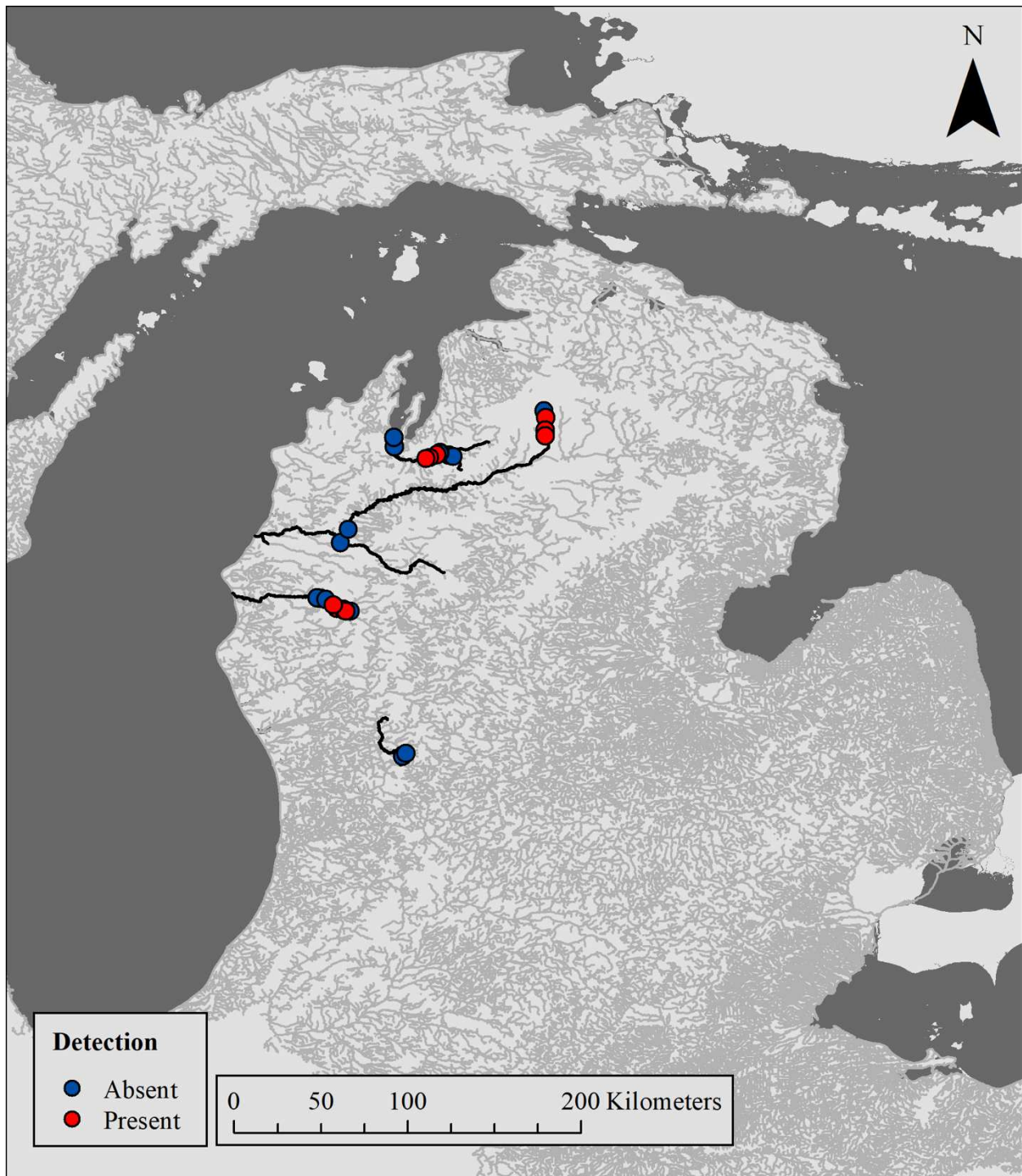


Figure 12. Location and results of NZMS qualitative surveys, 2018.

New Zealand Mudsail Distribution within the Pere Marquette River

Despite the differences in sampling intensity across years, the distribution of NZMS in the Pere Marquette River appeared relatively constant throughout the 4-year monitoring period (Figure 13). Additional points of infestation were detected in 2017 above the previously documented maximum upstream extent. However, prior sampling was not as intensive within this upstream area. Overall, the distribution of NZMS appeared relatively continuous throughout the infested section of river. The area of highest relative density of NZMS was observed at a popular angling location. Wading anglers and guided fly fishing trips often access this location during the course of their fishing event. NZMS in this vicinity covered all available substrates and more than 100 snails were found per 20-minute timed survey.

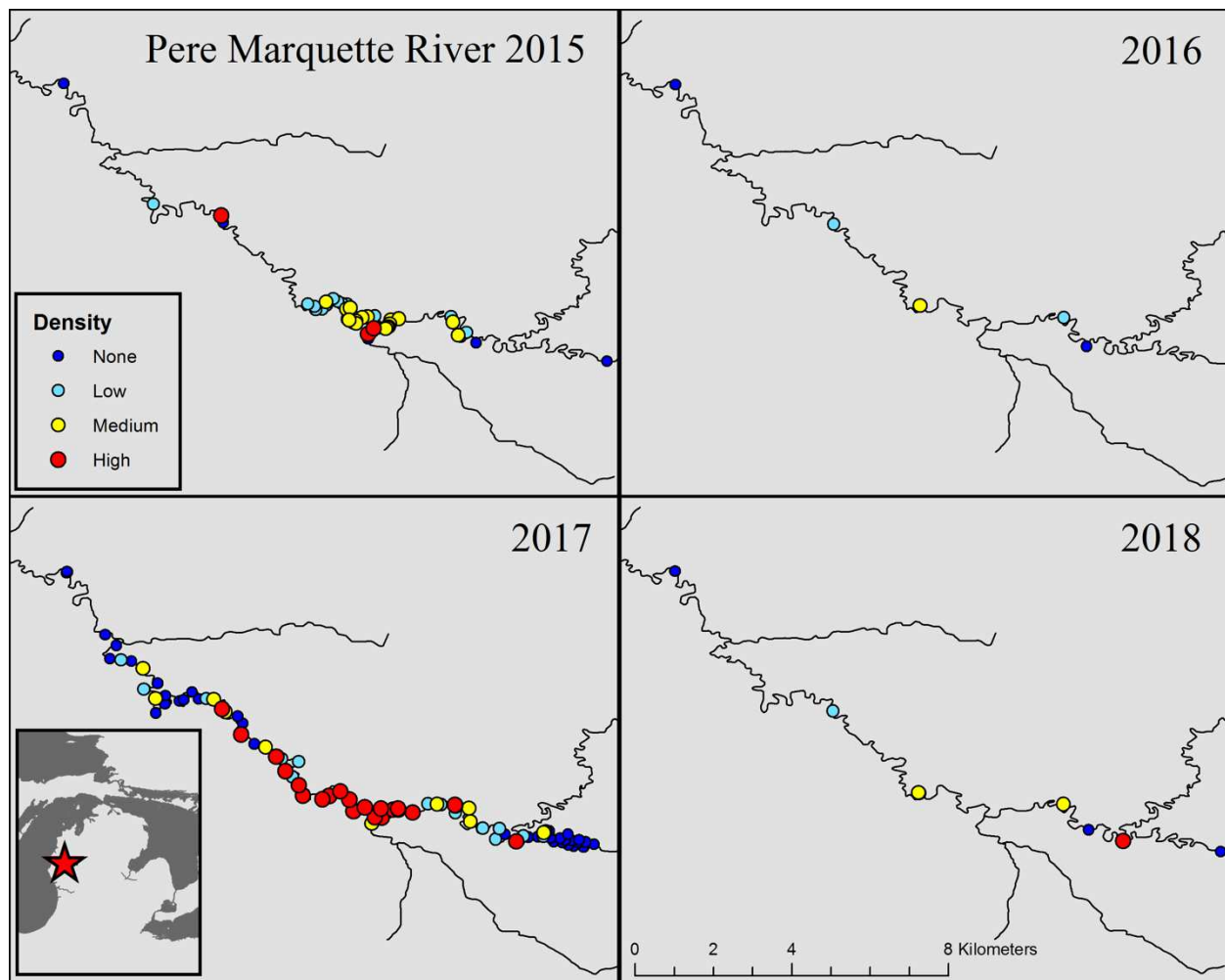


Figure 13. New Zealand mudsnail distribution and qualitative abundance in the Pere Marquette River between 2015 and 2018. Qualitative density levels include none (0), low (1-10), medium (11-100), and high (100+) found during a 20-minute timed search. Based on surveys conducted by Michigan State University.

New Zealand Mudsnail Distribution within the Boardman River

NZMS were first detected in the Boardman River in late 2016. The intensive survey in 2017 showed that NZMS were widespread in the Boardman River and had a continuous distribution, beginning slightly downstream of where the North and South Boardman branches meet to become the main stem and continuing downstream until the series of dams and reservoirs upstream of Boardman Dam (Figure 14). Sampling in 2018 focused on determining upstream range expansion as downstream dams and reservoirs limited my sampling ability. Based on these samples, no upstream range expansion was observed in 2018 (Figure 14).

NZMS showed a consistently high density through the main body of their infestation, with a tapering of density towards the upstream extent of their distribution. This is consistent with a diffusive type of spread process. NZMS were observed at the highest relative densities in the Boardman River compared to any other river surveyed in Michigan. Although this survey was not able to quantitatively describe density, surveyors observed $\geq 1,000$ NZMS per square meter covering all available substrate types. High density may be reflective of a longer-term history of infestation than previously documented.

A single detection was made in 2017 downstream of the Boardman Dam. In this instance, ≤ 10 NZMS shells were recovered during a survey event, but no live NZMS were found. NZMS may be present in the reservoir in higher abundances than the survey detected due to the survey's inability to accurately detect relative densities in deep, lentic environments.

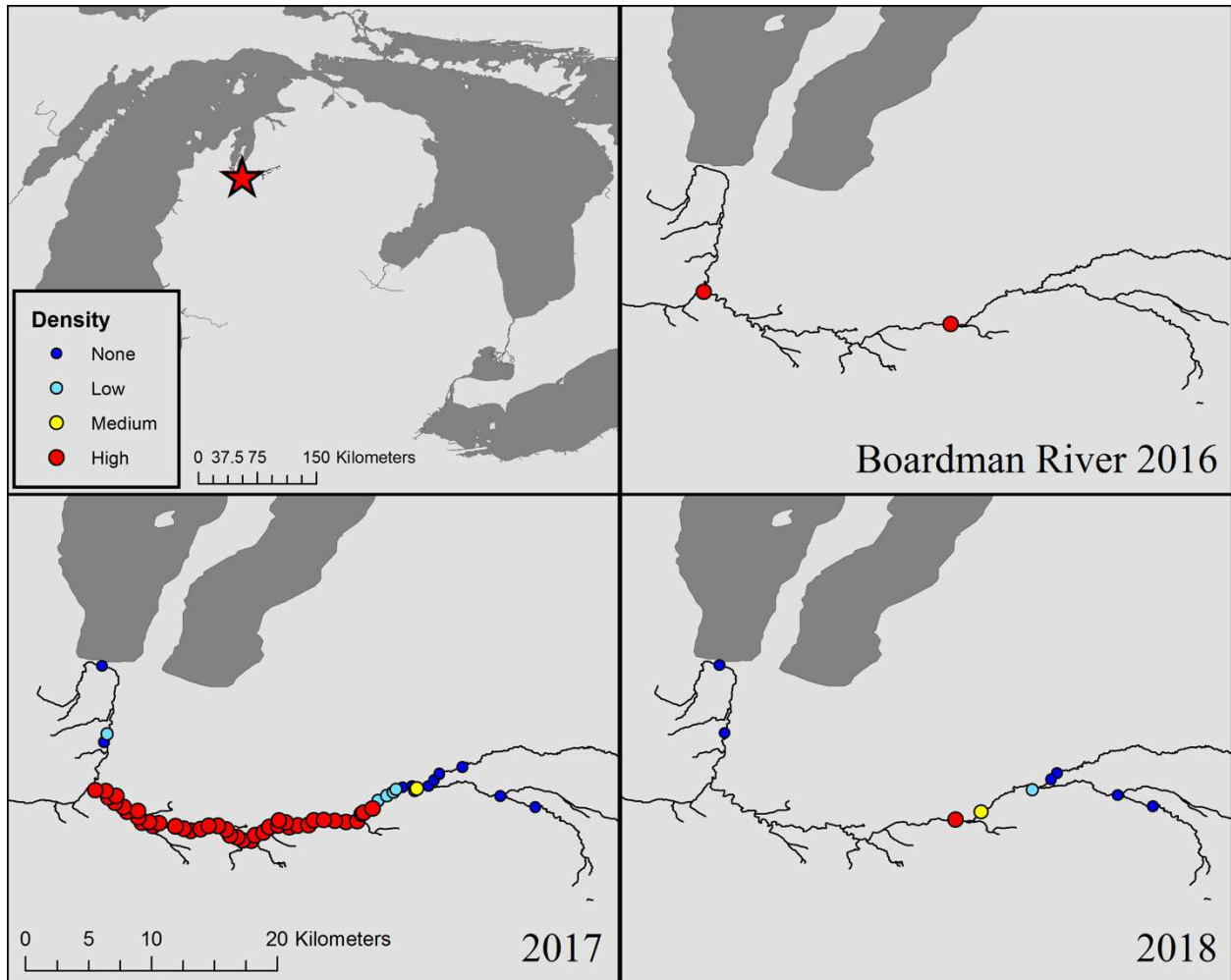


Figure 14. NZMS distribution and qualitative abundance in the Boardman River between 2016 and 2018. Possible density levels include none (0), low (1-10), medium (11-100), and high (100+). Based on surveys conducted by Michigan State University.

