diversity

MDPI

Article

# Tracking the Recovery of Freshwater Mussel Diversity in Ontario Rivers: Evaluation of a Quadrat-Based Monitoring Protocol

**Scott M. Reid [1,*] and Todd J. Morris [2]**

[1] Aquatic Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Trent University, DNA Building, 1600 West Bank Drive, Peterborough, ON K9L 0G2, Canada

[2] Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON L7S 1A1, Canada; todd.morris@dfo-mpo.gc.ca

* Correspondence: reid.scott@ontario.ca; Tel.: +1-705-755-2267

Academic Editor: Thilina Surasinghe

Received: 11 November 2016; Accepted: 6 January 2017; Published: 13 January 2017

**Abstract:** Watershed inventories and population monitoring are essential components of efforts to conserve and recover freshwater mussel diversity in Canada. We used two datasets to assess the efficacy of a quadrat-based sampling protocol for: (1) detecting mussel species at risk; (2) characterizing species composition; (3) providing accurate estimates of abundance; and (4) detecting changes in density. The protocol is based on a systematic design (with random starts) that samples 20% of monitoring sites with visual-tactile surface searches and excavation of 1 $m^2$ quadrats. The first dataset included 40 sampling sites in five Ontario rivers, and the second dataset consisted of complete census sampling at two 375 $m^2$ sites that represented contrasting mussel assemblages. Our results show that the protocol can be expected to detect the majority of species present at a site and provide accurate and precise estimates of total mussel density. Excavation was essential for detection of small individuals and to accurately estimate abundance. However, the protocol was of limited usefulness for reliable detection of most species at risk. Furthermore, imprecise density estimates precluded detection of all but the most extreme changes in density of most individual species. Meeting monitoring objectives will require either substantially greater sampling effort under the current protocol, or a fundamental revision of the sampling approach.

**Keywords:** unionids; endangered species; systematic sampling; population monitoring

## 1. Introduction

Freshwater mussels are considered one of the most imperiled faunal groups in North America [1]. In Canada, there are 55 native freshwater mussel species, with 41 of those occurring in the province of Ontario [2]. Almost a third of Ontario mussel species are listed as Endangered, Threatened, or of Special Concern under the federal *Species at Risk Act* and the provincial *Endangered Species Act* [3,4]. Catastrophic declines to the Ontario mussel fauna occurred after the introduction (and subsequent spread) of non-native dreissenid mussels (Zebra Mussel, *Dreissena polymorpha*; and Quagga Mussel, *D. bugensis*) to the Laurentian Great Lakes [5]. In contrast, most Ontario rivers are not heavily infested by dreissenids and the historical mussel diversity is largely intact [6,7]. Recovery strategies have been developed to conserve remnant mussel diversity [8,9]. Actions undertaken include surveys and the establishment of population index monitoring stations in rivers. Data collected through these activities are essential for delineating protected habitat, assessing population status and trends, and evaluating recovery actions [10,11].

Since 2002, the Ontario Freshwater Mussel Recovery Team (OFMRT) has implemented a quadrat-based protocol to assess the current status, distribution, and demographics of mussel species

at risk (SAR) in Ontario rivers. The protocol is based on a systematic design (with random starts) [12] that samples 20% of the monitoring site with surface searches and excavation. Systematic designs such as this approach are considered efficient for sampling freshwater mussels where populations are expected to be clustered and rare [13]. In Ontario, the protocol has been implemented at more than 40 sites across five rivers. Information collected relevant to recovery efforts includes: locations of species and populations; descriptions of biophysical habitat attributes for different life-stages; population status (i.e., density, size and age structure, sex-ratio); and the presence of invasive species [10,11].

Monitoring protocols for imperiled mussels need to provide accurate, precise, and cost-effective information. Given the long-term nature of monitoring objectives, it is important that biologists and resource managers are confident that investments in sampling will provide data that is useful for detecting trends in species distribution and abundance. The application of the protocol has been demonstrated at riverine sites in Pennsylvania and West Virginia (United States) [14,15]. However, the protocol's performance has not been evaluated for freshwater mussels in more northern, and less productive, rivers. As the likelihood of collecting individuals is a function of local abundance, species detection rates are expected to lower. In this study, we used two datasets to assess the efficacy of the OFMRT protocol at a site level to: (1) detect mussel SAR; (2) characterize species composition; (3) provide accurate estimates of density; and (4) detect long-term changes in density. The effort required to implement monitoring can be reduced by collecting mussels with surface searches at all selected quadrats and only excavating the substrate at a random subset of selected quadrats (i.e., double-sampling, [14]). However, the approach can be limited by observation bias when only a low percentage (i.e., <40%) of mussels are detected at the surface [14]. Therefore, we also compared the composition of mussel data (species presence and shell length) collected using surface searches and excavation.

## 2. Materials and Methods

The first dataset (multi-river dataset) included information from the Ausable, Grand, Maitland, Sydenham, and Thames rivers (Figure 1). Collection data from 40 sites within these rivers were available (Appendix A). Informed by previous timed-search surveys, we located sites in areas of substantial mussel occurrence and diversity [16] (Morris unpublished data). The dataset was used to: (1) evaluate the effectiveness of previous sampling efforts to estimate species richness; (2) evaluate whether density estimates are sufficiently precise to detect future changes; and (3) estimate species detection probabilities.

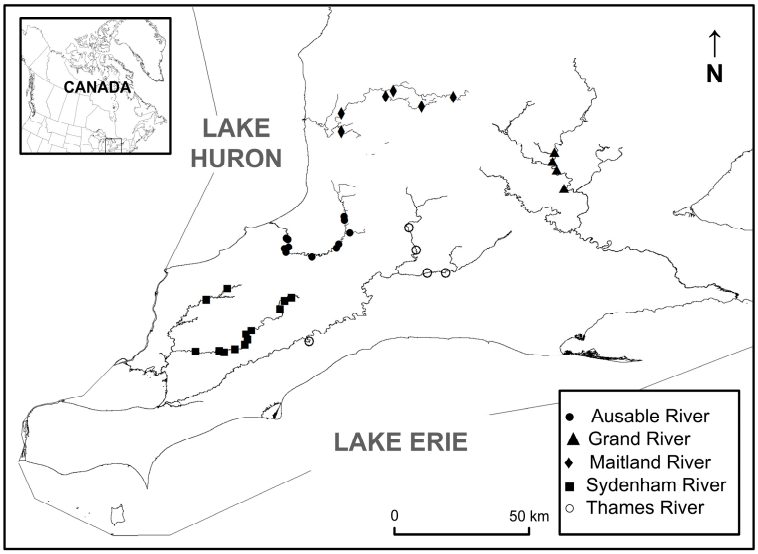


**Figure 1.** Distribution of 40 quadrat sampling locations across five southwestern Ontario rivers. Location of the study area within Canada is provided in the inset map.

The multi-river dataset was collected following the OFMRT protocol. This protocol is based on a systematic sampling design with three random starts [15,17]. Each site was delineated into 3 × 5 m blocks (15 $m^2$; Figure 2). The number of blocks at a site depended on the extent of suitable mussel habitat; the median number of blocks at each site was 24 (range: 16–31; see Appendix A). Wetted channel widths at sites ranged from 7 to 59 m (median = 21 m). We randomly selected three 1 $m^2$ quadrats, and the same quadrat locations were sampled in each block. This protocol resulted in sampling 20% of the area of each site. In each quadrat, mussels were initially collected from the surface visually (aided by an underwater viewer) and by touch. Afterwards, the substrate in each quadrat was excavated to a depth of 10–15 cm to improve detection of juveniles and small species. Live mussels were identified to species [2], and shell lengths were measured. Collection method (at the surface by visual-tactile detection or by excavation) was recorded during sampling of Grand and Maitland river sites. Sampling occurred during the summer low-flow period (mid-June through early September). Two days of sampling effort with a three-person crew was typically required at each site. Each site was sampled only once over the years 2002 to 2013. Although the status of individual species may have changed over 11 years of monitoring, the pooled dataset provided a larger sample size and geographic coverage than if a shorter timeframe was used.

