

Research Article

Evaluation of a qualitative survey for early detection monitoring of New Zealand mudsnail

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Citation: Tank SN, Herbst SJ, Hayes DB (2021) Evaluation of a qualitative survey for early detection monitoring of New Zealand mudsnail. *Management of Biological Invasions* 12(2): 344–362, <https://doi.org/10.3391/mbi.2021.12.2.09>

Received: 18 April 2020

Accepted: 9 July 2020

Published: 20 October 2020

Handling editor: David Wong

Thematic editor: Matthew A. Barnes

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Abstract

Early detection of an invasive species is the first critical step to managing their invasion. New Zealand mudsnails (*Potamopyrgus antipodarum*; hereafter NZMS) are a small gastropod native to New Zealand and a documented worldwide invader. Although many approaches for sampling NZMS have been used, no protocol has emerged as a standard for early detection monitoring in lotic environments. In order to document the occurrence of NZMS, we developed a qualitative sampling methodology and evaluated its effectiveness. The sampling methodology involved conducting two searcher visual surveys along stream margins in a 50-meter search range for a standard 20-minute search time. Qualitative estimates of abundance and the time when the first detection was made were documented by each searcher. We conducted a total of 227 surveys in 12 rivers in Michigan, USA in 2017. Survey data were analyzed using an occupancy model framework, resulting in a per survey detectability exceeding 96%. We ran the occupancy model in an atypical fashion to explore the impact that relative abundance had on detectability. As relative abundance increased, detectability of NZMS increased. We modeled shorter survey durations (i.e., 15, 10, and 5 minutes) to determine their impact on detectability and found that less than 2% of detections were lost when reducing the survey length from 20 to 5 minutes. We developed a novel decision support tool to help understand how the number of sites surveyed, the site level detection probability, and the occupancy level of a rare species interact to determine the overall probability of detection of a new invader. The decision support table can help guide sampling design choices by giving insight into what combination of choices provides the overall highest probability of detection across all sites combined.

Key words: sampling, occupancy, invasive species, detectability

Introduction

Expanding globalization has come with ecological risks. One such risk is the introduction of non-native and invasive species, which have increased with the expansions of human-mediated modes for transporting goods (Hulme 2009; Meyerson and Mooney 2007). The introduction and establishment of invasive species has resulted in drastic economic, environmental, and social costs that are estimated in the hundreds of billions of dollars annually (Lovell et al. 2006; Pimentel et al. 2005).

Therefore, effective management tools are critical for reducing costs to address the ongoing threat of invasive species.

Invasive species management is a dynamic, time sensitive, and complex task that requires resource agencies to consider the many aspects related to the invasion process. The primary management goal for invasive species is to prevent the introduction because it is the most cost-effective approach (Ruesink et al. 1995). Prevention, however, is not always feasible and therefore an increased emphasis needs to be placed on early detection. Early detection is desired because it is presumed that effective control can be implemented prior to widespread establishment of an invasive species that would result in ecological and economic harm (Anderson 2005). An introduced species is generally rare in the early stages of invasion and may only occur at a limited number of locations, making early detection of the target species challenging (Dejean et al. 2012). As such, it is important to identify effective early detection monitoring strategies that result in immediate or rapid detection, high detection probability per sampling effort, efforts that are cost conscious and efficient, and practical to implement (Morissette et al. 2020; Reaser et al. 2020; Hoffman et al. 2016). Optimal surveillance strategies to determine presence also requires knowledge of the target species' ecology and invasion pathways. This information can inform strategic detection efforts that can be implemented at sites with high perceived risk of introduction using methods that provide a high probability of detection, if the species is present (Morissette et al. 2020).

The New Zealand mudsnail (*Potamopyrgus antipodarum* (Grey, 1843); hereafter NZMS) is a gastropod native to New Zealand that has been introduced and established throughout the world (Bowler 1991). NZMS infestations 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). Introduced NZMS populations in rivers in the western United States have reached densities upwards of 500,000/m² (Hall et al. 2006). These high densities are likely the result of the species ability to reproduce asexually with individual females reproducing during a large proportion of the year (i.e., spring-fall) and brooding up to 70 offspring per reproductive event (McKenzie et al. 2012).

Early detection methods for NZMS can be challenging because the species is small (approximately 4–6 mm) and cryptic in appearance, making detection difficult. The detection of the species, however, is critical because it has a high potential to spread to novel environments via hitchhiking on gear and equipment of recreational users. Specifically, 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 2008, 2012). No large-scale treatments are available for open-water environments to effectively control NZMS

populations, so early detection results would be most beneficial to inform strategic and place-based education/outreach campaigns focused on increasing decontamination practices among recreational users to prevent the introduction and spread to other non-infested waters.

Although NZMS monitoring has occurred broadly for decades, no standard qualitative or quantitative traditional survey methods for early detection have emerged from the scientific literature (Tank 2020). This is likely because previous NZMS studies have primarily focused on estimating population densities with the goal of determining ecological impacts, and therefore methods consisted primarily of D-framed kick-nets, Hess and Surber samplers with various mesh sizes, standard sized viewing buckets, and ponar dredges that are better suited for quantitative analyses (Moffitt and James 2012; Bennett et al. 2015; Levri et al. 2008; Schreiber et al. 2003). These survey methods are well suited for traditional macroinvertebrate sampling, but less desirable when early detection is the goal. The key distinguishing characteristic being that early detection is focused on maximizing efficiency and detections (Morissette et al. 2020) to determine presence, which differs from the quantitative focus of traditional macroinvertebrate methodologies conducted to estimate species-specific densities. The existing professional opinion in the literature is that traditional survey methods are limited in their capabilities for early detection of NZMS (Levri et al. 2007; Trebitz et al. 2010). Although Goldberg et al. (2013) has partially addressed the need for NZMS early detection methods through the development and evaluation of a species-specific environmental DNA assay, there continues to be a need for the development and evaluation of traditional survey methods for the purpose of NZMS early detection.