New Zealand Mudsnail Distribution within the Manistee River

Surveys in 2016 focused on the river reach below Tippy Dam, the most downstream dam in the Manistee river system. This area was selected because it is one of the most heavily fished sites in the region. No NZMS were detected in this area in 2016 (Figure 15). In early 2017, I sampled the downstream reach of the Manistee River, and NZMS were detected. In late 2017, an anecdotal report of NZMS in the upstream reaches of the Manistee River was received. I conducted additional sampling within the reported reach late in 2017 and confirmed the presence

of NZMS at a limited number of sites. Surveys in 2018 focused on the upstream reach and documented some range expansion. However, the distribution of sampling sites was limited (Figure 15).

A tributary of the Manistee River named the Pine River was reported to have NZMS in a reservoir upstream of a dam in late 2017. I surveyed the Pine in 2018 but was unable to access the exact reported location due to a lack of deep water sampling gear and found no NZMS in our other survey sites on the Pine. However, conditions (high water levels, high discharge, and turbid waters) made for non-ideal sampling conditions either year (2017 and 2018).

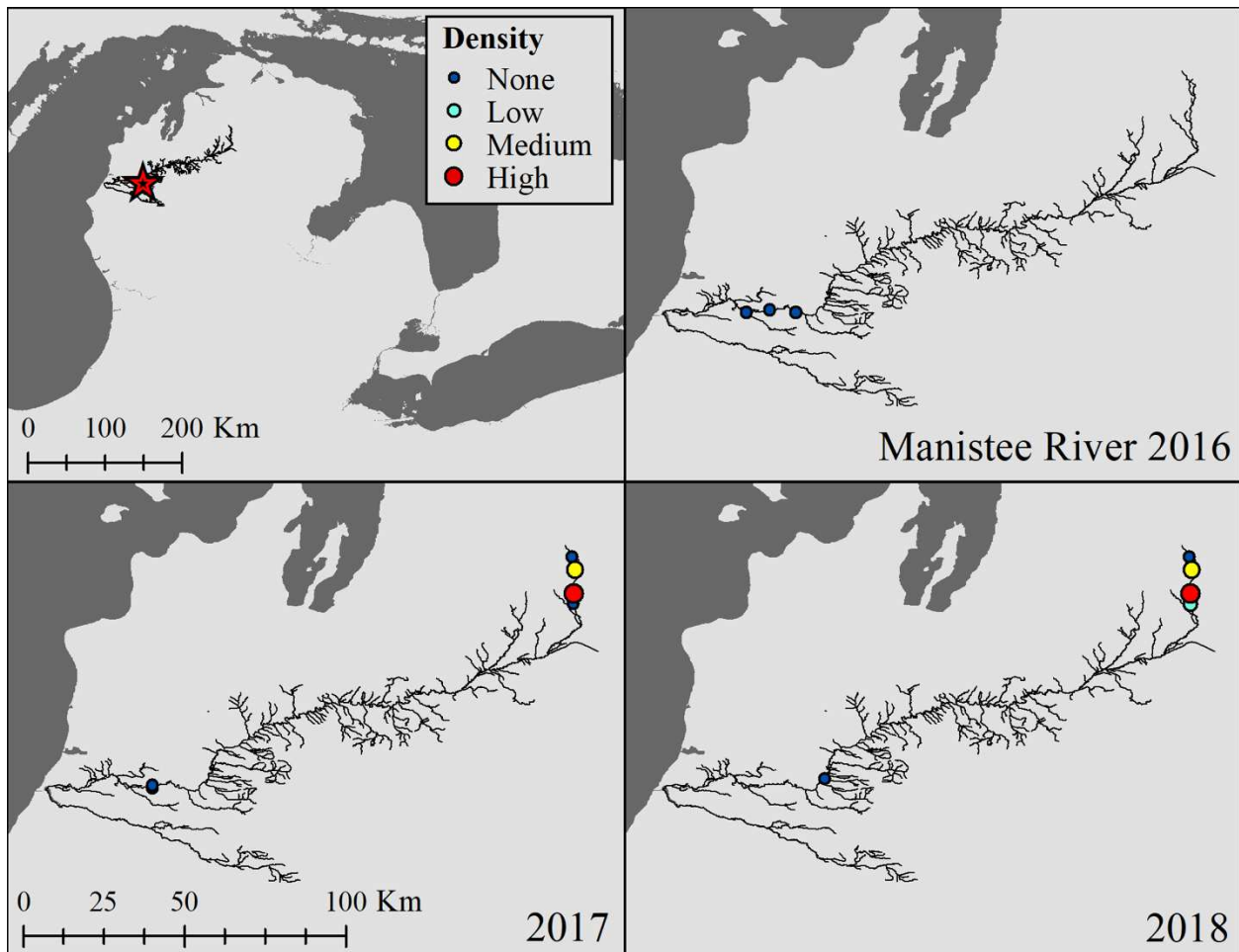


Figure 15. New Zealand mudsnail distribution and qualitative abundance in the Manistee River between 2016 and 2018. Qualitative density levels are none (0), low (1-10), medium (11-100), and high (100+).

DISCUSSION

As NZMS were not detected in Michigan's rivers until 2015, my initial assumption was that it was a relatively new invasion. However, given the relative densities observed and the extent of the infested area, it is more likely that NZMS had been established in some of Michigan's rivers for several years prior to initial detection. It is possible that the invasion went undetected for some time due to the low densities and small size of NZMS. NZMS are subject to the Allee effect where higher population densities resulted in a higher embryo production (Neiman et al. 2013). This suggests that the rate of spread and population growth may increase more rapidly over time. The highest relative densities recorded were much less than that of found in the western United States, where NZMS have been established for nearly a half century. Presuming that NZMS were recently established within Michigan's rivers and that the rivers provide a suitable habitat, the densities of NZMS are likely to increase. Future research is needed to track the quantitative densities of NZMS in the infested streams to determine if the current rate of increase is similar to what was experienced in the western U.S. rivers.

The broad-scale distribution of NZMS in Michigan rivers was discrete and disjointed, suggesting multiple introduction events. Invaded rivers are separated by multiple rivers where NZMS were not detected (Figures 10 and 11), even though the habitat appeared similar to sites with established populations. Based on the disjoint distribution and that the infested rivers are highly used by anglers, it is likely that the primary vector for the component of the invasion process are the anglers themselves. Uninvaded and presently invaded streams in this study share many characteristics. All are classified as coldwater fisheries and often used by trout or salmon anglers. Further, all appeared to have suitable habitat to support NZMS populations (personal observation, William Keiper with the Michigan Department of Environment, Great Lakes, and

Energy: personal communication). As discussed in Chapter 3, anglers commonly fish multiple rivers within the same trip. Thus, rivers in this study are at high risk of NZMS infestation.

Spread appears minimal on each of the three rivers where NZMS have been detected. NZMS spread can occur via two sources: natural and human mediated. Natural spread is defined as spread that occurs without the influence of people. Natural spread can take different forms including NZMS movement due to their own volition, transport by other species (fish, birds, other invertebrates), or drift (free drift or as a result of attachment; e.g., leaf packs or woody debris). NZMS has been documented moving up to 1 m/hour (Richards 2002 as cited in Proctor et al. 2007). Given their limited mobility, it is likely that long-distance travel within a river system is not due to their own active movement. In other areas where NZMS have established invasive populations, spread has been documented to increase exponentially (Loo et al. 2007). While spread is likely occurring each year, the granularity of this survey was not able to capture significant spread. The observed rate of spread across 2015-2018 appears to be relatively low, however this is based on a short time frame. Further, the shape of the NZMS distribution in the Pere Marquette and Boardman rivers suggests that NZMS dispersed from the point of initial introduction. The density and range of distribution in these rivers could have also resulted from multiple points of introduction that coalesced. The process of disjoint introductions becoming a single wider distribution was observed in the invasion dynamics of the beech scale insects in Michigan (Wieferich et al. 2011), quagga mussels in western Europe (Heiler et al. 2013), round goby in the Great Lakes basin (Sard et al. 2019), and crayfish in northern Wisconsin (Puth and Allen 2005). Given that I was unable to document significant range expansion during the course of this study, I hypothesize that the current range of NZMS within Michigan's inland waters is a result of both human mediated transport and natural diffusion.

I occasionally observed infested sites that were separated from the main body of infestation by sites where no NZMS were detected. Such sites could represent a new introduction or conversely could represent a lack of contiguous suitable habitat. As an example, the distribution of NZMS on the Boardman river ends abruptly after approximately 36 river kilometers of infestation from the upstream edge of their distribution. This sudden end to their apparent distribution is due to a series of dams and reservoirs that have been constructed along the river until it enters Lake Michigan. A low abundance of NZMS shells (not alive) were found downstream of one of the dams at the edge of a reservoir. It is possible that more snails were present in the reservoirs downstream of the dams, however it was not possible to sample the majority of those areas with the above listed protocols due to water depth.

One of my goals was to document spread within the river systems over a short period of time. I encountered difficulties achieving this goal. Setting up points based on the limited initial survey sites made it difficult to observe NZMS localized intra-river spread over the short timeframe of this study. The timing of 2018 survey events, which took place in May of that year, may have prevented me from capturing any spread that took place later in the year due to the seasonally low abundance of NZMS. Given the appropriate conditions, NZMS can reproduce year-round but are most fecund in early fall (McKenzie et al. 2012).

Based on my experience, I recommend that future efforts at detected spread of NZMS should focus sampling in late summer and early fall sampling as NZMS populations are at peak density during this time of year. I would also offer that detecting change would typically take a minimum of three years with consistent sampling. The first year is critical for establishing the approximate outer bounds of infestation. In the second year, sites should be located with

sufficient density expanding the range boundaries. In the third year, second year sites can be resurveyed to establish change in infestation status.

Where NZMS were found at high densities, I observed them utilizing all available substrates. At low relative densities, I observed NZMS to mainly occupy areas of deposition, generally attached to submerged aquatic vegetation, leaf packs, or woody debris, even when other substrates were available. This indicates that these habitats are potentially preferred by NZMS, and at high densities they broaden their habitat utilization.

No management options for control of NZMS are currently available. As such, this information may be used to guide outreach and engagement efforts to prevent further human mediated spread within and between rivers. Additional monitoring efforts may be useful to inform where additional outreach activities should focus and may inform future management activities. Outreach and engagement should be targeted to river recreationalists including anglers and boaters. There have likely been several introduction events leading to the disconnected distribution observed in several rivers, suggesting anglers or paddlers are moving this species. There remain a number of high risk rivers that appear un-infested. Working with anglers and paddlers to engage in best practices to minimize spread appears to be the best hope for containing NZMS, which does not have a currently approved treatment method.

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WORKS CITED

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CHAPTER 3: THE RELATIONSHIP BETWEEN ANGLER AWARENESS OF NEW ZEALAND MUDSNAIL INVASION AND ACTION TAKEN TO PREVENT SPREAD

INTRODUCTION

New Zealand mudsnails *Potamopyrgus antipodarum* (hereafter NZMS) are an invasive species recently detected in Michigan. Native to New Zealand, NZMS have established populations in Australia, Asia, Europe, and most recently North America (Loo et al. 2007, Hamada et al. 2013, Ponder 1988, and Bowler 1991). The species was first detected in the United States in the Snake River, Idaho in 1985 and have since been detected in lotic and lentic environments across the country (Bowler 1991; Figure 16). The biology of the NZMS makes this organism a successful invader. In the introduced range, NZMS populations consist of entirely parthenogenetic females that produce clones asexually (Dybdahl and Kane 2005). Two genetically distinct clones are known to exist within North America (Levri and Jacoby 2008). The ability to reproduce asexually along with their high fecundity (McKenzie et al. 2012) allows the species to establish a population with a single individual, which is of particular concern to resource managers as even low propagule pressure could result in a new infestation. Furthermore, NZMS are small in size averaging approximately 12 mm in their native range and are typically even smaller (2-6mm) in areas of invasion because of genetic mutations (McKenzie et al. 2012; Alonso and Castro-Diez 2008; Winterbourne 1970)). Their small size makes the species difficult to detect. Therefore, when NZMS attach themselves to recreational gear or equipment, the likelihood of being unknowingly transported to other waters via human vectors such as anglers and other river recreationalists is increased.