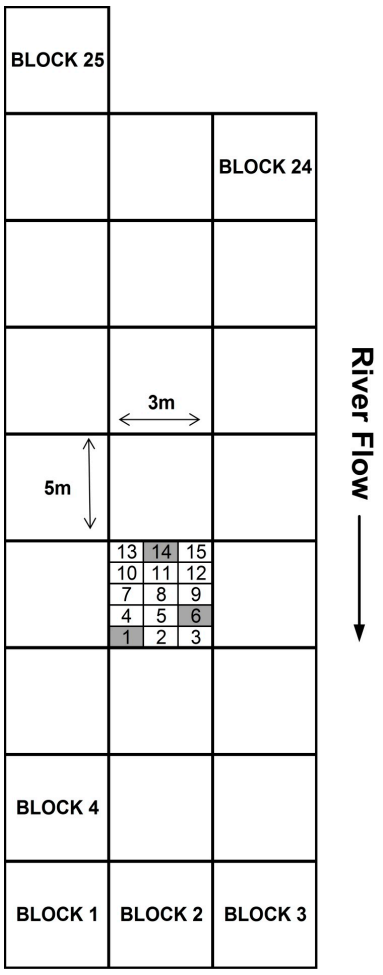


**Figure 2.** Representative diagram of the systematic sampling design used in Ontario rivers. The location of random starts (quadrats) within each block is highlighted in grey.

The second dataset (census dataset) consisted of data from the complete sampling of one 375 $m^2$ site in each of two streams, Rawdon Creek and the Sydenham River. Rawdon Creek is a tributary of the Trent River, and the census site (44°16′08″ N; 77°33′12″ W) was located 17 km north of Trenton.

The Trent River drains into Lake Ontario. The mean wetted channel width was 8.3 m and the mean water depth was 0.29 m. The Sydenham River drains into Lake St. Clair, and the census site (42°36′20″ N; 82°02′40″ W) was located 11 km east of Dresden. The mean wetted channel width was 20.0 m and the mean water depth was 0.34 m. Substrate at both sites was a relatively even mix of sand, gravel and cobble. These watercourses represent contrasting densities, species richness, and number of SAR; all of these measures were high in the Sydenham River and lower in Rawdon Creek. Site selection was informed by previous quadrat (Sydenham River) and timed-search (Rawdon Creek) surveys [17,18]. The second dataset was used to: (1) compare results at the current level of sampling effort (three random starts) with a census dataset; and (2) model the extent to which increased sampling effort could improve mussel population and assemblage estimates.

The census dataset was collected by sampling all 15 quadrats in each block at Rawdon Creek and the Sydenham River; both sites had 25 blocks, yielding a total of 375 quadrats at each site. Mussels were collected using the previously described visual-tactile and excavation methods. Sampling Rawdon Creek took five days with a four to five-person crew, and sampling the Sydenham River took six days with a four to eleven-person crew. Census sampling was conducted at Rawdon Creek between 27 August and 4 September 2013, and at the Sydenham River between 7 and 14 August 2012.

Both datasets were archived in the Lower Great Lakes Unionid Database (Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries, and Oceans Canada).

## 3. Data Analysis

### *3.1. Multi-River Dataset*

The probability of detecting each species at a site was estimated using the following equation:

$$p(\text{detection}) = 1 - e^{-mn} \quad (1)$$

where $m$ is the mean number of individuals in a sample (quadrat), $n$ is the number of samples, and $e$ is the base of natural logarithms [19]. By linking species detection to local population size, the equation provided an approach to: (1) evaluate the influence of representative mussel densities on the likelihood of species detection during future sampling at other Ontario river sites; and (2) assess whether mussel SAR are less likely to be detected than other mussel species. The Kolmogorov-Smirnov test [20] was used to test for differences in detection probability between mussel SAR and other mussels. Statistical analyses were completed using PAST version 1.94 [21].

We evaluated the efficiency of our protocol for estimating species richness by comparing observed species richness ($S_{OBS}$) to estimated richness ($S_{EST}$) following Chao et al. [22]. $S_{EST}$ provides a non-parametric estimator of total richness (accounting for undetected species) based on the number of rare species in the sample. For each site, $S_{EST}$ was derived using

$$S_{EST} = S_{OBS} + \frac{(1 - \frac{1}{t})Q_1^2}{(2Q_2)} \quad (2)$$

where $Q_1$ is the number of singletons (species represented by only one individual in a sample), and $Q_2$ is the number of doubletons (the number of species represented by exactly two individuals in a sample). Due to a lack of doubletons, $S_{EST}$ could not be calculated for 23% of sites.

Accuracy of species richness estimates was further evaluated by calculating: (1) sampling intensity (the total number of individuals sampled divided by the number of species encountered) and (2) the proportion of singletons (number of species represented by one individual divided by total species number) [23]. There is an intuitive connection between singletons and species inventory completeness: the proportion of singletons is expected to decrease with increasing sampling efforts as the true proportion of singletons in the assemblage is approached. High singleton frequencies indicate that species richness is underestimated, likely as a result of under-sampling [24]. Lopez et al. [23]

proposed that species richness can be reliably estimated from samples with sampling intensities of >50 individuals/species, and less than 14% singletons.

For each species at each site, mussel density and variance were calculated using unbiased estimators developed for systematic sampling designs with multiple random starts [12,14,25]. The Mann-Whitney Test was used to test for differences between density estimates for SAR and other species. 90% and 95% confidence intervals (CI) associated with density estimates were calculated to assess the likelihood of the protocol detecting future changes in mussel density [26]. In each case, the minimum percent change in mussel density detectable by the protocol ($\delta$) was calculated as:

$$\delta = \frac{|\chi_1 - \chi_2|}{\chi_1} \times 100\% \quad (3)$$

where $\chi_1$ is the mussel density and $\chi_2$ is the upper CI value. To be consistent with the Committee on the Status of Endangered Wildlife in Canada assessment process [27], values of $\delta$ were evaluated in regard to listing criteria thresholds of 30%, 50%, and 70% change in future population size. The 90% and 95% CIs represent differing levels of uncertainty associated with these assessments (i.e., $p = 0.10$ and $p = 0.05$, respectively); a $p = 0.05$ is typical of traditional hypothesis testing, but $p = 0.10$ is recommended for monitoring programs [28].

### *3.2. Census Dataset*

A bootstrap re-sampling procedure (sampling with replacement from the complete dataset and 5000 randomizations) [29,30] was used to estimate: (1) the probability of detecting an individual species based on three random starts in each block (as applied in the protocol); and (2) the number of random starts required to obtain mussel community data equivalent to the full census. The bootstrap procedure was used to simulate mussel count data associated with 1, 2, 3, . . . , 14 random starts (i.e., number of quadrats sampled within each block). Probabilities were defined as the percentage of randomizations where a species was successfully detected. Equivalence was assessed using the Jaccard (species presence/absence data) and Bray-Curtis (species abundance data) similarity measures [31]. Data were simulated using the Excel Add-in PopTools version 3.2.5 [32].

The bootstrap method was also used to estimate: (1) the percent difference between mussel density estimates using three random starts and the census count; and (2) the number of random starts required to be within 15%, 10%, and 5% of the census count. We viewed these measures as evaluating the accuracy of estimates. The bootstrap procedure was used to simulate mussel count data associated with 3, 4, 5, . . . , 14 random starts. Mean mussel density and variance were calculated using simulated data and the previously identified estimator for systematic samples. Obtaining accurate parameter estimates can be difficult for populations that exhibit high spatial variability [33]. To characterize the spatial distribution of individuals within each site, the variance to mean ratio was calculated for each species. This ratio is used as an indicator of spatial clustering; values >1 are interpreted as supporting a clumped distribution [34]. Analyses were done using PASSaGE 2.0 [35].

The Kolmogorov-Smirnov test was used to test for differences in size of mussels collected at the surface and by excavation.

## 4. Results

### *4.1. Multi-River Dataset*

Thirty-two mussel species were detected across all five rivers. Species richness ($S_{OBS}$) at individual sites ranged from 2 to 23 species (Table 1). Ten SAR were collected, and most of these species were found at less than one-third of sites. The most widespread species were *Alasmidonta marginata*, *Amblema plicata*, *Lampsilis cardium*, *Lampsilis fasciola*, and *Lasmigona costata*; only one of these was an SAR (*Lampsilis fasciola*; Threatened). Mussel density and species richness were greatest in the Ausable and Sydenham rivers (Figure 3).