The purpose of this study was to develop and evaluate a visual qualitative survey method for NZMS early detection that could be broadly implemented by resource agencies and citizen scientists. The survey methodology for this study was designed to evaluate how NZMS detectability was influenced by duration of search time, number of searchers, and the number of sites surveyed, which are all critical aspects of an early detection survey program.

Materials and methods

Study sites

This study focused on rivers in Michigan, USA with established NZMS populations and rivers that were presumed 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 historical creel surveys, and proximity to known infested rivers. The first reported detection of NZMS in Michigan was in the Pere Marquette River in 2015 through an incidental observation by a recreationist (Sarah LeSage, Michigan

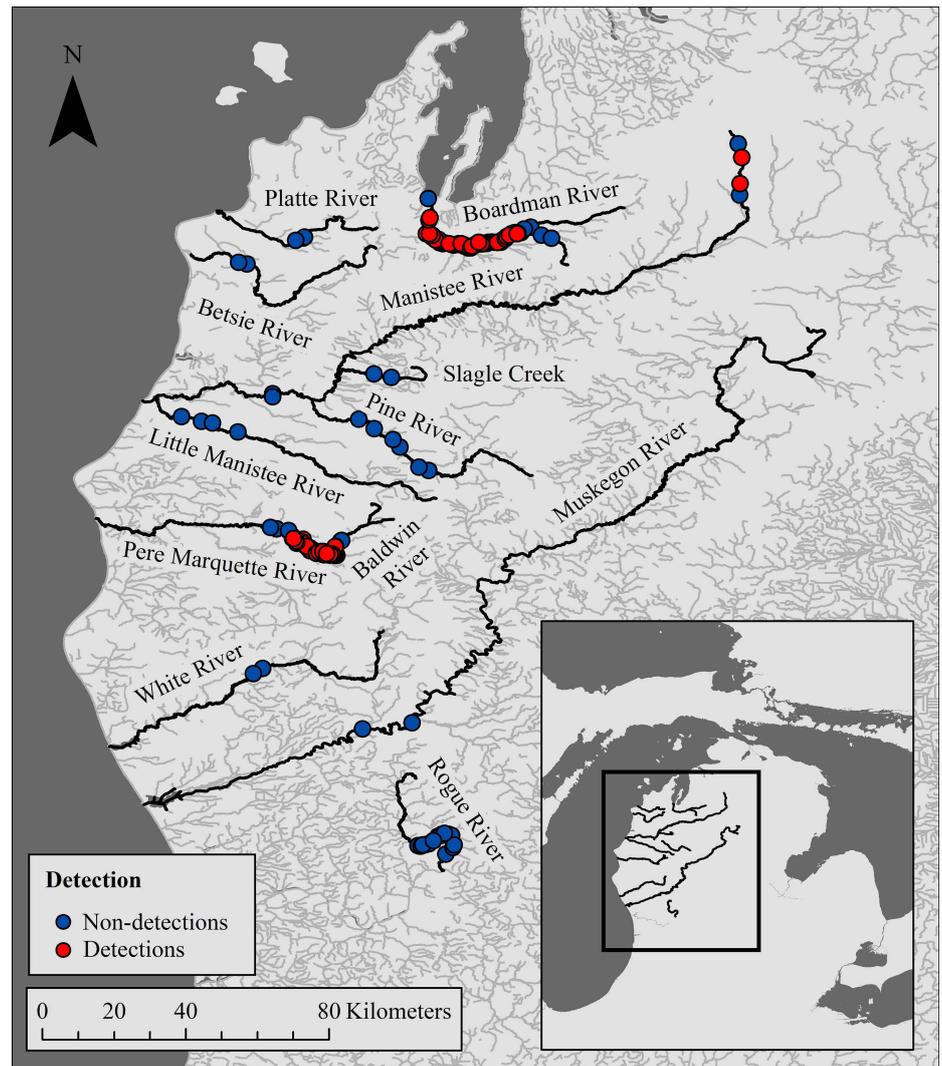


Figure 1. Sampling locations and results of NZMS qualitative surveys conducted in the Lower Peninsula of Michigan, USA in 2017.

Department of Environment Great Lakes and Energy, *pers. comm.*). 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 establishments have been detected in five Michigan 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).

The study rivers surveyed included the infested rivers, but also other Michigan rivers within the Lake Michigan drainage basin. Specifically, early detection surveys were conducted in wadeable reaches of each of the following rivers: Pere Marquette, Manistee, Boardman, Baldwin, White, Rogue, Pine, Muskegon, Little Manistee, Platte, and Betsie Rivers and Slagle Creek (Figure 1). All surveyed rivers are located on the western half of Michigan's Lower Peninsula and are considered coldwater fisheries.