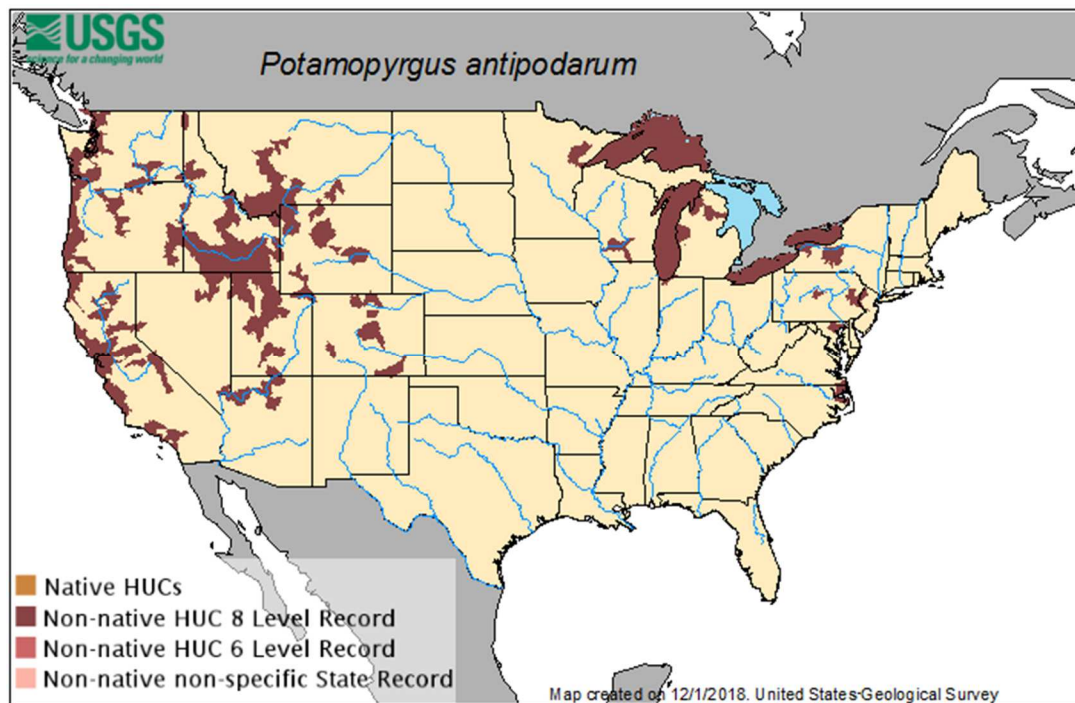


Figure 16. Distribution of the New Zealand Mudsnail in North America. Credit: USGS 2018.

The initial detection of NZMS in Michigan's inland waters occurred in 2015. Invaded rivers in the western United States have reported NZMS densities in the hundreds of thousands per square meter (Bowler 1991; Hall et al. 2006). At these densities, NZMS has been reported to have negative impacts on par with those of zebra mussels (Hall et al. 2003) and can change the structure of the invaded ecosystem. At such high densities, NZMS outcompete native macroinvertebrates that are an important food source for sport fishes (Maret et al. 2008). Currently the NZMS invasion within Michigan is isolated to 5 rivers across the Lower Peninsula and generally occur at relatively low densities but increases in densities and future introductions seem inevitable if vectors of spread remain unimpeded.

The distribution of introduced populations combined with population characteristics and an understanding of historic pathways of introductions are useful information to consider when attempting to determine potential risk of spread. NZMS are known to exist in the Laurentian

Great Lakes and several of Michigan's rivers. However, genetic analysis indicated that the clone found in the Laurentian Great Lakes differs from that found in Michigan's inland waters (Zaranko et al. 1997). The population within the Great Lakes is a clone that matches one found in Europe, suggesting introduction via ballast water (Levri et al. 2008). The inland clone is the same as one found in the Western United States (personal correspondence with Michigan Department of Natural Resources 2016) suggesting that introduction into Michigan's inland waters occurred directly from that region.



Figure 17. NZMS attached to trash pulled out of the Pere Marquette River in Michigan. Photo credit: William Keiper.

Limiting the spread of aquatic nuisance species (ANS) is a societal task that requires broad-scale cooperation and will reduce the future costs of management. Many vectors of ANS transport have been identified including shipping (e.g. ballast water [Ricciardi 2006] and

packaging materials), accidental and intentional release from the pet trade (Padilla and Williams 2004), accidental introduction concurrent with other intentionally transported aquatic organisms (e.g. fish stocking programs, bait, and aquaculture [Kerr et al. 2005]), and recreational users (e.g. boaters and anglers [Anderson et al. 2014, Johnson et al. 2001]). In the case of NZMS, anglers likely play a significant role in their movement and introduction. As evidence of this, A strong correlation has been found between angling activity and the presence of NZMS (Lee et al. 2007), where anglers are a likely vector of long-distance movement. This long-distance transport is possible due to the ability of a NZMS to seal its operculum, allowing it to survive nearly 2 days on a dry surface and up to several weeks with limited moisture (Alonso and Castro-Diez 2012; Oregon State University 2010; Haynes et al. 1985). During this study, we observed NZMS attached to a variety of surfaces including skin, clothing, waders, and trash (Figure 17). This suggests that anglers can actively, yet unintentionally, transport NZMS within and between water bodies.

The initial or primary introduction of an invasive species into a waterbody, and the subsequent or secondary introductions into a waterbody that already been invaded, even on a local scale, increases the rate of ANS spread and establishment, making management of these species less predictable and effective (Vander Zanden and Olden 2008). To help reduce the probability of accidental transport of ANS by the angling community, awareness and subsequent behavioral changes must occur. Anglers are a likely source of spread within and between Michigan rivers and additional information is needed to identify the magnitude of risk (i.e., awareness NZMS invasion, angler movement, and what if any decontamination techniques are being implemented). This effort can be made easier by identifying the groups of anglers that are

most likely to spread NZMS and developing targeted engagement specific to each of those groups.

Under the assumption that anglers are a source for spreading NZMS, the first goal of this study was to understand the relationship between angler awareness and action taken by anglers to reduce the spread of NZMS. Determining the relationship between awareness and action will provide insights to guide angler outreach and engagement efforts to positively influence behavioral change and enhance natural resource protection from the introduction of invasive species. The secondary research goal was to document the actions anglers are presently taking to prevent the spread of non-native species. To achieve this goal, I identified the types of actions anglers who are aware of the NZMS infestation are taking to prevent their spread, specifically what wader decontamination techniques are being implemented. Wader boot type was also evaluated in accordance with this goal. Felt vs. rubber surfaces (boot soles) have a different risk for harboring ANS and may influence the effectiveness of decontamination efforts (Bothwell et al. 2009). Additionally, I conducted a literature review to determine the effectiveness of the decontamination techniques that anglers reported implementing. My final goal was to understand angler movements between rivers to identify rivers that were susceptible to NZMS introduction. Angler movement, especially travel immediately prior and following fishing trips in an infested river, can inform which other rivers are at an increased risk of future infestation. This information can then guide early detection monitoring efforts for rivers at-risk of future NZMS invasion. To date, NZMS have been detected in the Pere Marquette, Au Sable, Boardman, Manistee, and Pine rivers, totaling greater than 65.6 documented river kilometers (Figure 18). These rivers support thousands of angler days per year, hold both economic and intrinsic value for the state, and four of which (the Pere Marquette, Au Sable, Manistee, and Pine Rivers) are

federally recognized as National Wild and Scenic Rivers. All NZMS infested rivers in Michigan support economically important trout fisheries (the Pere Marquette River also supports a popular salmon fishery) that draw anglers from across the country. Un-infested Michigan rivers are currently at risk of introduction from NZMS in infested rivers within the state and in other areas of the country. The Western United States has desirable trout and salmon fisheries that draw similar anglers as Michigan. Understanding angler movement between rivers in the West can help us to identify additional at-risk rivers within Michigan. Achieving these goals will 1) help refine current gear decontamination recommendations based around which techniques anglers are already implementing and 2) identify the risk of NZMS spread as the survey provides information on the proportion of anglers taking decontamination steps along with their movements from infested to un-infested locations.

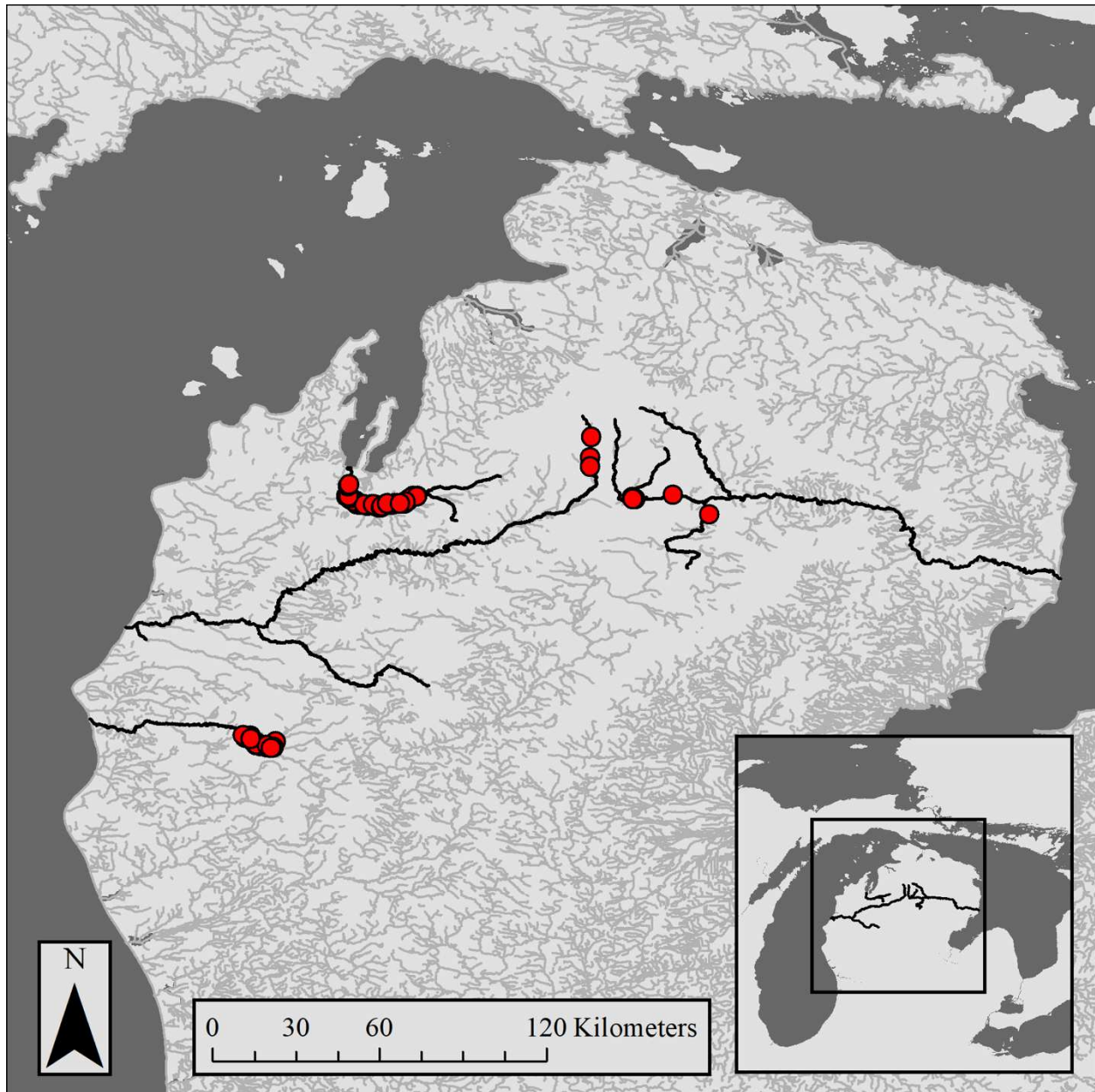


Figure 18. NZMS have been detected in 5 Michigan rivers including the Pere Marquette, Au Sable, Boardman, Manistee, and Pine Rivers. Red dots indicated points of detection as found by a series of detection surveys conducted by Michigan State University (MSU) and the Michigan Department of Environmental Quality (MDEQ; now the Michigan Department of Environment, Great Lakes, and Energy). Locations of detection on the Pine River are not indicated on this map as they were not confirmed by MSU/MDEQ surveys.

METHODS

Study Sites

My study site encompassed United State Forest Service angling access sites located along approximately 32 river kilometers of the Pere Marquette River, Lake County, Michigan. The Pere Marquette was selected for the study as it was the site of first detection for NZMS in Michigan's inland waters. The upstream boundary of the surveyed river reach began at 72nd Street in Baldwin, MI and continued downstream to the Upper Branch in Branch Township, MI (Figure 19).