**Table 1.** Mussel densities (number·m$^{-2}$), precision of density estimates (coefficient of variation, CV), and detection probabilities (*p*detection) from across five Ontario rivers (2002–2013). Mean (±SD) values shown were calculated using estimates from each site where a species was collected. Species at risk are identified by an asterisk (*).

| Tribe | Species | Rivers [1] | Sites | Density | CV | *p* Detection |
|---|---|---|---|---|---|---|
| Alasmidontini | *Alasmidonta marginata* | A,G,M,S,T | 30 | 0.09 (0.21) | 0.27 (0.17) | 0.62 (0.30) |
| | *Alasmidonta viridis* | G,M,S | 5 | 0.06 (0.06) | 0.48 (0.09) | 0.45 (0.27) |
| | *Anodontoides ferussacianus* | M,S | 1 | 0.07 | | 0.18 |
| | *Lasmigona complanata* | A,S,T | 15 | 0.24 (0.38) | 0.26 (0.17) | 0.64 (0.33) |
| | *Lasmigona compressa* | A,G,M | 9 | 0.03 (0.01) | 0.49 (0.08) | 0.21 (0.10) |
| | *Lasmigona costata* | A,G,M,S,T | 35 | 0.58 (0.78) | 0.16 (0.15) | 0.82 (0.27) |
| | *Pyganodan grandis* | A,G,M,S | 14 | 0.08 (0.14) | 0.36 (0.17) | 0.47 (0.29) |
| | *Simpsonaias ambigua* * | S | 7 | 0.03 (0.01) | 0.43 (0.16) | 0.31 (0.15) |
| | *Strophitus undulatus* | A,G,M,S,T | 18 | 0.09 (0.08) | 0.27 (0.15) | 0.52 (0.28) |
| | *Utterbackia imbecillis* | S | 1 | 0.01 | | 0.32 |
| Amblemini | *Amblema plicata* | A,S,T | 24 | 1.11 (2.28) | 0.14 (0.14) | 0.84 (0.27) |
| Lampsilini | *Actinonaias ligamentina* | M,S,T | 16 | 0.53 (0.61) | 0.19 (0.16) | 0.79 (0.31) |
| | *Epioblasma torulosa rangiana* * | A,S | 10 | 0.07 (0.08) | 0.31 (0.16) | 0.50 (0.27) |
| | *Epioblasma triquetra* * | A,S | 13 | 0.07 (0.14) | 0.38 (0.15) | 0.43 (0.27) |
| | *Lampsilis cardium* | A,M,S,T | 24 | 0.16 (0.16) | 0.25 (0.18) | 0.69 (0.28) |
| | *Lampsilis fasciola* * | A,G,T | 26 | 0.15 (0.18) | 0.24 (0.16) | 0.62 (0.26) |
| | *Lampsilis siliquoidea* | A,G,S | 17 | 0.10 (0.09) | 0.24 (0.19) | 0.67 (0.31) |
| | *Leptodea fragilis* | A,S,T | 17 | 0.17 (0.14) | 0.22 (0.16) | 0.69 (0.32) |
| | *Ligumia recta* | A,S,T | 21 | 0.12 (0.11) | 0.22 (0.15) | 0.63 (0.31) |
| | *Obliquaria reflexa* | S,T | 2 | 0.03 | 0.41 | 0.32 |
| | *Potamilus alatus* | A,S,T | 18 | 0.05 (0.05) | 0.35 (0.16) | 0.42 (0.27) |
| | *Ptychobranchus fascioloris* * | A,M,S | 14 | 0.19 (0.32) | 0.26 (0.16) | 0.69 (0.28) |
| | *Truncilla donaciformis* * | S | 2 | 0.06 | 0.22 | 0.57 |
| | *Truncilla truncata* | A,S,T | 12 | 0.08 (0.11) | 0.34 (0.14) | 0.49 (0.25) |
| | *Villosa fabalis* * | S,T | 11 | 0.74 (1.09) | 0.22 (0.18) | 0.69 (0.35) |
| | *Villosa iris* * | A,M,S,T | 12 | 0.4 (0.72) | 0.19 (0.14) | 0.66 (0.30) |
| Pleurobemini | *Cyclonaias tuberculata* | A,S,T | 19 | 0.91 (1.46) | 0.17 (0.15) | 0.82 (0.26) |
| | *Elliptio dilatata* | A,G,S,T | 21 | 0.47 (0.60) | 0.17 (0.12) | 0.82 (0.26) |
| | *Fusconaia flava* | A,S,T | 22 | 0.33 (0.55) | 0.22 (0.15) | 0.78 (0.29) |
| | *Pleurobema sintoxia* * | S,T | 8 | 0.05 (0.06) | 0.39 (0.14) | 0.44 (0.29) |
| Quadrulini | *Quadrula pustulosa* | A,S,T | 9 | 0.16 (0.32) | 0.27 (0.13) | 0.58 (0.25) |
| | *Quadrula quadrula* * | A,S,T | 13 | 0.22 (0.19) | 0.22 (0.15) | 0.79 (0.28) |
| All Mussels | | | | 3.51 (4.28) | 0.06 (0.04) | |

[1] A = Ausable River, G = Grand River, M = Maitland River, S = Sydenham River, T = Thames River.

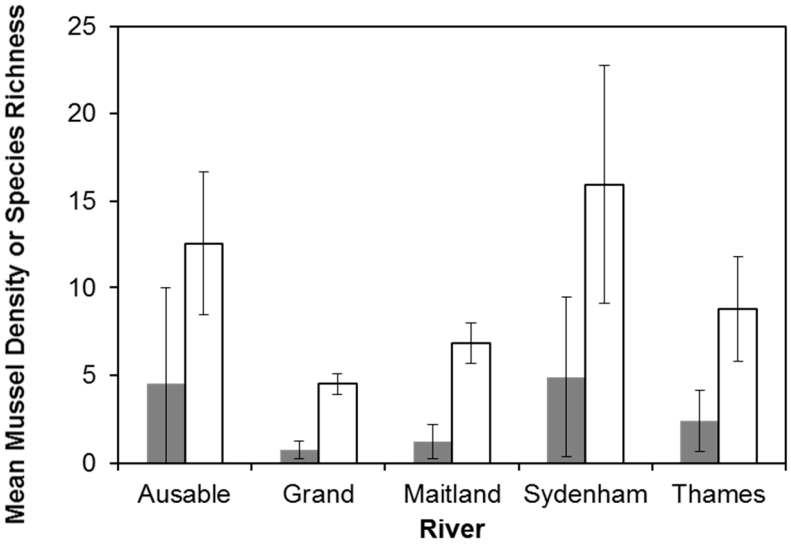


**Figure 3.** Mean (standard deviation, SD) mussel density (number·m$^{-2}$) (**grey**) and observed species richness (**white**) estimates from sites sampled along five Ontario rivers.

For most species, mean detection probabilities predicted from density estimates were between 0.4 and 0.7. The following species had mean detection probabilities >0.7: *Actinonaias ligamentina*, *Amblema plicata*, *Cyclonaias tuberculata*, *Elliptio dilatata*, *Fusconaia flava*, *Lasmigona costata*, and *Quadrula quadrula* (status: Threatened) (Table 1). Overall, detection probabilities were significantly lower for mussel SAR than other species (K-S test: D = 0.017, $p$ = 0.017), but values were similar between these two groups for all but detection probabilities >0.9 (Figure 4).

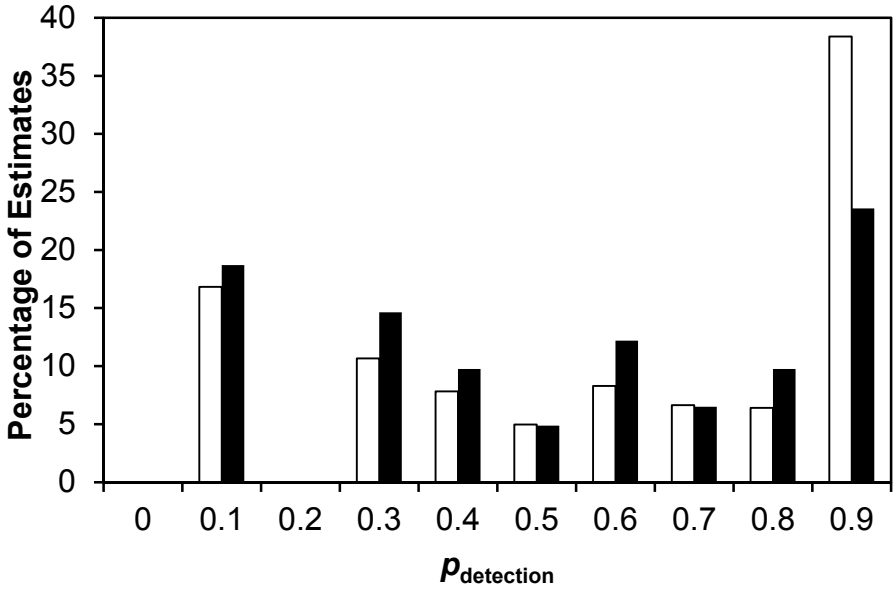


**Figure 4.** Comparison of detection probabilities for species at risk (**black**, $n$ = 10) and not at risk (**white**, $n$ = 22) mussels found in southern Ontario rivers. Probabilities were estimated using the equation developed by Green and Young [19], and the multi-river quadrat sampling dataset.