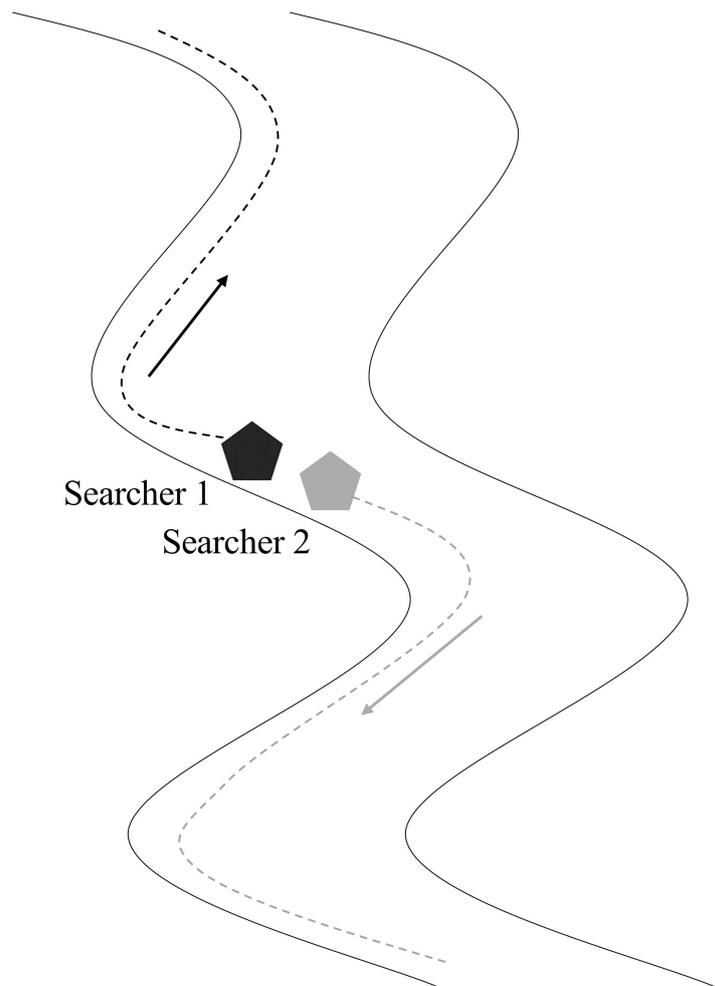


Figure 2. Schematic of the qualitative surveys that involved two searchers independently surveying up to a 50-meter stretch of river without overlapping when possible for a 20-minute search time.

The rivers cover a collection of habitat types but are all generally low grade, tree lined streams. These rivers were selected for surveys because they are popular destinations among trout and salmon anglers, supporting thousands of angler days per year (Michigan Department of Natural Resources 2019). In addition, angler movements among these rivers over short time periods (i.e., hours or days) are perceived to be occurring routinely, and therefore these rivers are perceived to have a greater risk for NZMS introduction via secondary spread from recreational users. The access points along these rivers were selected due to their accessibility as most access points were on federal or state-owned land with public access. Some points fell on private property, where permissions were easy to obtain.

Qualitative early detection survey protocol

Qualitative visual surveys were developed and implemented for the purpose of NZMS early detection. A unique sampling event consisted of two to four individuals searching up to 50 meters of river (determined using a rangefinder) for 20 minutes. However, our standard survey

protocol called for two individual searchers (Figure 2). The methods used by each searcher consisted of a visual survey aided by the use of an underwater viewing chamber (Aquavue Underwater Viewer, Fieldmaster™). During each sampling event, each searcher would enter the site at a central access point and determine their approximate 50-meter search range. When two searchers conducted the sampling event, searchers would wade in opposite directions from the site's central access point. Each searcher would travel along the bank while wading in the river, examining all available substrates and habitat types. This involved picking up and sorting through submerged vegetation, leaf litter, submerged woody debris, etc. from the river's margins and visually inspecting for the presence of NZMS. Each searcher kept track of their search time using a digital watch. An individual sampling event ended after 20 minutes of searching. In the event that the searcher reached the 50-meter extent of their search range prior to the 20 minutes of search time, they would move back towards the access point and continue searching the same side of the river.

To ensure searcher independence, communication regarding observed snails was prohibited during the active search. 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. If searchers were uncertain about snail identification, the snails in question were preserved and properly identified in the laboratory. In a further attempt to standardize search effort, each searcher would pause their 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 search 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]). The time to first NZMS detection was recorded to evaluate detection probability and survey duration needed to detect NZMS across a gradient of relative abundance.

Surveys were conducted in each of the available habitat types to avoid potential sampling bias within a site. Sampling events were performed simultaneously with each searcher covering a different section of river without spatial overlap.

Data analysis

Survey detection data were analyzed within an occupancy framework to estimate site occupancy and detection probability (e.g., Mackenzie et al. 2002, 2004; MacKenzie and Royle. 2005). Each searcher was treated as an independent replicate for each sampling event. As such each independent searcher was analogous to a repeated sampling event at the same site

during a different time period, which is the traditional approach used for occupancy analyses. The occupancy model (MacKenzie et al. 2002, 2004) used for this study was:

$$L(\psi, p) = (\psi^n \prod p_t^{n_t} (1 - p_t)^{n - n_t}) \times (\psi \prod (1 - p_t) + (1 - \psi))^{N - n}$$

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 (i.e., by the t^{th} searcher). The occupancy model was implemented using the package *unmarked* in R (R Core Team 2018).

We used assumptions consistent with other applications of occupancy analyses (e.g., MacKenzie et al. 2004). For example, we assumed that if NZMS were present, the organism was present throughout the entire search area at a given site. This assumption implied 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. Additionally, we assumed that sites were closed; meaning no NZMS immigration or emigration occurred during the survey. Finally, detection probability was assumed to be consistent across all sites within a river system, and that NZMS were identified accurately.

Detection probability is a critical metric for an early detection program. For this study, this parameter was assumed constant across sites, which is typical within this modeling framework. A primary goal of effective early detection is to detect a species before it becomes abundant and widespread. Therefore, we evaluated how detection probability varied as a function of relative abundance. Within the occupancy modeling framework any covariates included need to be sampled separately from the detection process, however, relative abundance was not a covariate that was observed independently. As such, we created *a posteriori* designations of relative abundance to approximate detection probability across categorical levels of relative abundance (i.e., low, medium, and high as described above). At each site we used the maximum level of relative abundance detected as the true site abundance.