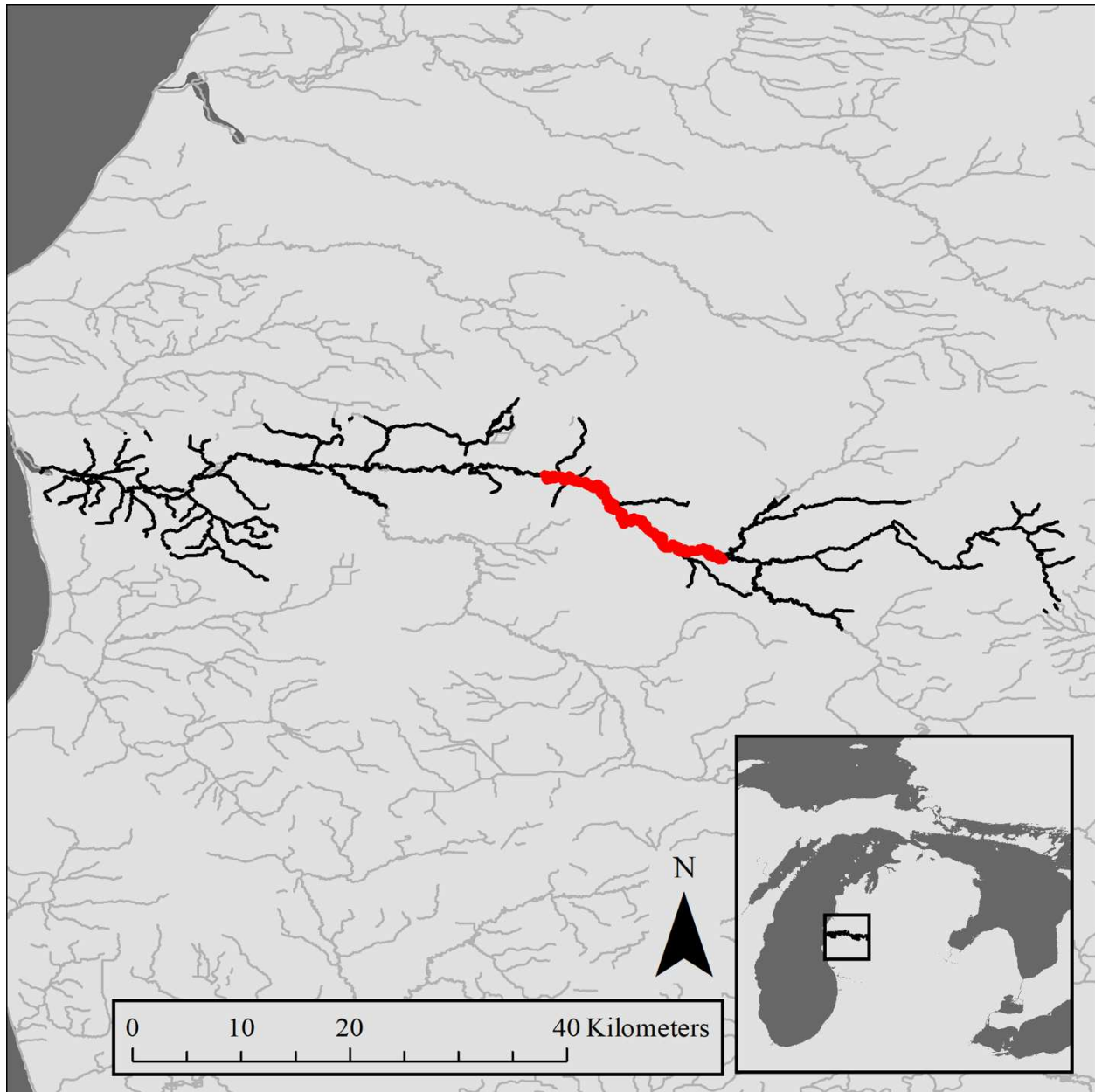


Figure 19. Map of the survey range on the Pere Marquette. Red indicates extent of survey locations.

Angler Survey

The survey was designed collaboratively between Michigan State University and the Michigan Department of Natural Resources. Prior to implementation, the survey was reviewed by the Internal Review Board at Michigan State University and was granted an exempt status (IRB# x16-944e; category: exempt 2; approval date: August 1, 2016; Appendix C). An angler

survey was conducted at high use locations on the Pere Marquette River near Baldwin, Michigan between July 2016 and October 2017. A roving survey design was implemented that consisted of interviewers seeking out anglers by walking trails along the river or within the river until at least one angler was encountered. This method was used to maximize the number of responses per time spent conducting the survey. Students from Michigan State University were hired to conduct surveys. Surveys during the first month of sampling were distributed to all anglers encountered, but after this time, when a group of anglers was encountered, a single angler from the party was surveyed to decrease potential bias in their answers. We identified ourselves to anglers as students from Michigan State University, told them we were conducting an angler survey, and asked if they would like to participate. If they agreed to participate, we would read the consent form and have them initial the survey as a sign of consent. The survey questions were read in order to the anglers and their responses recorded.

We designed a series of questions to better understand the connections between NZMS distribution, movement, wader type, wader decontamination, and angler awareness. Questions were phrased without judgment and no correct answer was implied. All leading questions (e.g. “*How do you decontaminate your waders in between fishing trips?*”) were avoided.

The survey took approximately 5-10 minutes to conduct, during which anglers were asked the 8 following questions (Appendix D):

1. Which zip code do you live in?
2. What type of footwear [wader type] will you be using during your fishing trip?
3. Have you fished another river within the last 2 days? Which river(s)?
4. Do you plan to fish another river within the next 2 days? Which river(s)?
5. Have you fished in a Western State? When and where?

6. Do you disinfect or clean your waders in between fishing trips? How?
7. Were you aware that invasive New Zealand mudsnails were detected in Michigan Rivers in 2015? How did you hear about the infestation?
8. Have you taken this survey during a previous fishing trip?

For question 2, angler wader type was visually observed by the interviewer or anglers were asked to respond when visual observation was insufficient. Five wader types were identified during survey development (Table 7).

Table 7. Five wader types were determined prior to the survey; each angler surveyed was evaluated for wader type.

Wader Type	Definition
Boot-Foot Felt Sole	Waders with boot bottoms attached; boots with a felt sole
Stocking-Foot Felt Sole	Waders with stocking feet attached and removal boots; boots with a felt sole
Boot-Foot Other Sole	Waders with boot bottoms attached; boots with a sole that was another material than felt (e.g. rubber)
Stocking-Foot Other Sole	Waders with stocking feet attached and removal boots; boots with a sole that was another material than felt (e.g. rubber)
Other	Non-wader foot wear or different than described above (e.g. rubber boots)

Analysis

I coded survey responses to organize the open-ended responses into discrete categories, then summary statistics were computed using SAS (SAS Institute 2005). To code the survey responses, I created categories that could house a variety of the open-ended response (i.e., when asked how did you hear of the NZMS infestation, responses of “fellow angler” and “friend” were grouped in the “Word of Mouth” category; Table 8).

Table 8. Qualitative survey responses were coded for quantitative analysis.

Question	Do you disinfect or clean your waders in between fishing trips? How?	Were you aware that invasive New Zealand mudsnails were detected in Michigan Rivers in 2015? How did you hear about the infestation?
Code	409 Soap Vinegar Wash Rinse Hot Water Bleach Cleaner Scrub Dry Wader Wash Station Lysol Powerwash Wipe Alcohol	Access Point Sign Fishing Community Word of Mouth Department of Natural Resources Trout Unlimited Internet Print Media (Magazine/Newspaper) Other Social Media Radio Sign Out West Class at Michigan State University

Certain assumptions had to be made when categorizing the responses. For instance, when anglers responded to the “How?” portion of the wader disinfection question, a response of “use soap and water” and “wash” were lumped together and a similar level of effectiveness (at inducing NZMS mortality) was assumed. These grouping assumptions allowed for discrete categories that could more easily summarize the range of responses that anglers provided.

Angler-provided zip codes were used to establish the home range of anglers and to understand angler movement. GPS coordinates were established from the center of zip code location and used to estimate a distance traveling to the fishing location using Google Maps. The estimated distance traveled was based on the shortest distance (in miles) that the angler would have traveled by car between their zip code established location and the fishing access point.

I determined the relationship between awareness of NZMS infestation and action taken to decontaminate using the Fisher's Exact Test on the contingency table. A hierarchy of risk was then established given the various levels of awareness and action that each angler reported.

RESULTS

A total of 308 surveys were conducted between the summer of 2016 and fall 2017. The majority of anglers were approached and surveyed before or during their fishing trip (n=251), and most anglers fished via wading (n=227). Nine anglers reported taking the survey during a prior fishing trip. Of the 308 anglers surveyed, 78% were from Michigan. Anglers also originated from 14 other states (Figure 20).

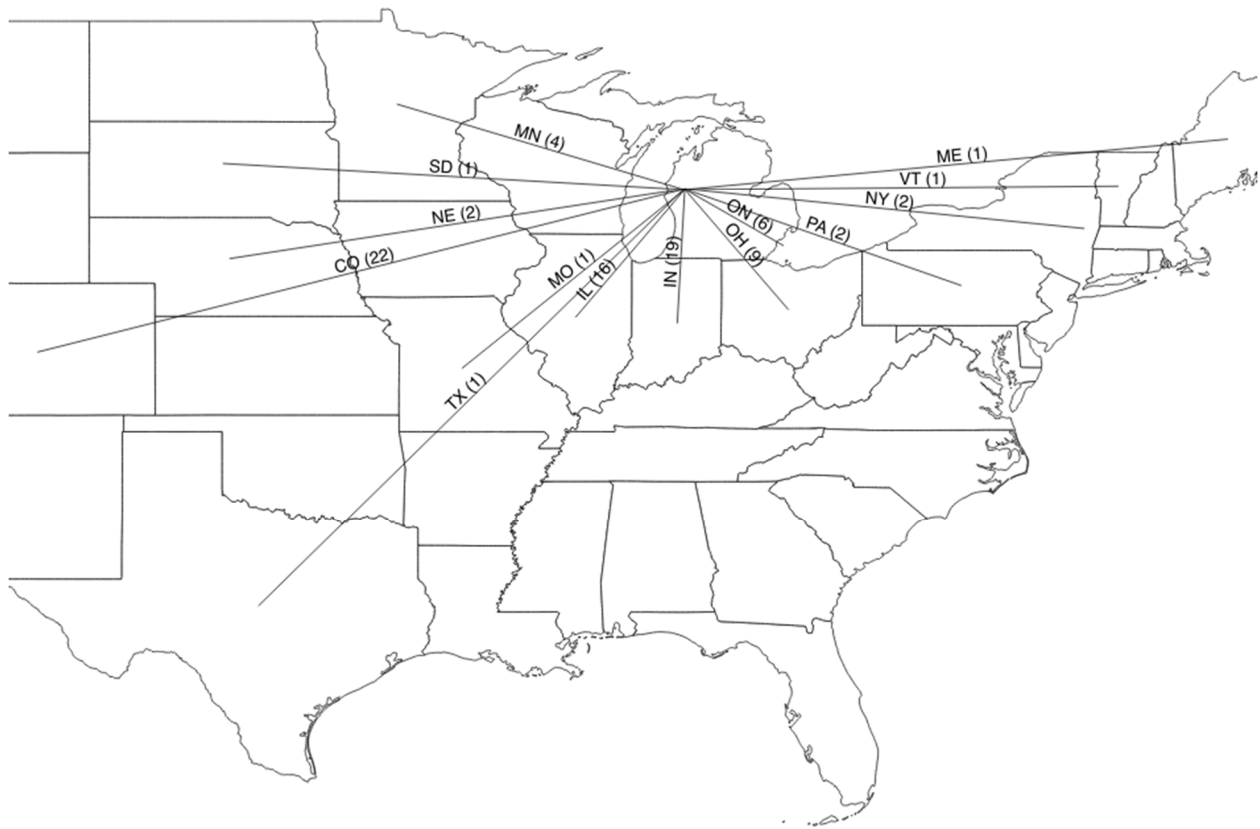


Figure 20. Home states of anglers surveyed, outside of Michigan. Of the 308 anglers surveyed, 240 were from Michigan. Numbers in parentheses indicate number of respondents from the given state.

Anglers travelled an average of 191 miles and traveled as far as 1,364 miles and as nearby as Baldwin, MI (<5 mi). The majority (76 %) of anglers travelled between 51 – 300 miles to reach the Pere Marquette (Figure 21).

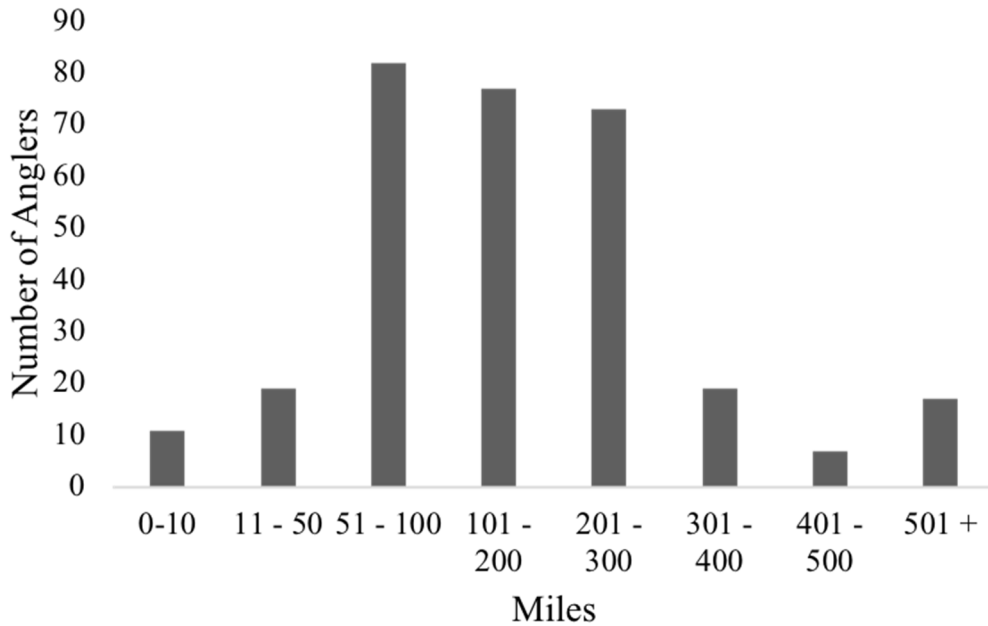


Figure 21. Distance traveled by anglers to reach the Pere Marquette from their home.