Mean $S_{OBS}$ across all sites was 11.7 (range: 2 to 23), which was 83% of mean $S_{EST}$ (14.1; range: 5.5 to 29.3) (Appendix A). At half the sites, $S_{OBS}$ was within one species of $S_{EST}$, and it was within two species at 66% of sites. The mean percentage of singletons was 24.5% (range: 0 to 60) and the mean number of individuals per species was 9.4 (range: 1.8 to 24.1). Based on the singleton threshold of Lopez et al. [23], 70% of samples provided reliable estimates of species richness, but none of the samples met the sampling intensity threshold.

Total (all species) mussel density ranged from to 0.12 to 18.1 $m^{-2}$, and most of the high-density sites (>3 $m^{-2}$, [36]) were in the Ausable and Sydenham rivers (Figure 3). The most abundant species (mean density >0.5 $m^{-2}$) were *Amblema plicata*, *Lasmigona costata*, *Cyclonaias tuberculate*, and *Villosa fabilis*. Mean densities of 50% of mussel SAR were less than 0.1 $m^{-2}$. The mean density of SAR (0.045 $m^{-2}$) was slightly lower than other species (mean = 0.066 $m^{-2}$) (Mann-Whitney Test: U = 2.05, $p$ = 0.037). Seventy-five percent of species density estimates were considered precise (coefficient of variation, CV < 0.3, [25] (Table 1). Less precise estimates were associated with low density (<0.09 $m^{-2}$) species, including four SAR (*Epioblasma torulosa rangiana*, *Epioblasma triquetra*, *Pleurobema sintoxia*, and *Simpsonaias ambigua*), and four other species. All total mussel density estimates were precise (CV $\leq$ 0.2).

More than 80% of systematic samples from river sites provided data sufficiently precise to detect at least a 30% change in future total mussel density at both significance levels ($p$ = 0.05 or 0.10). For individual species, the detection of $\geq$50% changes in future density are expected for less than half the samples at both significance levels. Less than one-third of sampling events allowed for the detection of a 30% change in the density of individual species. At both significance levels, detection of future changes in density of any magnitude was 10 to 17% lower for mussel SAR than other species (Figure 5).

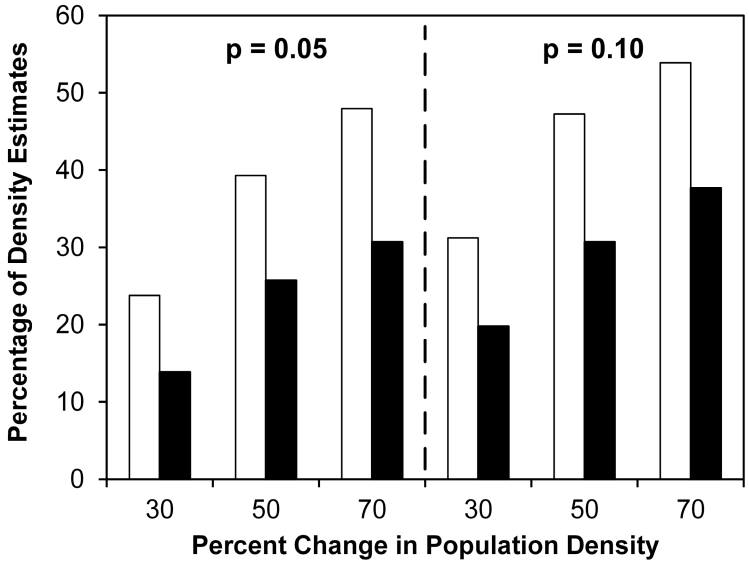


**Figure 5.** Comparison of the changes in mussel density (number·m$^{-2}$) predicted to be detectable under two levels of statistical significance for species at risk (**black**) and not at risk (**white**). Percentage was calculated from pooled datasets of individual species density estimates at each site.

At the Grand River, 81% of mussels were collected by excavation, but only 19% were collected from the surface using visual-tactile methods. At the Maitland River, 70% of mussels were collected by excavation, and 30% were collected from the surface. Three species in the Grand River (*Alasmidonta viridis*, *Elliptio dilatata*, *Pyganodon grandis*) and three in the Maitland River (*Alasmidonta viridis*, *Anodontoides ferussacianus*, *Pyganodon grandis*) were detected only by excavation. Mussels collected by excavation were smaller than those detected by visual-tactile searches. Individuals <50 mm were predominantly collected by excavation (Figure 6).

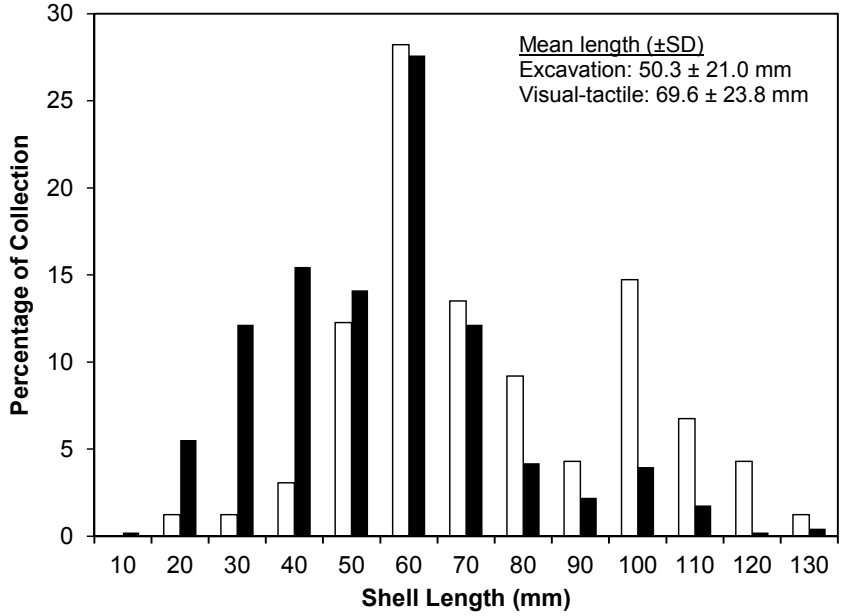


**Figure 6.** Length-frequency distributions of mussels collected by visual-tactile (**white**) and excavation (**black**) methods during quadrat sampling at Grand and Maitland river sites.

### *4.2. Census Dataset*

#### 4.2.1. Rawdon Creek

Mussels were collected from 74% of the 375 quadrats sampled, and >92% of individuals were collected by excavation. Mean total mussel density was 2.3 $m^{-2}$; densities of individual species ranged from <0.1 to 2.0 (Table 2). Seven species, including one SAR (*Villosa iris*), were collected. Variance/mean ratios provided statistical support for a clumped spatial distribution for the two most abundant species, *Elliptio complanata* and *Villosa iris* were clumped (*Elliptio complanata*: $s^2/x = 2.5$, Chi-square test: $p < 0.0001$, df = 374; *Villosa iris*: $s^2/x = 1.3$, $p < 0.0001$, df = 374). No other species showed evidence of spatial clumping, but the power of these tests was low due to small sample sizes.

Most species were collected by both visual-tactile sampling of the surface and excavation methods, but the percentage of individuals (all species) collected by excavation (92.8%) was much higher than by visual-tactile sampling (7.2%). Ninety-two percent of *Villosa iris* were collected by excavation. The mean lengths of individuals collected using visual-tactile and excavation methods were similar. However, size distributions were significantly different, with excavation yielding more small (<70 mm) individuals than visual-tactile searches (K-S test: D = 0.20, $p < 0.0001$) (Figure 7).