Our application of the occupancy model in a non-traditional way led to a violation in one of the assumptions. Preliminary analysis indicated that how sites where no NZMS were detected were handled (i.e., whether they are treated as having no abundance or simply non-detected at other levels of abundance) affected estimates of detection probability. As it was not possible to determine which sites had NZMS even when both searchers failed to detect their presence, it was possible that some of the non-detection sites may have indeed been occupied. Therefore, we ran the occupancy model two ways: including and excluding events where no NZMS were detected to determine the sensitivity of detection probability estimates to how data were incorporated into the model. The model that

included all of the non-detect events assumed that the non-detect events were actually occupied at each of the various levels of abundance. The second model excluding the non-detect events, treated the non-detects as uninformative relative to the question of how detection probability differed with relative abundance.

We simulated the impact of differing survey durations by including additional iterations of the occupancy model where detection was truncated at shorter time intervals. We modeled the detection process for three scenarios: 1) any detection occurring after 15 minutes was treated as a non-detect, 2) detections occurring after 10 minutes were treated as non-detects, and 3) detections occurring after 5 minutes were treated as non-detects.

Detection probability was estimated for each independent searcher, however, we used two searchers per site working independently. Therefore, the combined detection probability for the sampling event was calculated 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 estimated using the occupancy analysis.

A decision support table was created to determine how detection probability was influenced after systematically adjusting 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:

$$P(x) = \binom{k}{x} (\psi p)^x (1 - \psi p)^{(k-x)}$$

where k is the number of sites, ψ is the probability of occupancy, p is the detection probability for a single searcher, $1 - \psi p$ 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 (i.e., $x=0$), then the formula becomes:

$$P(0) = \binom{k}{x0} (\psi p)^0 (1 - \psi p)^k$$

which reduces to $P(0) = (1 - \psi p)^k$

Since this equation provides the probability of zero detections across a sample of k sites, the probability can be subtracted from 1.0 to give the probability of detecting NZMS in at least one site. In addition, this equation can be rearranged to determine the required sample size (number of survey sites) to achieve a given detection probability $P(x)$:

$$k = \frac{\ln(P(x))}{\ln(1 - \psi p)}$$

Table 1. Survey events and subsequent number of detections of New Zealand mudsnails among relative abundance categories and across the 12 rivers sampled in 2017.

| River | Survey Events | Detections | Relative abundance | | |
|-----------------|---------------|------------|--------------------|--------|------|
| | | | Low | Medium | High |
| Pere Marquette | 118 | 64 | 18 | 16 | 30 |
| Boardman | 61 | 49 | 6 | 1 | 42 |
| Manistee | 6 | 2 | 0 | 1 | 1 |
| Rogue | 17 | 0 | – | – | – |
| Pine | 6 | 0 | – | – | – |
| Baldwin | 4 | 0 | – | – | – |
| Little Manistee | 4 | 0 | – | – | – |
| Platte | 3 | 0 | – | – | – |
| Betsie | 2 | 0 | – | – | – |
| Muskegon | 2 | 0 | – | – | – |
| Slagle Creek | 2 | 0 | – | – | – |
| White | 2 | 0 | – | – | – |

This analysis resulted in a range of detection estimates to inform early detection sampling strategies.

Results

In 2017, 227 sampling events consisting of 512 independent replicates were implemented across 12 rivers. These efforts resulted in NZMS detections at multiple sampling sites within three rivers: Pere Marquette, Boardman, and Manistee (Table 1). NZMS were detected during 280 of the 512 total replicates, totaling 180 unique detection sites. The discrepancy between total detections and site level detections was because a number of sites were sampled multiple times throughout the field season, particularly on the Pere Marquette, as that river was the focus of other NZMS research (Tank 2020). The majority of the NZMS detections occurred near the stream margins, with many individuals being detected on submerged woody debris, vegetation, and leaf litter within the river.

Number of searchers

Detection probability was relatively high for a single searcher but increased with the use of additional searchers per sampling event. The detection probability for a single independent searcher was 0.82 ± 0.03 , using the full data set of detections that included non-detection events. More simply stated, each independent searcher was estimated to detect NZMS 81% of the time when the species was present. The detection probability for two searchers, which was our standard protocol for each sampling event, was higher at 0.97, meaning that the standard protocol used in this study resulted in detecting NZMS > 96% of the time when NZMS were present at a site.

Relative abundance

Relative abundance directly influenced estimates of detection probability. Specifically, detection probability increased with greater relative abundance

Table 2. 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 ± 0.03 | 0.82 ± 0.03 | 0.97 |
| Low | 306 | 0.40 ± 0.10 | 0.34 ± 0.08 | 0.57 |
| Low | 74 | 1 | 0.55 ± 0.06 | 0.80 |
| Medium | 281 | 0.19 ± 0.04 | 0.68 ± 0.08 | 0.89 |
| Medium | 49 | 1 | 0.74 ± 0.06 | 0.93 |
| High | 389 | 0.43 ± 0.04 | 0.97 ± 0.01 | > 0.99 |
| High | 157 | 1 | 0.97 ± 0.01 | > 0.99 |