The most common wader type of the anglers surveyed was Boot-Foot Other Sole (40%), where “other sole” was being defined as not felt (Figure 22). The majority of these anglers used boot-foot waders with rubber sole bottoms. Stocking-Foot Other Sole was the second most popular wader type, also with the majority having rubber bottom boots. Boot-Foot Felt Sole and Stocking-Foot Felt Sole followed next in popularity. In combination, 27% of anglers used waders with felt soles, and 73% of anglers had waders with other soles or did not wear waders at all.

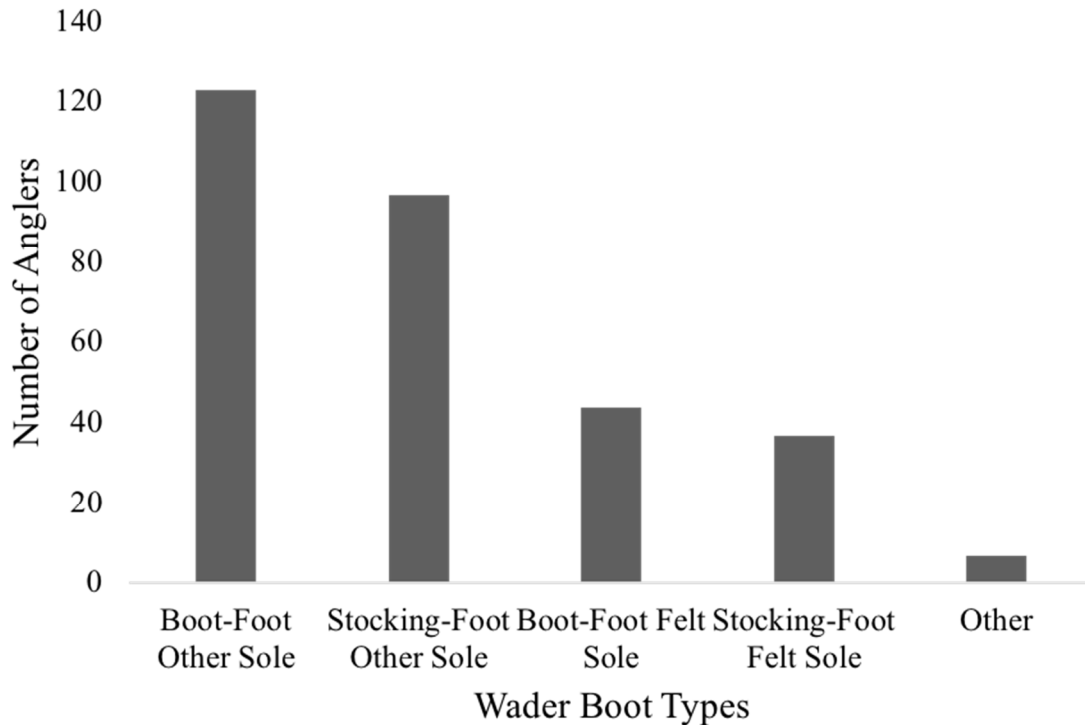


Figure 22. Distribution of usage of different wader types among anglers surveyed on the Pere Marquette River.

Only 11% of anglers reported traveling to another river within the two days prior to fishing in the Pere Marquette. Similarly, 13% of anglers reported planning to travel to other rivers within the two days following their current fishing trip. Anglers listed 14 rivers they planned to visit, including the Boardman and Manistee Rivers which are known to already have NZMS (Figure 23). When anglers were asked if they had ever fished in a western state, 41% responded that they had. The specificity of the responses to where the anglers had fished in the western U.S. varied. Some anglers simply provided the state they had fished and an approximate date, while others provided specific rivers and exact dates. Of the nine western states anglers reported traveling to, eight have known established populations of NZMS (Figure 24). Anglers who reported fishing in western states, traveled to another river two days prior and/or following

their trip on the Pere Marquette 25% of the time. This is in contrast to the 16% of anglers that have not fished in a western state but did report traveling to or planning to travel to another river two days prior and/or following their current trip.

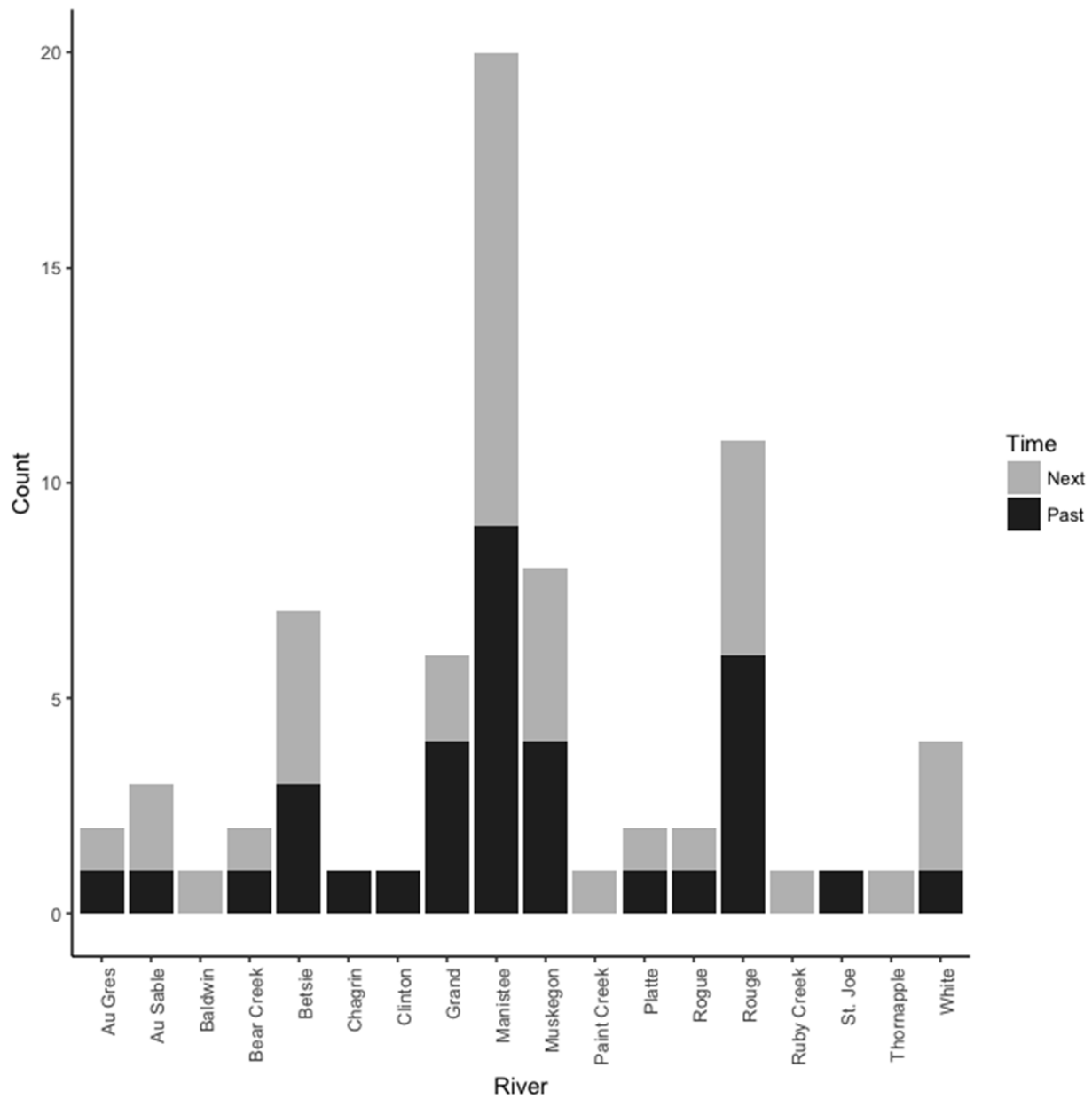


Figure 23. Rivers traveled within 2 days pre and post fishing in the Pere Marquette.

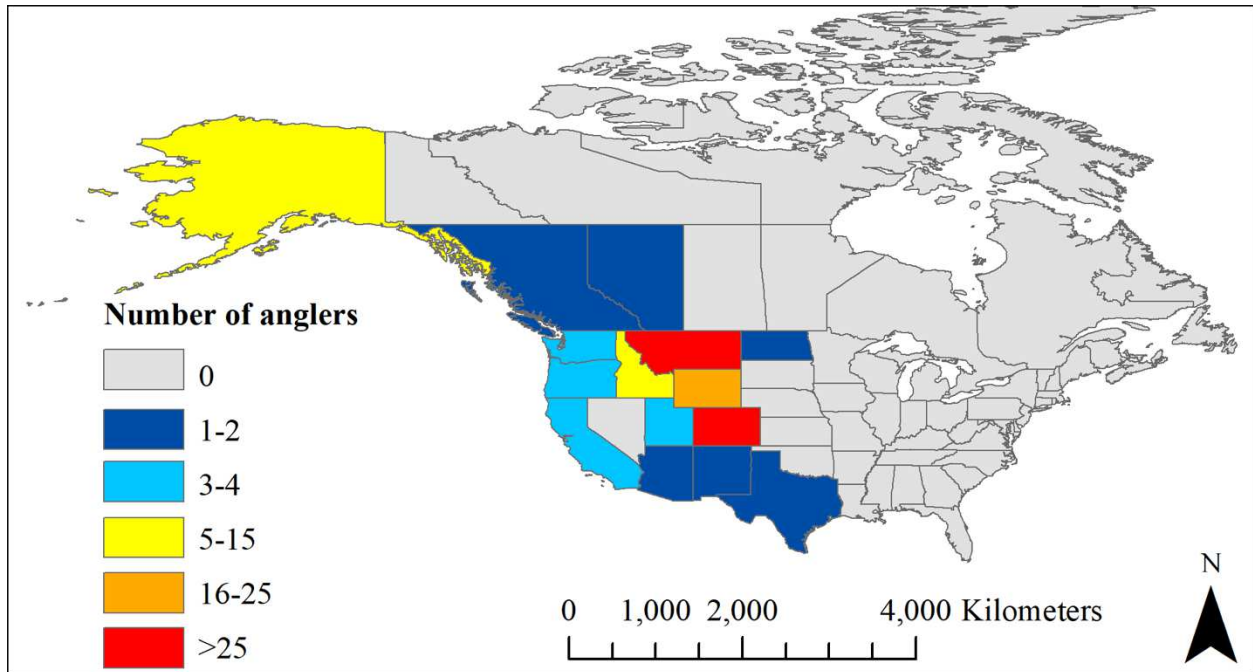


Figure 24. Map of western states that surveyed anglers have fished at some point prior to fishing the Pere Marquette River. Anglers were asked if they had ever fished in the western United States. Of the nine western states anglers reported traveling to, eight have known established populations of NZMS.

Approximately 52% of anglers reported they were aware of NZMS prior to the survey. The largest percentage of anglers with previous knowledge of the NZMS infestation reported learning of NZMS from the NZMS signage that was posted at river access points across the state (38%; Figure 25). Other common methods of learning included information from the Michigan DNR (8%), Trout Unlimited materials (7%), and word of mouth/the general angling community (18%).

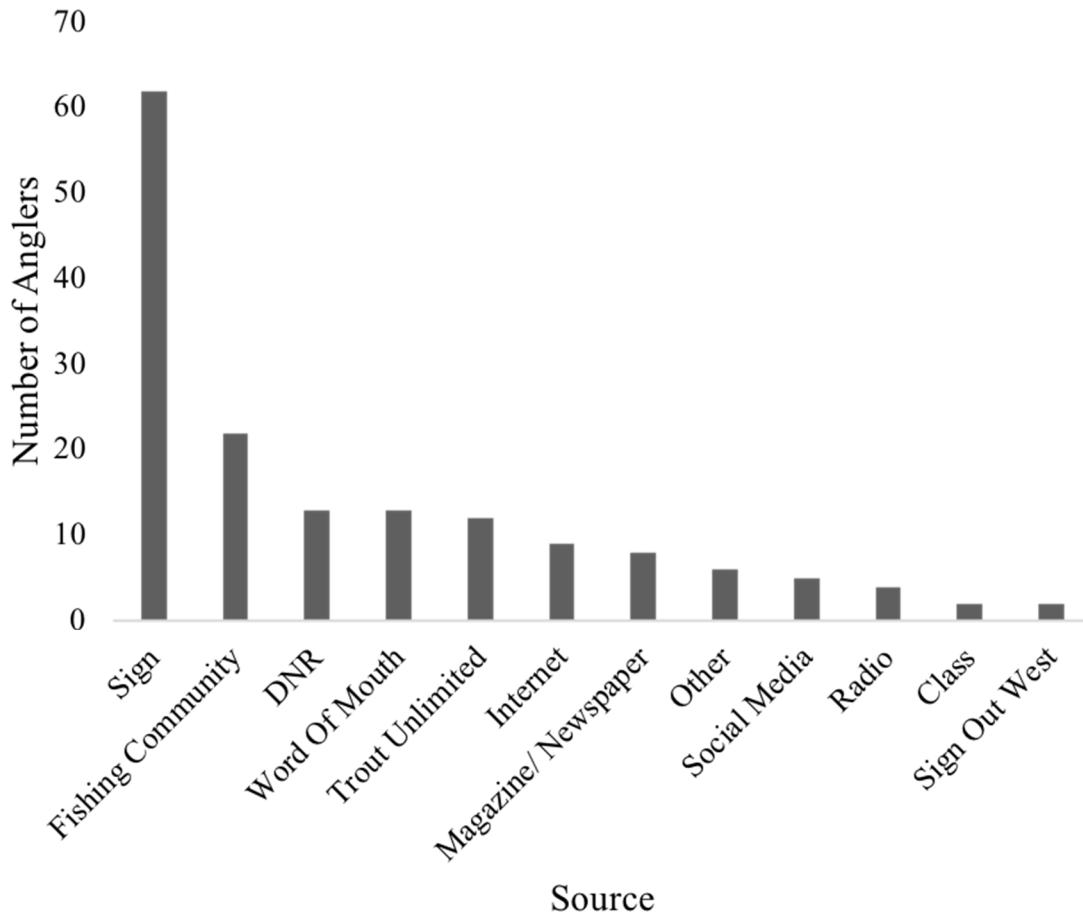


Figure 25. The source where anglers first learned of NZMS invasion in Michigan.