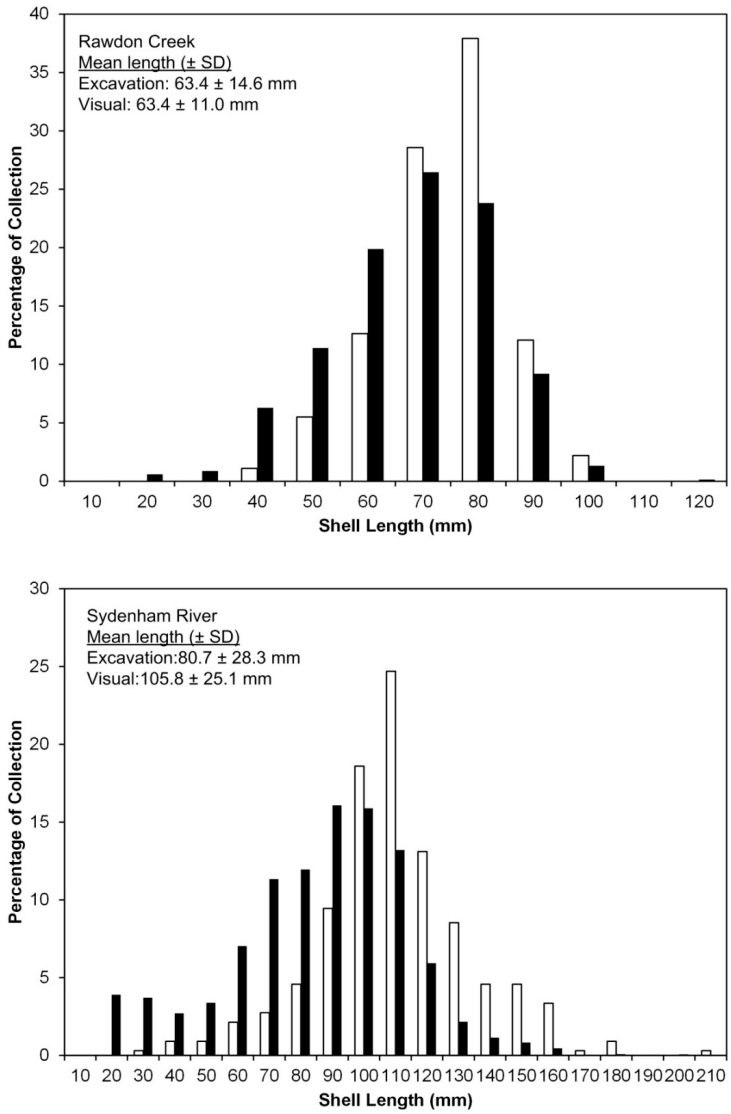


**Figure 7.** Length-frequency distributions of mussels collected by visual-tactile (white) and excavation (black) methods at Rawdon Creek (**upper**) and Sydenham River (**lower**) census sites.

**Table 2.** Mussel density (x), variance to mean ratio ($s^2/x$), frequency of occurrence (FO), and the percentage of individuals collected by either visual or excavation methods from two census sampling sites. FO is the percentage of all quadrats in which a species was collected. Species at risk are identified by an asterisk (*).

| Species | Rawdon Creek (Total = 866 Mussels) | | | | | Sydenham River (Total = 6180 Mussels) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | x | $s^2/x$ | FO (%) | Visual (%) | Excavation (%) | x | $s^2/x$ | FO (%) | Visual (%) | Excavation (%) |
| *Actinonaias ligamentina* | | | | | | 0.54 | 1.8 | 32.0 | 9.3 | 90.7 |
| *Alasmidonta marginata* | | | | | | 0.17 | 1.1 | 0.5 | 3.1 | 96.9 |
| *Amblema plicata* | | | | | | 1.78 | 2.1 | 70.7 | 8.8 | 91.2 |
| *Cyclonaias tuberculata* | | | | | | 6.98 | 5.0 | 96.0 | 5.3 | 94.7 |
| *Elliptio complanata* | 1.97 | 2.5 | 68.3 | 6.8 | 93.2 | | | | | |
| *Elliptio dilatata* | | | | | | 1.40 | 1.5 | 68.3 | 0 | 100 |
| *Epioblasma torulosa rangiana* * | | | | | | 0.16 | 1.1 | 14.1 | 1.6 | 98.4 |
| *Epioblasma triquetra* * | | | | | | 0.19 | 1.1 | 16.8 | 0 | 100 |
| *Fusconaia flava* | | | | | | 0.43 | 1.2 | 31.2 | 2.5 | 97.5 |
| *Lampsilis cardium* | 0.06 | 1.0 | 5.6 | 23.8 | 76.2 | 0.02 | 1.0 | 1.6 | 16.7 | 83.3 |
| *Lampsilis siliquoidea* | 0.02 | 1.0 | 1.9 | 37.5 | 62.5 | | | | | |
| *Lasmigona complanata* | | | | | | 0.12 | 1.1 | 10.7 | 15.9 | 84.1 |
| *Lasmigona costata* | 0.01 | 1.0 | 1.1 | 40 | 60 | 0.99 | 2.8 | 68.5 | 5.9 | 94.1 |
| *Leptodea fragilis* | | | | | | 0.18 | 1.1 | 16.3 | 2.9 | 97.1 |
| *Ligumia recta* | 0.003 | 1.0 | 0.3 | 0 | 100 | 0.24 | 1.2 | 19.5 | 15.7 | 84.3 |
| *Obovaria subrotunda* * | | | | | | 0.003 | 1.0 | 0.3 | 0 | 100 |
| *Pleurobema sintoxia* * | | | | | | 0.08 | 1.1 | 6.9 | 6.9 | 93.1 |
| *Potamilus alatus* | | | | | | 0.08 | 1.1 | 6.9 | 14.2 | 85.8 |
| *Ptychobranchus fuscioloris* * | | | | | | 0.46 | 1.1 | 36.0 | 1.2 | 98.8 |
| *Pyganodon grandis* | | | | | | 0.01 | 1.0 | 1.1 | 0 | 100 |
| *Quadrula pustulosa* | | | | | | 0.17 | 1.2 | 14.4 | 3.2 | 96.8 |
| *Quadrula quadrula* * | | | | | | 0.74 | 1.7 | 42.1 | 9.9 | 90.1 |
| *Simpsonaias ambigua* * | | | | | | 0.02 | 1.0 | 1.3 | 0 | 100 |
| *Strophitus undulatus* | 0.01 | 1.0 | 0.8 | 66.7 | 33.3 | 0.04 | 1.0 | 4.0 | 0 | 100 |
| *Truncilla donaciformis* * | | | | | | 0.03 | 4.3 | 1.1 | 0 | 100 |
| *Truncilla truncata* | | | | | | 0.01 | 1 | 0.5 | 0 | 100 |
| *Villosa fabalis* * | | | | | | 0.69 | 1.6 | 41.9 | 0 | 100 |
| *Villosa iris* * | 0.24 | 1.3 | 19.5 | 7.8 | 92.2 | | | | | |
| All Mussels | 2.31 | 2.7 | | 7.2 | 92.8 | 16.48 | 11.5 | | 5.3 | 94.7 |

With three random starts, we estimated the probability of detecting 95% of all species present at the Rawdon Creek site to be only 0.05. The probability of detecting 70% of species present was much greater at 0.79. Compared to the census dataset, simulated data based on three random starts provided a Jaccard similarity index of 0.73. The Jaccard index increased slowly with increasing sample size and reached 0.8 when five quadrats were sampled within each block (Figure 8). The Bray-Curtis index showed little similarity to the census dataset in species abundance with three random starts (0.33), but increased rapidly with increasing sample size. However, Bray-Curtis similarity $\geq$0.8 was not reached until 11 quadrats were sampled within each block.

The estimated density of *Villosa iris* from three random starts (0.03 $m^{-2}$) was 90% lower than density measured in the full census. For other species, estimated (from three random starts) densities were 9% to 72% lower (mean = 54%) than census counts. To be within 15%, 10%, or 5% of full census count of *Villosa iris*, sampling was predicted to require 6, 9, and 12 random starts. In contrast, three random starts were sufficient to provide estimates of total mussel density and *Elliptio complanata* density within 8% of that estimated from the full census.