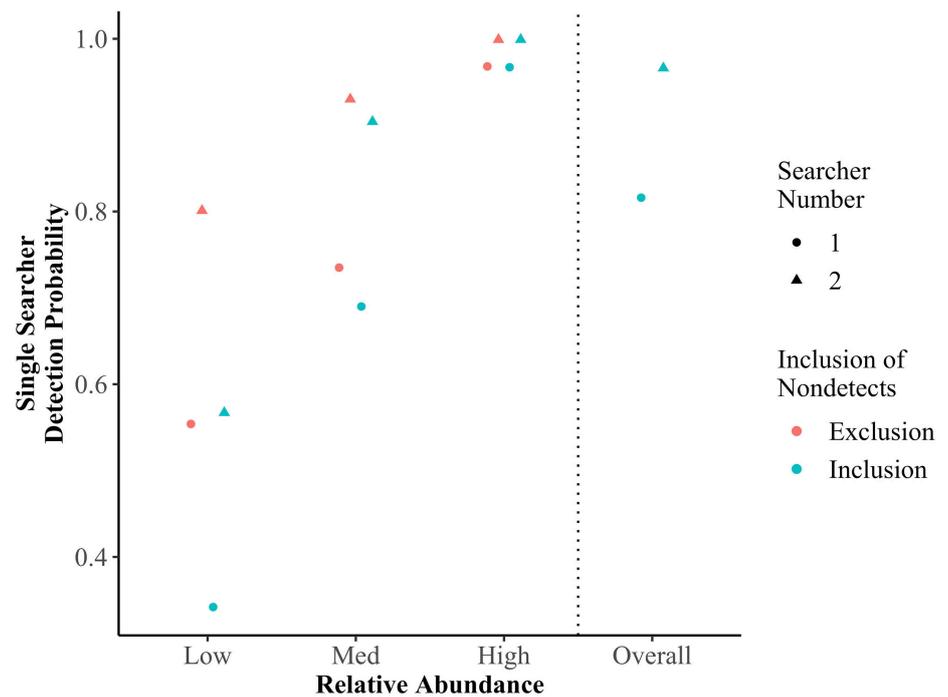


Figure 3. Single and two searcher detection probability of New Zealand mudsnails by relative abundance comparing the inclusion and exclusion of non-detections sites. In low abundance sites, 1–10 NZMS were detected; medium abundance sites, 11–100 NZMS were detected; high abundance sites, > 100 NZMS were detected by each searcher.

regardless of whether a single searcher was used or if the standard protocol consisting of two searchers was implemented (Table 2). The estimated detection probability also differed slightly depending on whether NZMS non-detection events were included or excluded from the analysis. For example, when events with NZMS non-detections were included with low relative abundance detections ($n = 360$) in the model, occupancy was 0.40 and detection probably was 0.57 when following survey protocols (Table 2). In contrast, when non-detections ($n = 74$ retained) were excluded, meaning the true occupancy equaled one (i.e., 100% of sites were known to be occupied under model assumptions), the detection probability using the standard protocol increased to 0.80 at sites with low relative abundance (Table 2, Figure 3). The same general pattern held true for estimates of occupancy and detection probability for the medium and high relative abundance categories (Table 2,

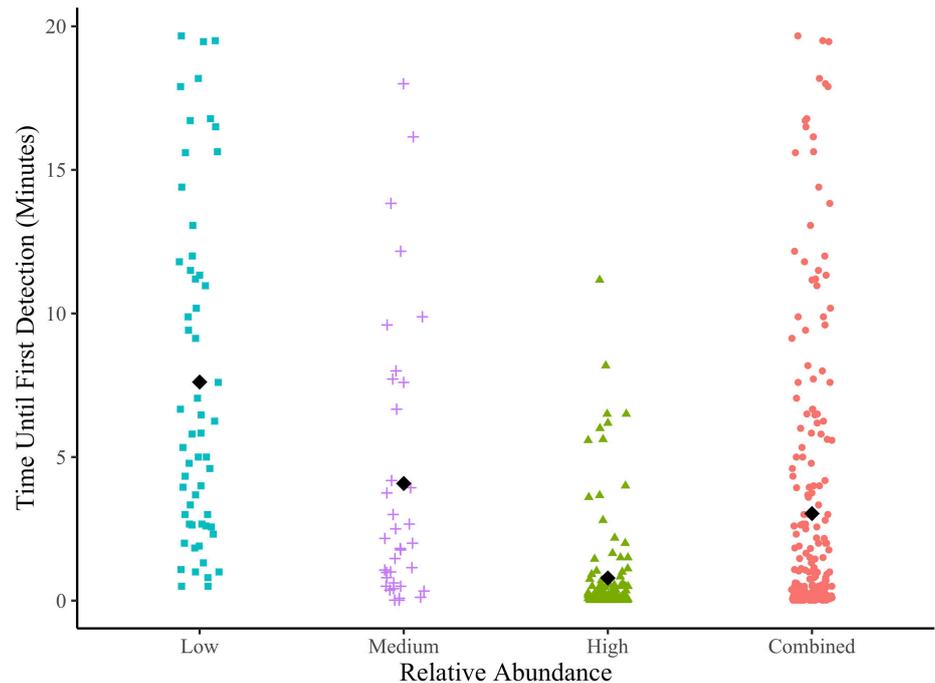


Figure 4. The mean time (minutes) until first detection of New Zealand mudsnails is represented by the black diamond for each of the three relative levels of abundance. The distribution of observed time to first detect are conveyed by the differing shapes for each of the relative abundance categories. 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 3. The time until first detection in minutes of New Zealand mudsnails summarized across three relative levels of abundance.

| Relative Density | N | Time (minutes) Until Frist Detection of NZMS | | | | | |
|------------------|-----|--|--------|----------|----------------|---------|---------|
| | | Mean | Median | Variance | Standard Error | Minimum | Maximum |
| High | 133 | 0.79 | 0.15 | 3.11 | 0.15 | 0.017 | 11.17 |
| Medium | 36 | 4.19 | 2.00 | 24.03 | 0.83 | 0.017 | 18.00 |
| Low | 57 | 7.74 | 5.82 | 34.14 | 0.78 | 0.500 | 19.67 |

Figure 3). The highest detection probability (> 0.99) was associated with high relative abundance and when the data was restricted to include only the sampling events with NZMS detections (Table 2, Figure 3).