Of the 134 total anglers reporting that they took steps to disinfect their waders, the most common method reported was rinse (47%; Figure 26). Other popular methods included using soap (23%), drying (23%) washing (12%), and applying bleach (12%). Forty-seven anglers reported taking multiple decontamination actions (e.g., scrub and rinse waders); both actions were coded and reported in the summary (Figure 26). Some anglers reported using different washing techniques for different fishing situations. For example, some anglers reported only taking steps to decontaminate when they were moving between rivers and not between sites on

the same river. Other anglers reported using a different pair of waders and boots during their next planned fishing trip to allow their recently used waders to dry for a number of days.

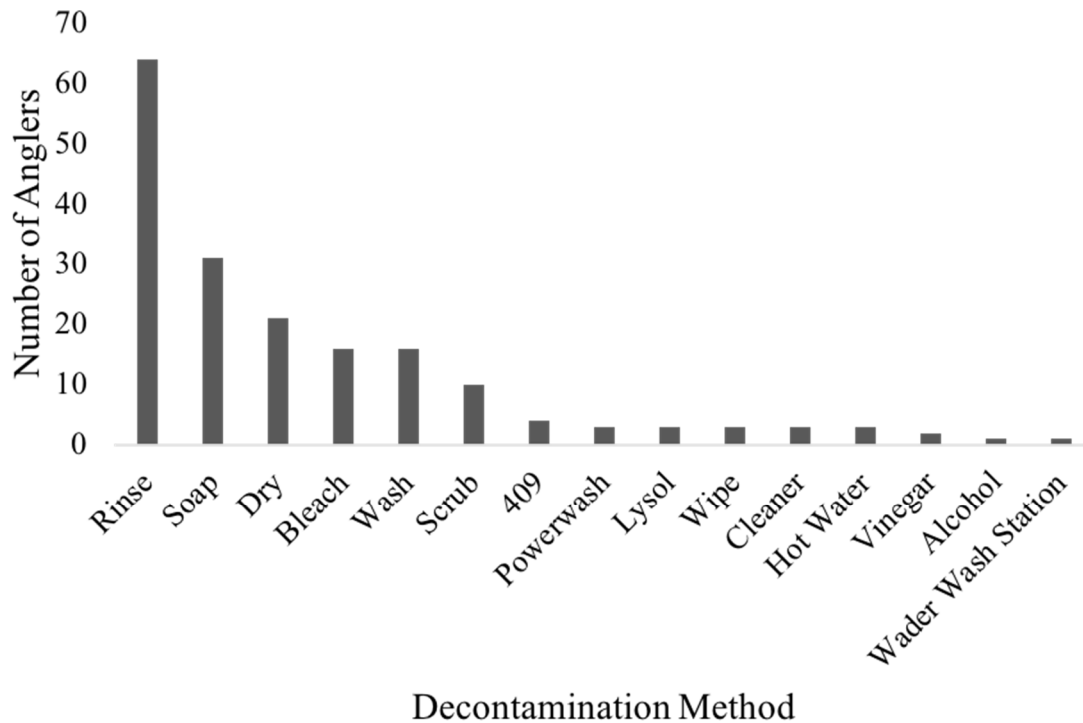


Figure 26. Wader decontamination methods of anglers.

No significant difference was found between the awareness of the NZMS infestation and action taken to decontaminate waders (Fisher's Exact Test, $P=0.30$; Figure 27). Of the 161 anglers that reported being aware of NZMS in the Pere Marquette, 55% stated that they do not decontaminate their waders between fishing trips (Figure 28). Among anglers reporting being unaware of the NZMS infestation, 60% reported not disinfecting waders between fishing event (Figure 29). Of the 55% of anglers that are aware of NZMS and not decontaminating, 28% ($n=24$) are aware, report traveling to another river within the two days prior to fishing in the Pere Marquette, and do not decontaminate (Figure 28).

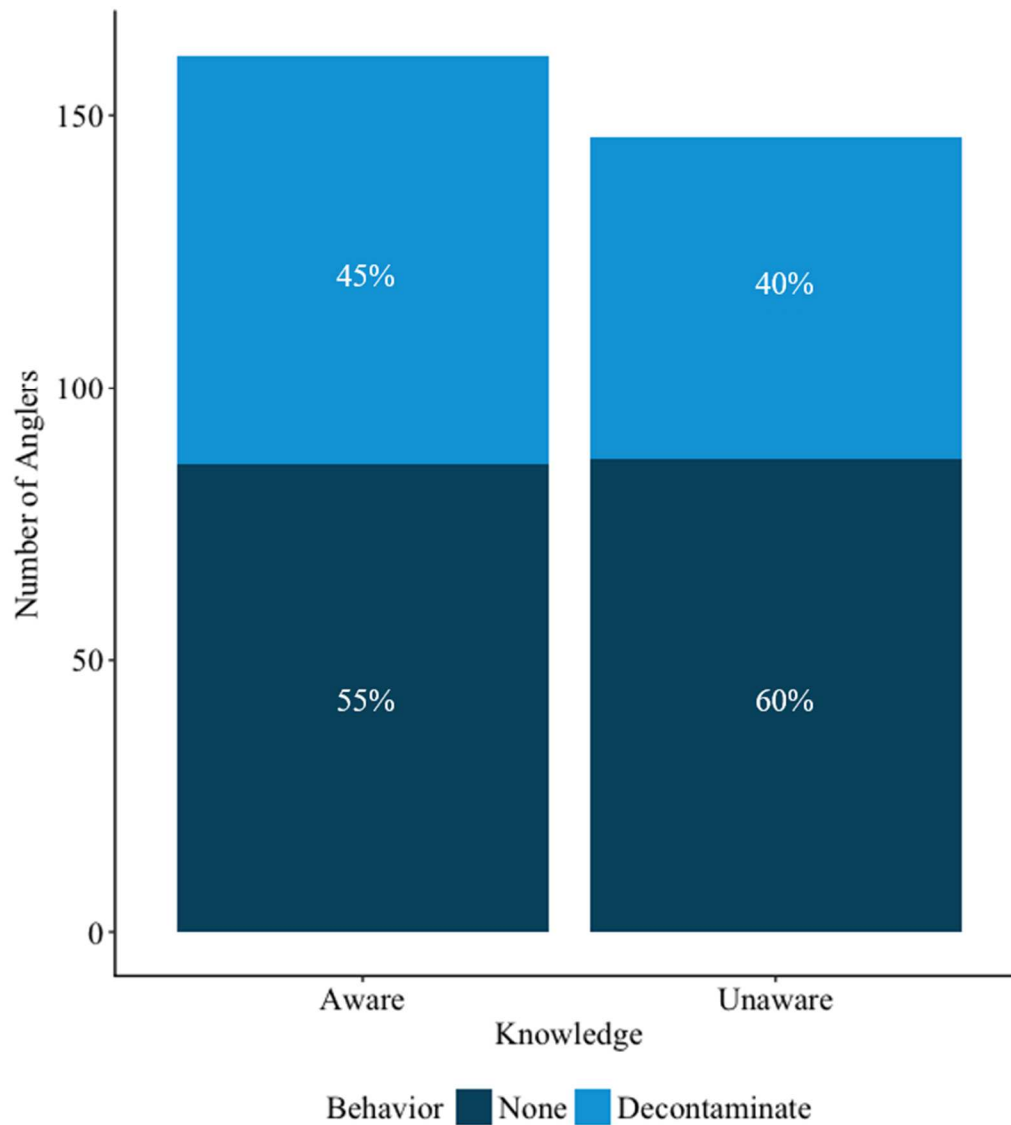


Figure 27. No significant difference was found between the awareness of the NZMS infestation and action taken to decontaminate waders (Fisher's Exact Test, $P=0.30$).

Not all anglers pose the same risk of incidental transport of ANS. I developed a series of flowcharts with the intent of characterizing the relative risks of transporting NZMS based on angler awareness, decontamination behavior, and travel behavior (Figures 28 and 29). The risk associated with aware anglers is likely less than those who are unaware. Likewise, risk

associated with anglers that take actions is likely lower vs anglers who do not. Of the 161 anglers that are aware, the 55% that do not decontaminate pose a higher risk of transporting NZMS than the 45% that do decontaminate (Figure 28). When we break that 55% of anglers that are aware but do not decontaminate down further, the 28% that reported traveling between rivers pose a higher risk of spreading NZMS to uninfected locations than those who do not travel. Aware anglers were ranked as being less risky than those who are unaware based on the assumption that aware anglers are more likely than unaware anglers to take action to remove NZMS if they are found on wading equipment. Of the 147 anglers that reported being unaware of NZMS, the 60% that do not decontaminate their waders are at a higher risk of transporting NZMS than the remaining 40% that do decontaminate for reasons other than NZMS (Figure 29). Going a step further, the unaware anglers that do not decontaminate but do travel to other rivers (14% of unaware anglers surveyed) pose the highest risk of spreading NZMS to un-infested rivers.

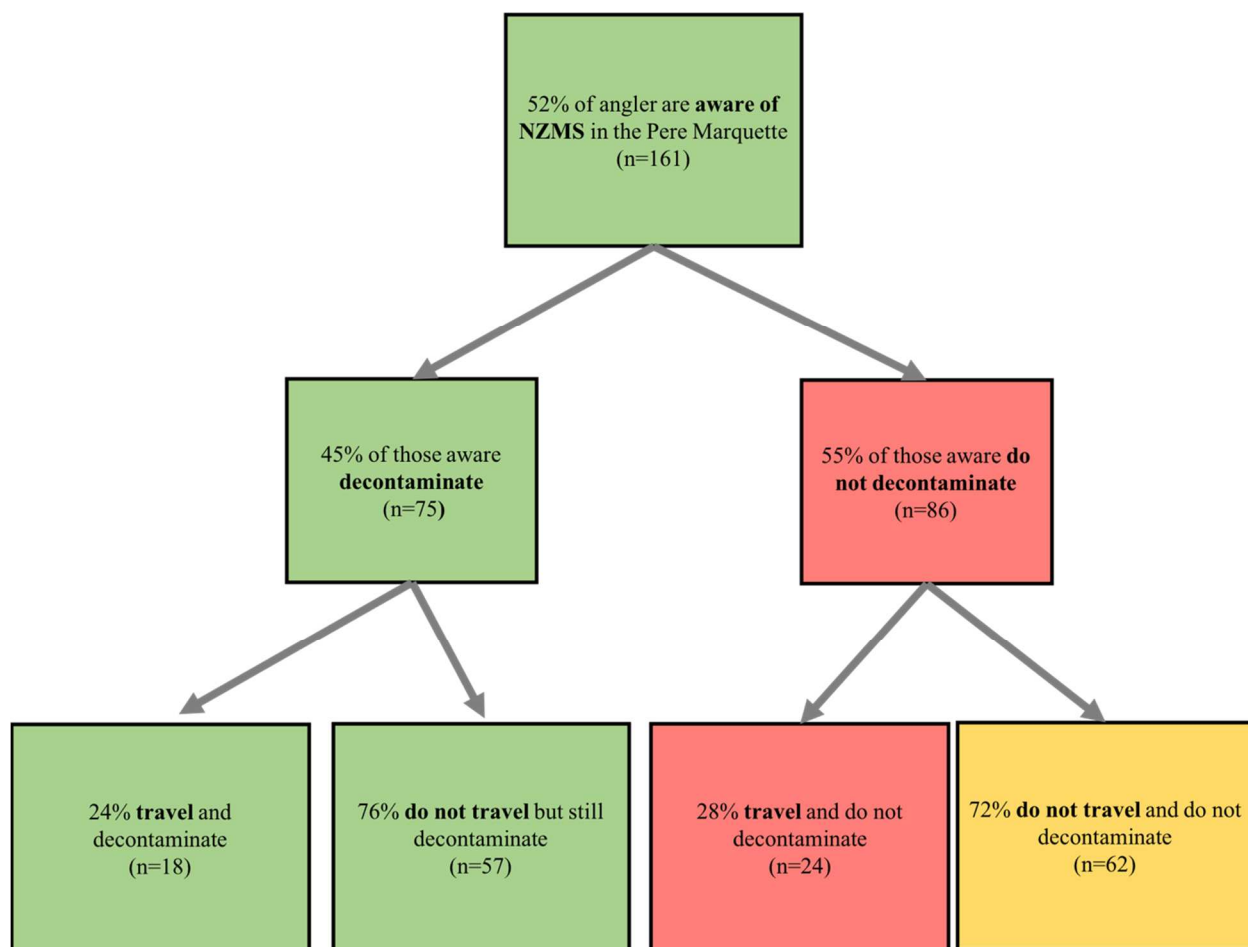


Figure 28. Anglers who are aware of the NZMS infestation in the Pere Marquette River exhibit behaviors with varying levels of risk. The green boxes are the least risky behavior and should result in the lowest likelihood of NZMS spread. The red boxes are the riskiest behaviors and are most likely to lead to NZMS spread. The yellow box represents a moderate level of risk.