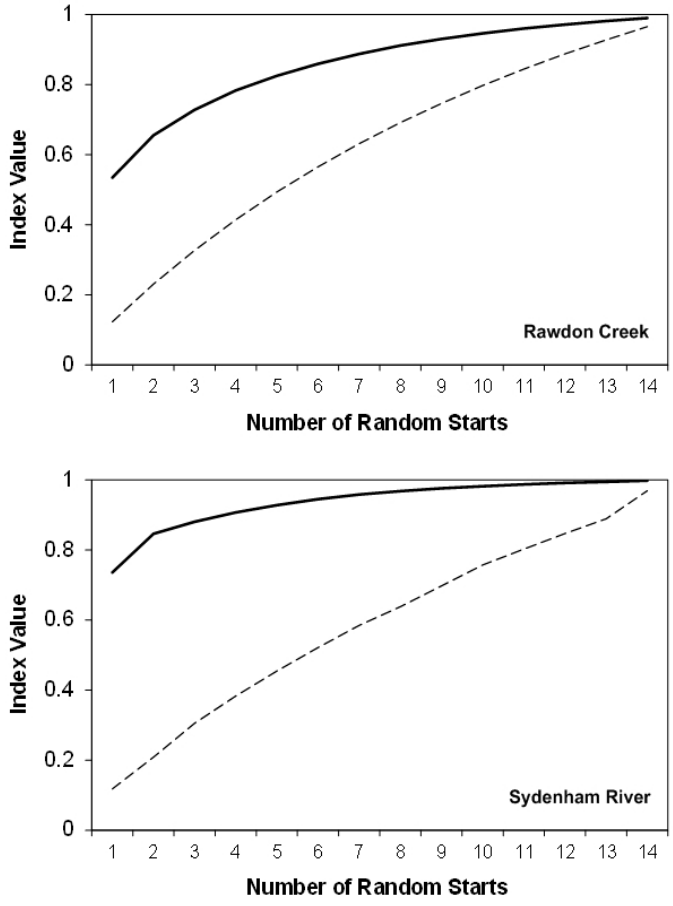


**Figure 8.** Relationships between sampling effort (number of random starts in each block) and mussel assemblage indices at the Rawdon Creek and Sydenham River census sites. The solid line is the Jaccard index of species richness, and dashed line is the Bray-Curtis similarity index; both indices are calculated in comparison to the full census dataset.

### 4.2.2. Sydenham River

Mussels were collected from all but one of the 375 quadrats, with more than 94% of individuals (and 96% of SAR mussels) collected by excavation. Total mussel density was 16.5 $m^{-2}$; densities of individual species ranged from 0.003 to 7.0 (Table 2). Twenty-five species (including nine SAR) were collected. Variance/mean ratios provided statistical support for a clumped spatial distribution for

14 species (Chi-square test: *P* range: <0.0001 to 0.039, df = 374). The variance/mean ratio was highest for *Cyclonaias tuberculata*, *Lasmigona costata*, and *Truncilla donaciformis*. In general, species with statistical support for clumping were the most abundant species. Mussel SAR were found in 83% quadrats sampled but only represented 14% of individuals collected.

Nine species (including four SAR species) were detected only by excavation. Mean length of mussels collected by excavation was significantly lower than visual-tactile methods (K-S test: D = 0.39, $p < 0.0001$). A greater number of small and juvenile mussels (<90 mm) were collected by excavation (Figure 7).

With three random starts, the estimated probability of detecting 95% of all species present was only 0.12. The probability of detecting 85% of all species present was much higher (0.93). Individually, 78% of mussel species (including six SAR) were predicted to be detected with three random starts. Probability of detection was lower for three other SAR (*Truncilla donaciformis*, *Obovaria subrotunda*, and *Simpsonaias ambigua*) but could be improved to varying extents with greater sampling intensity (Figure 9). Simulated data based on three random starts provided a Jaccard similarity index similar to the full census dataset (0.88). The Bray-Curtis index showed little similarity to the census dataset in species abundance when only three quadrats in each block were sampled (0.33), but increased rapidly with increasing sample size. However, Bray-Curtis similarity $\geq$0.8 was not reached until 11 quadrats were sampled within each block.

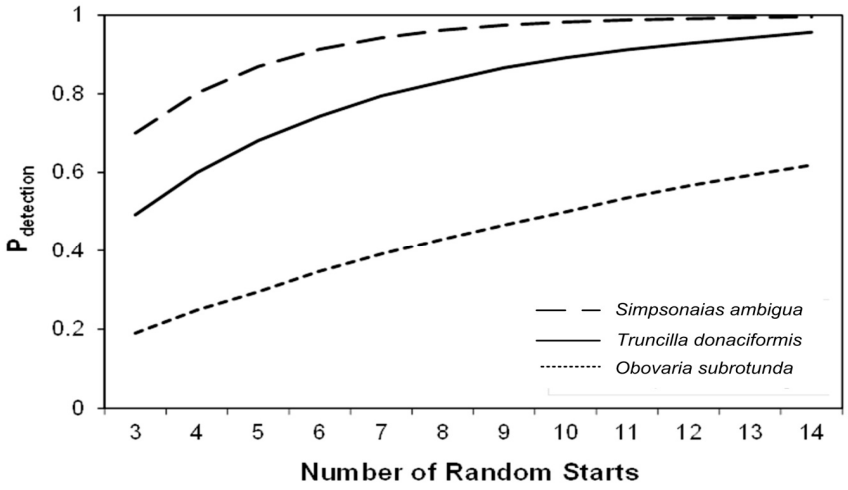


**Figure 9.** Relationships between sampling effort (number of random starts in each block) and probability of detection ($P_{detection}$) for three mussel species at risk occurring at low density ($\leq$0.02 $m^{-2}$) at the Sydenham River census site.

For SAR mussels, the mean percent difference between estimated and census population density was 49%. For other species, the difference was lower at 39%. To be within 15%, 10%, and 5% of full census density estimates for individual species, sampling is predicted to require two, three, and four times the current effort. In contrast, three random starts resulted in total mussel density estimates within 10% of full census value.

## 5. Discussion

Our results indicate that the systematic, 1 $m^2$ quadrat-based sampling protocol can be expected to: (1) detect >80% of mussel species present at a monitoring station; and (2) provide accurate and precise estimates of total mussel density that are suitable for population monitoring. The protocol is also likely to support monitoring objectives for detecting small changes in the density (30%) of relatively abundant species, including some SAR such as *Quadrula quadrula* and *Villosa fabilis*. However, rare species (i.e., densities < 0.1 $m^{-2}$) cannot be detected reliably, and the protocol is only expected to allow for detection of extreme changes in density (70%) for about 35% of SAR and slightly more than 50% of

other species. Detection of all species present at a site and detection of small changes in density of individual species will require substantially greater sampling effort (up to 12 random starts or 80% of the site). It may also require the application of different sampling designs such as adaptive cluster sampling [37]. The difficulty and expense required to collect accurate and precise density estimates is well recognized [38,39], especially for populations of rare species [40].

The consistent detection of freshwater mussels is challenging, because mussels are spatially clustered and cryptic, and many species are rare [41]. Timed search surveys are considered more efficient than quadrat sampling for detecting rare species (e.g., [7,42–44]). Visual-tactile timed searches are less labor-intensive than quadrat sampling, and this allows sampling a wider variety of habitats, a larger number of sites, and a greater number of individuals, all of which can improve species detection. Our results support the importance of excavation for improving species detection as reported by previous studies [14,40,45]. Excavation during quadrat sampling greatly improved the detection of mussel species (including several mussel SAR species). It was also essential for the detection of small individuals and to accurately estimate density. The importance of excavating to detect small individuals is also in agreement with other studies undertaken in eastern North American rivers [14,40,45]. It is uncertain whether gains in spatial coverage or number of sampling sites associated with less time-consuming timed searches can offset poor detectability associated with some species, seasons, or the low-densities characteristic of mussel populations in Ontario rivers. Repeated timed searches could improve detection if burrowed individuals of previously undetected species move to the surface. Repeat sampling data could also be used to develop an occupancy-based monitoring program that explicitly accounts for imperfect species detection [46]. Alternatively, double sampling approaches that combine timed searches along river reaches to identify mussel aggregations with excavation might provide a balance between improved species detection and greater cost [25,40].