Survey duration

The survey duration needed to initially detect NZMS was associated with the relative abundance of the infestation. The average time to first detect an individual NZMS followed a general pattern of increased time with decreased relative abundance. Specifically, the time until first detection ranged from 1 second at sites with relatively high abundance to 19 minutes 43 seconds at sites with low abundance. The average time until first detection with high relative NZMS abundance was approximately 47 seconds (0.8 minutes \pm 0.2; Figure 4, Table 3), followed by approximately 4 minutes and 11 seconds (4.2 minutes \pm 0.8) for medium relative abundance, and 7 minutes and 44 seconds (7.7 minutes \pm 0.8) for low relative abundance.

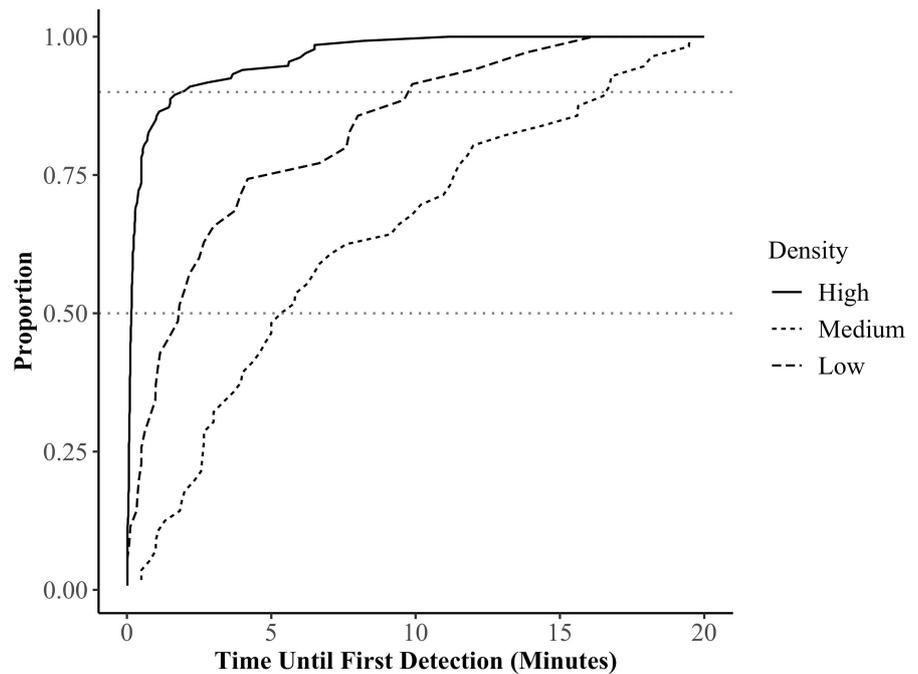


Figure 5. Cumulative proportion of the time (minutes) until first detection of New Zealand mudsnails calculated at three levels of relative abundance.

Table 4. Detection probability for New Zealand mudsnails given different survey durations of 5, 10, 15, and 20 minutes.

| Survey Length (Minutes) | Detectability | |
|-------------------------|--------------------------|---------------|
| | Single Searcher \pm SE | Two Searchers |
| 20 | 0.82 \pm 0.03 | 0.97 |
| 15 | 0.79 \pm 0.03 | 0.96 |
| 10 | 0.79 \pm 0.03 | 0.96 |
| 5 | 0.77 \pm 0.03 | 0.95 |

At a high level of abundance, all detections were made within 11 minutes and 10 seconds from the start of the sampling event; medium abundance detections made within 18 minutes; and low abundance detections made within 19 minutes and 43 seconds (Figure 5).

When the survey duration was reduced below 20 minutes, a decrease in NZMS detection probability was observed. Specifically, detection probability increased with longer search times regardless of whether a single searcher was used or if our standard protocol consisting of two searchers was implemented. The overall detection probability using the standard protocol (i.e., not taking NZMS relative abundance into consideration and utilizing a two-searcher approach) was reduced from 0.97 to 0.96 when the survey duration was reduced from 20 minutes to 15 minutes. This resulted in the loss of $\leq 1\%$ of detections (Table 4). When the survey duration was further reduced to 10 minutes, the overall detection probability subsequently reduced to 0.96. When survey time was reduced to 5 total minutes of search time, then the overall detection probability was reduced to 0.95. 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 changes with different survey durations.

Table 5. Decision support tool to assist balancing limiting resources across a gradient of detection probabilities. The probability of detection at a system level is a function of site level detectability, true occupancy within a system, and the number of survey events. For example, the probability of detecting New Zealand mudsnails within a defined system that has 5% true occupancy is 0.397 (approximately 40%) when 20 sites within a system are surveyed with a 0.5 (5%) site level detection probability.