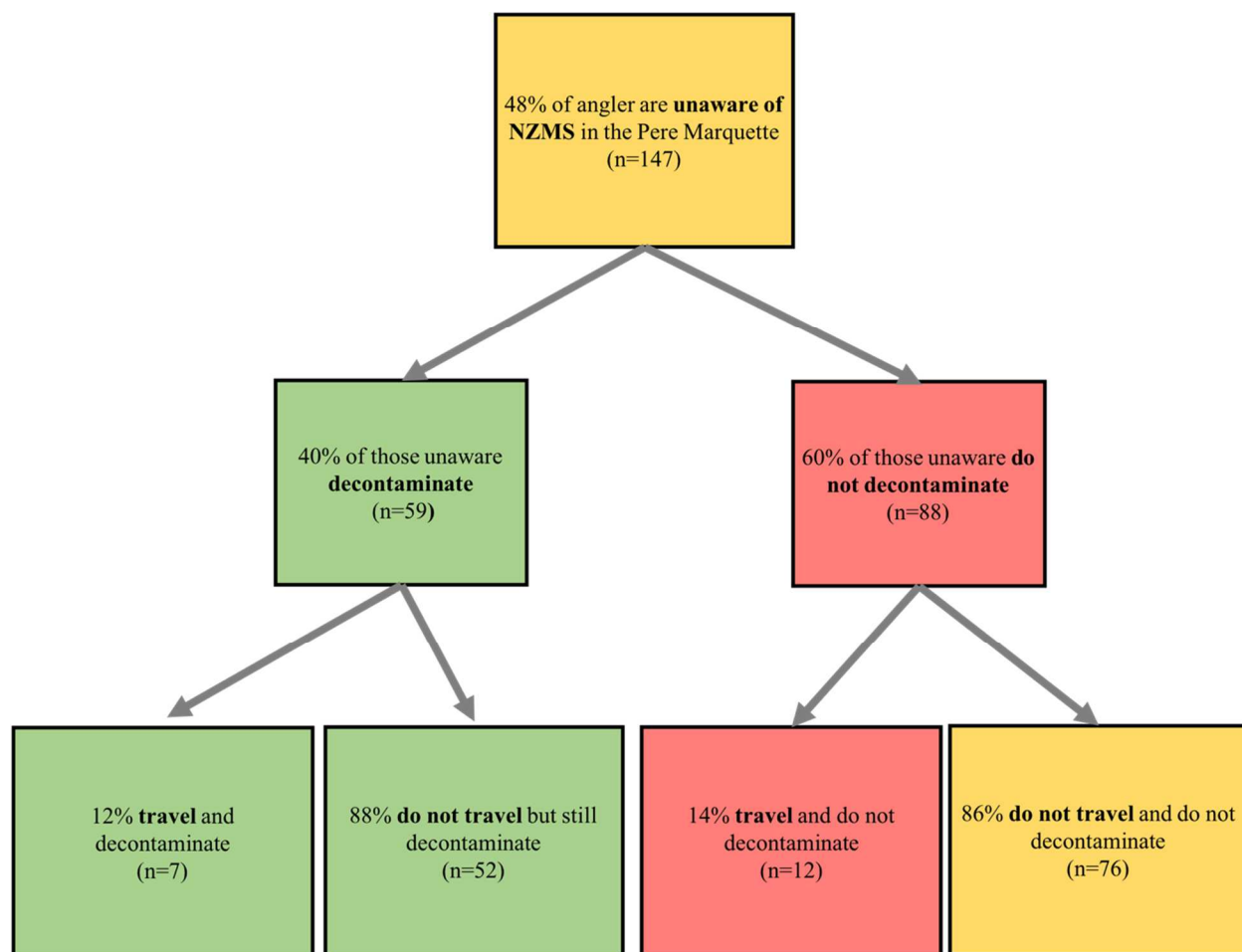


Figure 29. Anglers who are unaware of the NZMS infestation in the Pere Marquette River exhibit behaviors with varying levels of risk. The green boxes are the least risky behavior and should result in the lowest likelihood of NZMS spread. The red boxes are the riskiest behaviors and are most likely to lead to NZMS spread. The yellow boxes represent a moderate level of risk.

DISCUSSION

One of my major findings was that wader decontamination behavior did not vary with the level of angler awareness of NZMS. The disconnect between knowledge and behavior is a significant concern for management agencies tasked with informing stakeholders of environmentally responsible behaviors. I feel that examining the disconnect between knowledge and behavior in a broader social science perspective is useful toward addressing the problem. Theories of human behavior posit that knowledge of an issue alone is often not the driving force

behind action; instead the action (or behavior) is more closely linked to the attitude that the individual holds surrounding the issue (Ajzen 2001; Garrison 1995). Additionally, outside factors and the setting that issues are based in are thought to be more influential on behavior than knowledge of the issue (Heberlein 2012). This notion is supported by the results of my study; awareness of NZMS is not driving anglers to take proactive measures (decontaminating wading gear) to prevent further spread. This presents the question of what can managers do to influence the attitudes of anglers, so they are driven to action? I would argue that more attention needs to be paid to changing the attitude, and if possible, the culture and social norms around invasive species prevention. Effective engagement is not a singular one-size-fits-all activity. Instead, multiple points of engagement must occur with messages tailored to the various situations. This idea is demonstrated in Figure 30.

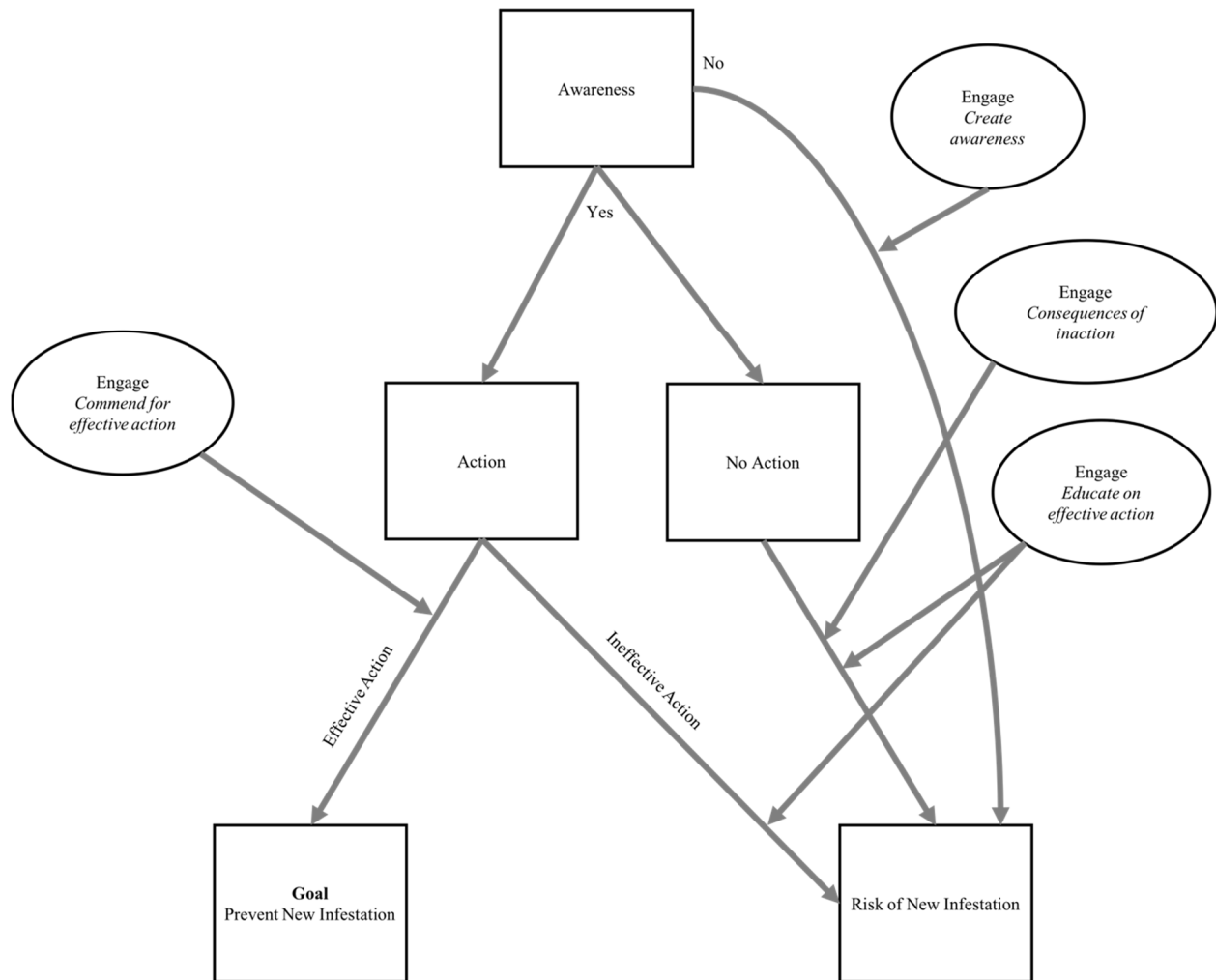


Figure 30. There are multiple steps that must occur for awareness of an issue to turn into effective the prevention of new infestations. Engagement is required to inform initial awareness and action for.

Awareness of a particular issue (in this case, awareness of NZMS presence in a Michigan river) first needs to be assessed. If the audience in question (anglers) is unaware, then engagement should focus on creating awareness. From the conceptual model, it is apparent that awareness alone is insufficient to drive action but is a necessary step in promoting behavior. If the audience is aware, then understanding what actions are being taken that could lead toward or away from the goal must be assessed. When the audience is aware but not taking action (e.g. not decontaminating wading equipment) then engagement should educate on the consequences of

inaction as well as what effective action they should be taking. If a desired action (effectively removing NZMS from wading equipment) is observed, then engagement should be focused on encouraging those taking the action to continue, thereby reinforcing positive social norms. When ineffective action is observed, the goal of engagement should be to educate those players on what effective actions they should be taking instead.

A study by Anderson et al. (2014) created a scheme to assign biosecurity hazard scores to different categories of anglers and canoeists. Through this, they were able to identify which groups were at highest risk of transporting ANS. I was able to construct a similar hierarchy of angler groups that pose a higher risk to accidental transport and introduction of ANS (Figures 28 and 29). This hierarchy should inform which messaging is more likely to reduce the overall risk of NZMS spread via angler vectors. I suggest focusing future engagement on educating anglers about effective decontamination techniques that will work across species.

Within the engagement-behavior process (Figure 30), the first step is awareness. A general lack of awareness of ANS has been identified as one of the major risks for their continued spread (Gates et al. 2009; Lindgren 2006). Anglers may unknowingly transport ANS hitchhikers on waders, boats, and other angling equipment, resulting in newly established invasive populations (Anderson et al. 2014; Gates et al. 2009; Johnson et al. 2001; Kelly et al. 2013). However, even those aware of an invasion may not understand the risk and associated consequences of transporting ANS between and within waterbodies.

On-site signage is a popular approach for enhancing awareness of a local issue. National outreach campaigns such as “Clean, Drain, Dry”; “Stop Aquatic Hitchhikers”; “Don’t move a mussel” are examples where information is provided with the intent of eliciting responsible behavior. Prior to implementation of my survey, signage designed through a collaborative effort

led by the State of Michigan and involving federal agencies, local conservation organization, and academic institutions was installed broadly along the Pere Marquette and at all USFS access points where I surveyed anglers. Despite the signage posted at all access points along the stretch of river where surveys were conducted, I found that only 52% of anglers were aware that NZMS were present in the Pere Marquette River. Unless the anglers were entering from private property, they would have had to pass by at least one sign designed to inform anglers of the presence of NZMS in the Pere Marquette River. Although signage was present and specifically designed with colors to draw attention, it was not noticed by over half of anglers surveyed. It is difficult to determine why anglers were unaware despite the presence of signage. I hypothesize that the exposure of anglers to the plethora of signage at access points, including posted fishing regulations, maps of the area, invasive species signage, can lead to eventual angler habituation. Habituated behavior can reduce the effectiveness of access point signage for increasing awareness. Snyder and Hamilton (2002) conducted a review of mass media campaigns and found that novelty of information shared in the campaign increased the desired behavioral change. A study by Gates et al. (2009) found magazines and newspapers to also be effective ANS communication tools. A mixed media outreach approach seems necessary to increase awareness throughout the angling community.