Census datasets, in combination with simulation approaches, can help to inform the design of freshwater mussel population monitoring programs. A census dataset from the Capacon River (West Virginia) was used to explore gains in efficiency associated with different quadrat sizes, sampling efforts, and sampling strategies [15,25,47]. In this study, our two census sites differed in species richness, mussel density, and patterns of spatial distribution. However, both datasets identified: (1) large differences in the size of mussels collected by visual-tactile and excavation methods; and (2) large biases in the characterization of mussel assemblages using species count data obtained with three random starts. In both cases, simulations indicate that large increases in sampling effort (i.e., number of random starts) are required to improve the accuracy of data collection. The two census datasets could be used in future research to explore whether adaptive clustering sampling [33] can improve mussel population data accuracy and precision in a more cost-effective manner. The low percentage of mussels detected at the surface of census sites, however, indicates that more efficient double-sampling designs (where only a subset of quadrats are excavated) are not suitable for low-density populations typical of Ontario river sites.

Over the past decade, a network of permanent monitoring stations has been established across southwestern Ontario to monitor mussel SAR populations, habitats, and threats. The OFMRT protocol was designed to monitor SAR density at individual stations. However, monitoring data from these stations are being used to infer status and trends at the population (i.e., within watersheds) and species (i.e., across Ontario) levels. For example, species status assessments use density estimates from individual stations along with the known distribution in a river to estimate total adult population size (e.g., [48]). This practice is not recommended for the following reasons. First, our results show that substantially more sampling effort is required to reliably detect rare species and generate accurate and precise population estimates. Second, the network was not established using a statistical-based design (i.e., using random site selection) or with the stated objective to generate population-level estimates for individual species. Instead, site selection was based on prior knowledge of the location of dense and diverse mussel aggregations and SAR occurrence, and the goal of distributing sites across southwestern Ontario. Data collection is also spatially unbalanced; some rivers (e.g., Sydenham River)

have a much greater density of monitoring stations than others. Consequently, estimates of total population size based on outstanding assemblages are likely biased and high, which could lead to inappropriate conservation decisions.

Our evaluation of the OFMRT protocol suggests needed improvements in monitoring the status of Ontario mussel SAR. At monitoring stations, sampling efforts need to be increased in order to consistently detect rare species, precisely estimate population densities, and to allow detection of small-moderate changes in density. However, the level of effort necessary to achieve these objectives likely exceeds available resources. Given these limitations, the scope and design of the monitoring protocol may require more fundamental revision. Due to the low densities of most species, the current design is best suited for monitoring trends in the overall density and species diversity of mussel beds. A statistical-based design has been implemented to sample mussels at each monitoring site. However, the design (i.e., number and location of stations) of the overall monitoring network needs to incorporate random site selection if population-level parameters are to be estimated, and if inferences over larger spatial scales are desired [25]. Two-phase sampling strategies successfully applied in Pennsylvania [45] and West Virginia [37] could provide such an approach.

**Acknowledgments:** Research was supported by Fisheries and Oceans Canada and Ontario Ministry of Natural Resources and Forestry species at risk program funds. We would like to acknowledge the efforts of the Ausable Bayfield Conservation Authority, Environment Canada, Fisheries and Oceans Canada, and Ontario Ministry of Natural Resources and Forestry staff that participated in sampling, and Jessica Epp, Jan Moryk, and Victoria Kopf who provided assistance with data entry and compilation. Earlier versions of the manuscript were improved by comments from Wendell Haag, Sarah Hogg, and three anonymous reviewers.

**Author Contributions:** Both authors conceived the project, participated in field data collection and contributed to manuscript preparation. Scott Reid analyzed the data.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Sampling details (number of blocks and quadrats), number of mussels (N), and number of species detected ($S_{OBS}$) at the multi-river, index monitoring sites. $S_{EST}$ was calculated using the non-parametric Chao2 species richness estimator. $Q_1$ and $Q_2$ are the number of singletons and doubletons.

| Site | Blocks | Quadrats | N | $S_{OBS}$ | $S_{EST}$ | $Q_1$ | $Q_2$ | $N/S_{OBS}$ | $Q_1/S_{OBS}$ [1] |
|---|---|---|---|---|---|---|---|---|---|
| Ausable River | | | | | | | | | |
| AR-12 | 25 | 75 | 298 | 16 | 19.9 | 4 | 2 | 18.6 | 25 |
| AR-13 | 25 | 75 | 82 | 9 | | 1 | 0 | 9.1 | 11 |
| AR-24 | 25 | 75 | 1356 | 18 | 18.5 | 1 | 1 | 75.3 | 6 |
| AR-27 | 31 | 93 | 122 | 12 | 16.5 | 3 | 1 | 10.2 | 25 |
| AR-28 | 27 | 81 | 379 | 16 | 16.7 | 2 | 3 | 23.7 | 13 |
| AR-29 | 24 | 72 | 9 | 5 | 9.4 | 3 | 1 | 1.8 | 60 |
| AR-33 | 29 | 87 | 24 | 7 | 11.0 | 4 | 2 | 3.4 | 57 |
| AR-34 | 22 | 66 | 133 | 11 | 11.2 | 1 | 2 | 12.1 | 9 |
| AR-5 | 23 | 69 | 114 | 13 | 15.2 | 3 | 2 | 8.8 | 23 |
| AR-7 | 23 | 69 | 870 | 19 | | 4 | 0 | 45.8 | 21 |
| AR-8 | 25 | 75 | 252 | 13 | 13.5 | 1 | 1 | 19.4 | 8 |
| Grand River | | | | | | | | | |
| GR-03 | 21 | 63 | 89 | 5 | 5.5 | 1 | 1 | 17.8 | 20 |
| GR-13 | 21 | 63 | 18 | 4 | 6.0 | 2 | 1 | 4.5 | 50 |
| GR-31 | 16 | 48 | 20 | 5 | 7.0 | 2 | 1 | 4.0 | 40 |
| GR-33 | 20 | 60 | 54 | 5 | | 1 | 0 | 10.8 | 20 |
| Maitland River | | | | | | | | | |
| MR-01 | 20 | 60 | 15 | 5 | 6.0 | 2 | 2 | 3.0 | 40 |
| MR-02 | 20 | 60 | 165 | 7 | 14.9 | 4 | 1 | 23.6 | 57 |
| MR-09 | 21 | 63 | 126 | 7 | 7.0 | 0 | 1 | 18.0 | 0 |
| MR-14 | 20 | 60 | 64 | 8 | 8.2 | 1 | 2 | 8.0 | 13 |
| MR-16 | 21 | 63 | 55 | 8 | 10.2 | 3 | 2 | 6.9 | 38 |
| MR-21 | 20 | 60 | 26 | 6 | 6.2 | 1 | 3 | 4.3 | 17 |

**Table A1.** *Cont.*

| Site | Blocks | Quadrats | N | $S_{OBS}$ | $S_{EST}$ | $Q_1$ | $Q_2$ | $N/S_{OBS}$ | $Q_1/S_{OBS}$ [1] |
|---|---|---|---|---|---|---|---|---|---|
| Sydenham River | | | | | | | | | |
| SR1 | 28 | 84 | 85 | 16 | | 8 | 0 | 5.3 | 50 |
| SR10 | 25 | 75 | 246 | 17 | 21.4 | 3 | 1 | 14.5 | 18 |
| SR12 | 26 | 78 | 233 | 20 | 20.0 | 0 | 3 | 11.7 | 0 |
| SR13 | 25 | 75 | 151 | 9 | | 4 | 0 | 16.8 | 44 |
| SR15 | 26 | 78 | 165 | 6 | 6.0 | 0 | 1 | 27.5 | 0 |
| SR17 | 27 | 81 | 318 | 19 | 19.0 | 0 | 3 | 16.7 | 0 |
| SR19 | 25 | 75 | 816 | 23 | 23.2 | 1 | 2 | 35.5 | 4 |
| SR2 | 26 | 78 | 276 | 15 | 27.3 | 5 | 1 | 18.4 | 33 |
| SR20 | 27 | 81 | 42 | 9 | | 4 | 0 | 4.7 | 44 |
| SR21 | 28 | 84 | 10 | 2 | | 1 | 0 | 5.0 | 50 |
| SR3 | 23 | 69 | 230 | 21 | | 7 | 0 | 11.0 | 33 |
| SR5 | 23 | 69 | 769 | 21 | 21.2 | 1 | 2 | 36.6 | 5 |
| SR6 | 26 | 78 | 761 | 23 | 24.5 | 3 | 3 | 33.1 | 13 |
| SR7 | 27 | 81 | 1138 | 23 | 29.2 | 5 | 2 | 49.5 | 22 |
| Thames River | | | | | | | | | |
| TM04-15 | 23 | 69 | 75 | 9 | 9.0 | 0 | 0 | 8.3 | 0 |
| TM-05-01 | 23 | 69 | 96 | 14 | 16.0 | 2 | 1 | 6.9 | 14 |
| TR 97-11 | 22 | 66 | 16 | 7 | 9.2 | 3 | 2 | 2.3 | 43 |
| TR 97-3 | 22 | 66 | 146 | 7 | 7.2 | 1 | 2 | 20.9 | 14 |
| TR 97-18 | 21 | 63 | 87 | 7 | | 3 | 0 | 12.4 | 43 |

[1]: value expressed as a percentage.