| Survey Events | Occupancy | Site Level Detection Probability | | | | | | | |
|---------------|-----------|----------------------------------|-------|-------|-------|-------|-------|-------|-------|
| | | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 0.95 | 0.99 | 1 |
| 10 | 0.01 | 0.049 | 0.058 | 0.068 | 0.077 | 0.086 | 0.091 | 0.095 | 0.096 |
| 10 | 0.05 | 0.224 | 0.263 | 0.300 | 0.335 | 0.369 | 0.385 | 0.398 | 0.401 |
| 10 | 0.1 | 0.401 | 0.461 | 0.516 | 0.566 | 0.611 | 0.631 | 0.647 | 0.651 |
| 20 | 0.01 | 0.095 | 0.113 | 0.131 | 0.148 | 0.165 | 0.174 | 0.180 | 0.182 |
| 20 | 0.05 | 0.397 | 0.456 | 0.510 | 0.558 | 0.602 | 0.622 | 0.638 | 0.642 |
| 20 | 0.1 | 0.642 | 0.710 | 0.766 | 0.811 | 0.848 | 0.864 | 0.876 | 0.878 |
| 50 | 0.01 | 0.222 | 0.260 | 0.296 | 0.331 | 0.364 | 0.380 | 0.392 | 0.395 |
| 50 | 0.05 | 0.718 | 0.782 | 0.832 | 0.870 | 0.900 | 0.912 | 0.921 | 0.923 |
| 50 | 0.1 | 0.923 | 0.955 | 0.973 | 0.985 | 0.991 | 0.993 | 0.995 | 0.995 |
| 75 | 0.01 | 0.313 | 0.363 | 0.410 | 0.453 | 0.492 | 0.511 | 0.526 | 0.529 |
| 75 | 0.05 | 0.850 | 0.898 | 0.931 | 0.953 | 0.968 | 0.974 | 0.978 | 0.979 |
| 75 | 0.1 | 0.979 | 0.990 | 0.996 | 0.998 | 0.999 | 0.999 | >.999 | >.999 |
| 100 | 0.01 | 0.394 | 0.452 | 0.505 | 0.552 | 0.595 | 0.615 | 0.630 | 0.634 |
| 100 | 0.05 | 0.920 | 0.952 | 0.972 | 0.983 | 0.990 | 0.992 | 0.994 | 0.994 |
| 100 | 0.1 | 0.994 | 0.998 | 0.999 | >.999 | >.999 | >.999 | >.999 | >.999 |
| 200 | 0.01 | 0.633 | 0.700 | 0.755 | 0.799 | 0.836 | 0.852 | 0.863 | 0.866 |
| 200 | 0.05 | 0.994 | 0.998 | 0.999 | >.999 | >.999 | >.999 | >.999 | >.999 |
| 200 | 0.1 | >.999 | >.999 | >.999 | >.999 | >.999 | >.999 | >.999 | >.999 |

Decision support tool

The decision support tool illustrated the influence of the number of sites surveyed, gradient of occupancy used to reflect presumed rare species presence, and detection probabilities on the confidence of detecting NZMS (Table 5). We found that 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 is reduced. For example, when 100 sites are surveyed with a 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 detecting NZMS is 0.55 (i.e., the probability of failing to detect NZMS is approximately 45%).

Discussion

Our objective was to develop and evaluate a method for rapid detection of NZMS in lotic environments. The criteria we used to evaluate the method included considerations such as speed of detection, probability of detection per sampling effort, practicality of implementation, as well as cost considerations (Morissette et al. 2020; Reaser et al. 2020; Hoffman et al.

2016). In summary, we found that the protocol we used met these criteria for being an effective early detection strategy. Other commonly used sampling techniques such as D-framed kick-nets, Hess and Surber samplers, and ponar dredges include a labor-intensive sample sorting process and are focused on quantitative analysis of existing populations (Moffitt and James 2012; Bennett et al. 2015; Levri et al. 2008; Schreiber et al. 2003). As such, they do not provide as rapid of a result, and are generally more time consumptive than our qualitative sampling protocol due to the need for post-sampling processing. Our methods were better suited for the early detection of NZMS as it allowed us to detect NZMS within a short survey duration, with a low number of searchers and limited equipment required.

The protocols developed within this study were effective for early detection, but broader implementation should take into consideration the assumptions we made, and potential limitations to application in other environments. For example, the analysis using an occupancy modeling framework required us to make a number of assumptions. As is typical with occupancy modeling, we assumed a closed population; and given the limited mobility of NZMS (Proctor et al. 2007) we feel that this assumption was met. In addition, we conducted species identification training for each searcher to address the assumption of proper identification. Furthermore, when identification was in question, we preserved specimens in the field to later be confirmed. An assumption that may not have been met is that the occupancy status is constant for all searchers. Since each searcher examined a different portion of the stream within the site, there is the possibility that NZMS are present in one search area and not the other. We observed that NZMS distribution was patchy within areas of infestation (sometimes high abundance patches were observed a few meters away from seemingly uninfested areas) and as a result, it is likely that search areas within a site did not have an equal occupancy status. The consequence of violating this assumption was that imperfect detection would bias the detection probability low (Hayes and Monfils 2015). Thus, true detectability is likely higher than estimated, and as such, estimated detection probabilities were likely conservative.

Although searchers likely vary in their search abilities and searcher-level random effects have been incorporated into other applications of occupancy modeling (e.g., McCarthy et al. 2013; Bornand et al. 2014; Crump and Forstner 2019), we did not model the effects of this potential factor. Our rationale for not including this is based on several analytical and practical considerations. First, consistent training and subsequent consistency in application of sampling protocols reduce much of the potential variation. The high overall detectability we observed implies that all searchers were effective and prior simulations (Crump and Forstner 2019) have shown that there is little benefit to modeling individual searcher effects when detectability is high. Another concern is that models including searcher as

a random factor imply that each searcher is static in their effectiveness over time, or if an searcher by time interaction is posited, it implies that each searcher's rate of increase occurs at a similar pace, both of which assumptions are also unlikely. Another statistical consideration is that searchers did not participate in sampling from each river or habitat equally. Thus, searcher-level effects may be confounded with differences in detectability across habitats, time or with the abundance of NZMS (Crump and Forstner 2019). Similarly, we did not incorporate habitat characteristics into our analysis of detectability as it is likely that confounding could occur with the abundance of NZMS, which we show has a strong influence on detectability.