In addition to increasing awareness, additional steps are needed to alter angler behavior to reduce the risk of NZMS spread. My survey indicated that of the anglers that were aware of the infestation, less than half took any steps towards decontamination, and further, rates of decontamination did not differ from anglers who were unaware of the presence of NZMS. This pattern of behavior is consistent with a survey conducted in Wyoming that examined angler awareness of the ANS *Myxobolus cerebralis* (the organism responsible for whirling disease) and

decontamination actions taken, finding no positive correlation (Gates et al. 2009). Another study looking at boats as a vector for ANS found that education alone was not enough to influence positive behavioral changes (Rothlisberger et al. 2010).

There are a number of potential reasons that awareness may not be correlating to productive actions, in this case, wader decontamination. Behavior is influenced by many factors working simultaneously including knowledge or awareness, motivation, intentions, etc. (Abroms and Miabach 2008). A conceptual model by Pradhananga et al. (2015) demonstrates that environmentally responsible behavior is value driven, specifically by environmental concern and past responsible behaviors. In the case of my study, anglers surveyed may be lacking environmental concern (value their own experience now vs other's experience in the future or environmental health), feel powerless to take part of any change (i.e. if they take steps to prevent the spread of NZMS, other's may not), or anglers do not understand the potential risks involved in the introduction of an invasive species. Of these potential factors, it appears unlikely that lack of value for the resource would be the reason for inaction. According to McMullin et al. (2007), there is a significant relationship between angling and general environmental activism (stewardship, etc.). Moreover, the fact that many anglers are willing to travel hundreds of miles to fish on the Pere Marquette River highlights the value they place on this activity. It seems more likely that anglers feel powerless to take action knowing that they are only a small part of a larger social system that must conform to taking steps to protect against the introduction and transport of non-native species. Additionally, it is possible that anglers do not understand the consequences of introducing an invasive species and therefore do not understand why action is needed. Invasive species management is a 'wicked problem' as described by Chapin et al. (2010). A 'wicked problem' is one in which the problem and subsequent solutions are difficult to

define, thus makes taking the right course of action difficult to identify. When the problem is largely not understood, stakeholders cannot be expected to understand how and why to take action.

In my survey, I found that anglers used a variety of methods to decontaminate their waders. A review of the literature on the effectiveness of decontamination methods (Appendix E) indicates that even when anglers did decontaminate, the majority of decontamination steps that were taken have been proven ineffective at completely reducing the risk of transport. Although my study did not try to evaluate why anglers selected particular decontamination methods, conversation with anglers pointed to a couple of concerns they have. First is the time and effort required for various methods, and perceived excessive time required for the most effective methods. The second set of concerns focused on issues surrounding chemical means of decontamination. Some anglers expressed concern that using chemicals other than those approved by the manufacturer would void the gear's warranty and potentially shorten the life of the gear. Studies looking at wader deterioration after disinfecting chemical decontamination showed accelerated deterioration of waders with repeated exposure (Stockton and Moffitt 2013; Hosea and Finlayson (2005), although the rate of breakdown varied greatly depending on the specific applied chemical. Future research is needed that focuses on methods for NZMS removal from gear. The second concern with chemical decontamination was anglers' worries that there would be no way to rinse off chemical decontaminates on-site, which demonstrates their concern over the potential negative impacts of chemicals being discarded in the environment.

Due to the concern of anglers on the potential for chemical decontamination treatments to damage wading gear, more effort should be dedicated to educating anglers on equipment safe decontamination measures that are available to them. I would also note that the literature that has

evaluated decontamination methods has focused on achieving high mortality rates on NZMS but has not considered practices whereby NZMS are effectively removed from wading equipment, thereby achieving the same goal of reducing spread.

Different types of angling equipment (waders and boot-types) may increase the likelihood of transport and consequential introduction of NZMS (Stockton and Moffitt 2013). Felt soled waders retain moisture longer than rubber-soled waders and are thus able to provide a short-term suitable environment for aquatic species that become attached (Gates et al. 2008; Bothwell et al. 2009). This allows for long-distance transport of ANS species both between and within waterbodies. Further, boot-type has implications on the colonization rate of NZMS (Stockton and Moffitt 2013). Stockton and Moffitt (2013) found that felt (and neoprene) waders were colonized more frequently than rubber, however all materials were actively colonized by NZMS. Felt soled waders made up 26% of the overall boot-type of anglers surveyed. The majority of the remaining anglers had rubber-bottom wading boots or shoes. Thus, the risk of transport is lessened due to the prevalence of rubber-soled boots, but this does not completely reduce the potential for long-distance transportation.

Anglers generally reported fishing only the Pere Marquette river in the two days prior and post being surveyed and do not often travel to other rivers during that time period. However, when anglers were fishing multiple rivers in the two days before and after their trip to the Pere Marquette, they reported visiting other coldwater trout streams and even two streams that have known infestations of NZMS. I identified 16 un-infested rivers that were used by the anglers that also fished NZMS infested waters and recommend that these un-infested rivers be targeted for angler outreach activities. If a continued monitoring effort is put forth by the state agencies, then these rivers should be monitored for NZMS presence. However, with the absence of an effective

control method, outreach to promote prevention activities is likely more worth the investment than extensive continued monitoring.

Conclusion

Too often we think more about how nuisance species impact society and disrupt valuable ecosystem services but not enough about the way society drives their introduction and movement. Exotic species are transported and introduced by humans and may become nuisance species because of certain life history characteristics and a lack of natural control. The rate of introduction of ANS is expedited due to anthropogenic vectors. Once established, ANS have the potential to irrevocably alter ecosystems, reduce species diversity, and adversely impact socially desired species. The idea that ANS are so interconnected with the actions and values of people should be encouraging to outreach specialists who are working to engage those stakeholders. Identifying potential pathways of introduction helps guide future management actions, regulations, and outreach efforts surrounding an invasive species. In the case of NZMS, the stakeholders that are impacted by the ANS (mainly anglers) are also one of the groups that has the ability to change their behavior (decontaminating wading equipment) in a way that could reduce the spread and subsequent impact of the species. When a vested interest in the resources impacted by ANS exists, engagement actions should seek to spread awareness and provide actionable steps.

The survey conducted for this research highlights the importance of effective and relevant engagement. Awareness alone is not enough to encourage a change in behavior. This study identified risks associated with low awareness, lack of action for decontamination (even among those aware), movement of anglers from infested to un-infested locations. There is a critical need to address these risks to prevent further spread of the NZMS and other ANS. I recommend

progress be made to identify hurdles that are currently limiting anglers from taking decontamination steps to reduce the risk of spreading ecologically harmful aquatic nuisance species. The shortcoming between awareness and action is not specific to ANS but seen throughout conservation related activities. A cultural shift needs to occur where general decontamination procedures are the normal course of action regardless of the documented presence of ANS. Such a shift has already been promoted by the “clean, rinse, dry” campaign and should be encouraged. In this study I outlined a conceptual model that highlights multiple necessary points of engagement and describes the locations where future management and research efforts should be focused. Deeper engagement, beyond awareness, is necessary to achieve the desired behavioral change.

APPENDICES

APPENDIX A: IRB exempt form.

MICHIGAN STATE UNIVERSITY

August 1, 2016

To: Daniel Hayes
334C Natural Resources Building
East Lansing, MI 48824-1222

Re: **IRB# x16-944e** Category: Exempt 2
Approval Date: August 1, 2016

Title: Angler Behavior in Relation to Transport of an Invasive Species, the New Zealand Mudsnail

Initial IRB Application Determination ***Exempt***

The Institutional Review Board has completed their review of your project. I am pleased to advise you that **your project has been deemed as exempt** in accordance with federal regulations.

The IRB has found that your research project meets the criteria for exempt status and the criteria for the protection of human subjects in exempt research. **Under our exempt policy the Principal Investigator assumes the responsibilities for the protection of human subjects** in this project as outlined in the assurance letter and exempt educational material. The IRB office has received your signed assurance for exempt research. A copy of this signed agreement is appended for your information and records.

Renewals: Exempt protocols do not need to be renewed. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: Exempt protocols do not require revisions. However, if changes are made to a protocol that may no longer meet the exempt criteria, a new initial application will be required.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects and change the category of review, notify the IRB office promptly. Any complaints from participants regarding the risk and benefits of the project must be reported to the IRB.

Follow-up: If your exempt project is not completed and closed after three years, the IRB office will contact you regarding the status of the project and to verify that no changes have occurred that may affect exempt status.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with the IRB office.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at IRB@msu.edu. Thank you for your cooperation.

Sincerely,



Harry McGee, MPH
SIRB Chair



Office of Regulatory Affairs Human Research Protection Programs

Biomedical & Health
Institutional Review Board
(BIRB)

Community Research
Institutional Review Board
(CRIRB)

Social Science
Behavioral/Education
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APPENDIX B: Anger survey response sheet.

Angler Behavior and Awareness Survey

Angler ID Number: _____ Angler initials indicating consent: _____

Year: _____ Month: _____ Day: _____ Time (24 hour clock): _____

River: _____ Access point: _____ Surveyor initials: _____

Survey Type (Circle one): Incomplete Trip Complete Trip

Fishing Mode (Circle one): Wading Boat Shore

1) Which ZIP code do you live in?

2) What type of footwear will you be using during your fishing trip?

Boot-foot felt sole Boot-foot other sole
Stocking-foot felt sole boot Stocking-foot other sole boot
Other _____

3) Have you fished another river within the past 2 days?

Yes No

If Yes, which river or rivers did you fish? _____

4) Do you plan to fish another river within the next 2 days?

Yes No

If Yes, which river or rivers: _____

5) Have you fished in a Western State?

Yes No

If Yes, where and when was your most recent trip _____

6) Do you disinfect or clean your waders between fishing trips?

Yes No

If yes, how: _____

7) Are you aware that invasive New Zealand mudsnails were detected in Michigan Rivers in 2015?

Yes No

If yes, how did you hear about the infestation? _____

8) Have you taken this survey during a previous fishing trip?

APPENDIX C: Decontamination effectiveness table.

Table 9. Decontamination methods used in the angler survey and their effectiveness at inducing NZMS mortality.

Decontamination Method Identified in the Survey	Percent Used in Survey	NZMS Mortality	Reference(s)
Rinsing (water only)	35%	0-2.5%	Hosea and Finlayson 2005
Soap (Dawn, 100% concentration)	17%	83.7%	Oplinger and Wagner 2011
Dry (24 hours; Completely dry with no residual moisture/desiccation)	11% *Unknown length of drying	50%	Alonso & Castro-Diez 2012
Dry (50+ hours; Completely dry with no residual moisture/desiccation)	11% *Unknown length of drying	99-100%	Alonso & Castro-Diez 2012
Wash	8%	Unable to interpret meaning of method	N/A
Bleach (+5%; 5-minute soak)	8%	2.5-70%	Hosea and Finlayson 2005
Scrub/manual removal	5%	Unlikely to increase NZMS mortality; focuses on removal	N/A
Lysol's active ingredient: Benzethonium Chloride 450 - 1,940 mg/L; shaken in dry sack or soak)	<2%	12.5-100% (100% at higher concentration)	Hosea and Finlayson 2005; Oplinger and Wagner 2011
409® (50% dilution, soak)	<2%	65-100%	Hosea and Finlayson 2005; Schisler et al. 2008

Table 9 (cont'd)

Hot water	<2%	100%	Richards et al. 2004 (>40°C, for >1 hour)
Power wash	1.5%	N/A	N/A
Cleaner	1.5%	Unable to interpret meaning of method	N/A
Alcohol	>1.0%	95% Ethanol “soak” will result in 100% mortality; spray is likely less effective	N/A
Wader wash station	>1.0%	Effectiveness depends on chemical used and upkeep of wader wash station	N/A
Disinfectant (ammonium-based; 500-2,000 mg/L)	>1.0%	99-100%	Oplinger and Wagner 2011
Additional Decontamination Methods	...	...	...
Virkon (2% concentration, 15-minute soak)	0%	100%	Stockton and Moffitt 2013
Copper Sulfate (252 mg/L; Soak)	0%	100%	Hosea and Finlayson 2005
Pine-Sol (50% dilution, 5-minute soak)	0%	100%	Hosea and Finlayson 2005
Germicidal Cleaner: Sparquat (4.7% dilution, 10-minute soak)	0%	100%	Cheng and LeClair 2011

Table 9 (cont'd)

Freezing (6-8 hours)	0%	80-100%	Richard et al. 2004; Bergendorf 2004; Hylleberg and Siegismund 1987; Siegismund and Hylleberg 1987; Cheng and LeClair 2011
Salt (35 ppt; 30-minute soak)	0%	90%	Acy 2015
Incomplete dry (25 days; incomplete drying with remaining moisture)	0%	50%	Winterbourn 1970b
Grapeseed Oil Extract (2,000 ml/L)	0%	37.5%	Hosea and Finlayson 2005

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