A general limitation of standard occupancy analysis is that the detection probability is assumed to be constant across sites, regardless of the abundance of the target species (MacKenzie et al. 2002; MacKenzie and Royle 2005). This limitation has been generally recognized as being particularly relevant to the design of an early detection strategy where detection probability at low levels of abundance are most relevant (e.g., McCarthy et al. 2013). In order to provide insight into how estimates of detection probabilities vary across a gradient of relative abundance, we implemented a novel ad hoc procedure categorizing sites based on qualitative measures of relative abundance. This analysis required us to make some assumptions on how to best categorize sites as to the relative abundance of NZMS, and importantly, how to categorize sites where no NZMS were detected, but which could have had individuals present. We used the higher of the relative abundance estimates from the two searchers as an approach that is logically consistent with the core assumption that the occupancy status of a site is constant. The issue of how to best incorporate non-detect sites proved to be a thorny issue. In preliminary analyses, we felt that excluding rivers where no NZMS were found from the analysis would provide more robust results, as these sites would not be informative to the detection process, but we found that their inclusion or exclusion resulted in substantial variation in the estimate of detection probability, particularly for sites categorized as having low abundance (Figure 3). We recommend that the more conservative estimate of detection probability be used for designing new surveys, but that more exploration of this topic is warranted, particularly as it pertains to the design of early detection programs for invasive species.

An emerging technology intended to provide greater sensitivity to detect invaders that are low in abundance, cryptic, or difficult to sample via traditional gears is environmental DNA (hereafter eDNA; Dejean et al. 2012; Piaggio et al. 2014; Goldberg et al. 2013). Specifically, eDNA sampling has been identified as an effective and sensitive tool for detecting NZMS (Goldberg et al. 2013). While this methodology is effective, it requires an investment in sophisticated equipment and greater level of training for

personnel involved. Further, interpretation of results from eDNA sampling is not always clear-cut. In some instances, inhibition of amplification has been observed in samples taken from natural waters, leading to imperfect detection (Thomas et al. 2020). As such, the benefits and tradeoffs associated with eDNA and our qualitative visual sampling approach should be considered when developing an effective early detection strategy.

Potential alterations to sampling protocol for enhancing early detection

The goal of detecting invasive species early in the invasion process is inherently difficult to achieve. These species are by definition rare and in low abundance early in the invasion process, and their distribution is unknown. Enhancing the likelihood of early detection can be accomplished in two general ways. First, the protocol used to collect samples can be altered to maximize the overall detection probability for the area of interest as a whole, or the sampling design (i.e., number of sites, site selection process) can be altered. We developed a decision support table that outlines the expected probability of detection (or failure to detect) an invasive species under a variety of conditions and levels of sampling effort (Table 5).

As others have observed (e.g., Hoffman et al. 2016) there is a high likelihood of missing an extremely rare species (where true occupancy is 0.01 or the species is present at 1% of survey locations) even when implementing a survey with high detectability at the individual site level if a limited number of locations are surveyed. As such, expectations for detecting new invaders need to be set to realistic levels, depending the number of sites that can be sampled.

In general, overall detection probabilities at low relative abundance can be enhanced by increasing the number of survey events or searcher replicates. This finding was consistent with other work focused on early detection that highlighted the need for increased sampling events to account for rare species (Hoffman et al. 2016). Another strategy to increase the detection rate in the early detection context is to focus sampling on habitats where the species is most likely to occur, based on the biology of the invader and the suspected vector of transport (Trebitz et al. 2010). For NZMS in specific, we found that survey efforts focused in near shore, low flow areas containing vegetation or woody debris often resulted in NZMS detections even when abundances were relatively low. By looking at locations where the propagule pressure is suspected to be highest, and in habitats where we expect the species to occur will likely make the most efficient use of limited sampling resources. Some effort should be allocated, however, to sites with a lower expectation of occurrence, as the problem of species invasion often involves a high level of uncertainty with many unknowns. Although the use of non-random site selection may be appropriate to the situation of early detection, we note that a consequence is that estimates of overall occupancy would be biased, and as such, such an

approach would not be appropriate for more routine monitoring efforts, or where quantification of range would be desired.

Alterations to on-the-ground protocols could also be altered to enhance overall detection probability. There are obvious tradeoffs between the number of searchers, the time spent searching, and the number of sites that can be sampled for a fixed cost. For example, if surveys were reduced to 5 minutes, approximately 4% of detections would be lost using the standard protocol, however the reduced cost of a shorter survey length may lead to the opportunity to add additional survey sites. We did not quantify how a shorter survey duration would affect the total number of sites that can be surveyed per day as travel time is a fixed cost and varied substantially among rivers. As such, the value of reducing survey time to sample more sites in total would need to be evaluated on a case by case basis. The decision support table we developed, however, can help guide these sampling design choices by giving insight into what combination provides the overall highest probability of detection across all sites combined (Table 5). Nett et al. (2012) used a similar approach to determine the most effective sampling methods for round goby, and to guide the sampling effort needed to achieve maximum detection rates. We recommend that similar approaches could be used to evaluate survey methods for other invasive species.

Acknowledgements

The authors gratefully acknowledge Billy Keiper for his intellectual and field support. We would also like to acknowledge Morgan Freebairn, James Beaubien, Jared Lepper, and Phillip Ankley for their assistance with fieldwork, Rob Hunter for his assistance with maps and figures, and Sarah LaSage, Michael Wagner, and Eric Benbow for their review and helpful comments. Finally, we would like to acknowledge and thank our reviewers, whose feedback helped us strengthen this manuscript.

Funding Declaration

The Great Lakes Restoration Initiative and United States Forest Service provided the funding for this project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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