## References


1. Williams, J.D.; Warren, M.L.; Cummings, K.S.; Harris, J.L.; Neves, R.J. Conservation status of the freshwater mussels of the United States and Canada. *Fisheries* **1992**, *18*, 6–22. [CrossRef]
2. Metcalfe-Smith, J.L.; MacKenzie, A.; Carmichael, I.; McGoldrick, D. *Photo Field Guide to the Freshwater Mussels of Ontario*; St. Thomas Field Naturalist Club Inc.: St. Thomas, ON, Canada, 2005.
3. Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Wildlife Species Search 2013. Available online: http://www.cosewic.gc.ca/eng/sct1/searchform_e.cfm (accessed on 17 September 2013).
4. Ontario Ministry of Natural Resources and Forestry (MNRF). Species at Risk in Ontario (SARO) List. 2014. Available online: http://www.ontario.ca/environment-and-energy/species-risk-ontario-list (accessed on 16 September 2014).
5. Schloesser, D.W.; Nalepa, T.F. Dramatic decline of unionid bivalves in the offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. *Can. J. Fish. Aquat. Sci.* **1994**, *51*, 2234–2242. [CrossRef]
6. Clarke, A.H. Ontario's Sydenham River, an important refugium for native freshwater mussels against competition from the zebra mussel, *Dreissena polymorpha*. *Malacol. Data Net* **1992**, *3*, 43–55.
7. McNichols-O'Rourke, K.A.; Robinson, A.; Morris, T.J. *Summary of Freshwater Mussel Timed Search Surveys in Southwestern Ontario in 2010 and 2011*; Canadian Manuscript Report Fisheries and Aquatic Sciences 3009; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2012.
8. Morris, T.; Burridge, J.M. *Recovery Strategy for Northern Riffleshell, Snuffbox, Round Pigtoe, Mudpuppy Mussel and Rayed Bean in Canada*; Species at Risk Act Recovery Strategy Series; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2006.
9. Department of Fisheries and Oceans Canada (DFO). *Report on the Progress of Recovery Strategy Implementation for the Wavyrayed Lampmussel, Northern Riffleshell, Snuffbox, Round Pigtoe, Mudpuppy Mussel and Rayed Bean in Canada for the Period 2006–2011*; Species at Risk Act Recovery Strategy Report Series; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2013.
10. Cudmore, B.; Mandrak, N.E.; Morris, T.J.; Edwards, A. *Allowable Harm Analysis Workshops for Freshwater Species at Risk in Central and Arctic Region*; Canadian Science Advisory Secretariat Proceedings Series 2006/026; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2006.

11. Department of Fisheries and Oceans Canada (DFO). *Assessment of Methods for the Identification of Critical Habitat for Freshwater Mussels*; Canadian Science Advisory Secretariat Science Advisory Report 2011/047; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2011.
12. Thompson, S.K. *Sampling*, 2nd ed.; John Wiley and Sons: New York, NY, USA, 2002.
13. Christman, M.C. A review of quadrat-based sampling of rare, geographically clustered populations. *J. Agric. Biol. Ecol. Stat.* **2000**, *5*, 168–201. [CrossRef]
14. Smith, D.R.; Villella, R.F.; Lemarie, D.P. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania. *J. N. Am. Benthol. Soc.* **2001**, *20*, 118–132. [CrossRef]
15. Pooler, P.S.; Smith, D.R. Optimal sampling design for estimating spatial distribution and abundance of a freshwater mussel population. *J. N. Am. Benthol. Soc.* **2005**, *24*, 525–537. [CrossRef]
16. Metcalfe-Smith, J.L.; Staton, S.K.; Mackie, G.L.; Lane, N.M. Changes in the biodiversity of freshwater mussels in the Canadian waters of the Lower Great Lakes drainage basin over the past 140 years. *J. Gt. Lakes Res.* **1998**, *24*, 845–858. [CrossRef]
17. Metcalfe-Smith, J.L.; McGoldrick, D.J.; Zanatta, D.T.; Grapentine, L.C. *Development of a Monitoring Program for Tracking the Recovery of Endangered Mussels in the Sydenham River, Ontario*; Water Science and Technology Directorate Contribution 07-510; Environment Canada: Burlington, ON, Canada, 2007.
18. Reid, S.M. Search effort and imperfect detection: Influence on timed-search mussel (Bivalvia: Unionidae) surveys in Canadian rivers. *Knowl. Manag. Aquat. Ecosyst.* **2016**, *417*, 17. [CrossRef]
19. Green, R.H.; Young, R.C. Sampling to detect rare species. *Ecol. Appl.* **1993**, *3*, 351–356. [CrossRef] [PubMed]
20. Zar, J.H. *Biostatistical Analysis*, 2nd ed.; Prentice-Hall: Engelwood Cliffs, CA, USA, 1984.
21. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 1–9.
22. Chao, A.; Colwell, R.K.; Lin, C.W.; Gotelli, N.J. Sufficient sampling for asymptotic minimum species richness estimators. *Ecology* **2009**, *90*, 1125–1133. [CrossRef]
23. Lopez, L.C.S.; Fracasso, M.P.D.; Mesquita, D.O.; Palma, A.R.T.; Riul, P. The relationship between percentage of singletons and sampling effort: A new approach to reduce the bias of richness estimates. *Ecol. Indic.* **2012**, *14*, 164–169. [CrossRef]
24. Coddington, J.A.; Agnarsson, I.; Miller, J.A.; Kunter, M.; Hormiga, G. Undersampling bias: The null hypothesis for singleton species in tropical arthropod surveys. *J. Anim. Ecol.* **2009**, *78*, 573–584. [CrossRef] [PubMed]
25. Strayer, D.L.; Smith, D.R. *A Guide to Sampling Freshwater Mussel Populations*; Monograph 8; American Fisheries Society: Bethesda, MD, USA, 2003.
26. Jones, N.E.; Petreman, I.C. The capacity to detect change stream fish communities characteristics at the site-level in the Lake Ontario basin. *Environ. Manag.* **2012**, *50*, 77–88. [CrossRef] [PubMed]
27. Committee on the Status of Endangered Wildlife in Canada (COSEWIC). COSEWIC Assessment Process, Categories and Guidelines. Version: November 2015. Available online: http://www.cosewic.gc.ca/eng/sct1/searchform_e.cfm (accessed on 17 October 2016).
28. Peterman, R. Statistical power analysis can improve fisheries research and management. *Can. J. Fish. Aquat. Sci.* **1990**, *47*, 2–15. [CrossRef]
29. Jackson, D.A.; Harvey, H.H. Qualitative and quantitative sampling of lake fish communities. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 2807–2813. [CrossRef]
30. Manly, B.F.J. *Randomization, Bootstrap and Monte Carlo Methods in Biology*, 3rd ed.; Chapman and Hall/CRC: Boca Raton, FL, USA, 2007.
31. Legendre, P.; Legendre, L. *Numerical Ecology*, 2nd ed.; Elsevier Science: Amsterdam, The Netherlands, 1998.
32. Hood, G.M. PopTools Version 3.2.5. 2010. Available online: http://www.poptools.org (accessed on 19 March 2014).
33. Morrison, L.W.; Smith, D.R.; Young, C.C.; Nichols, D.W. Evaluating sampling designs by computer simulation: A case study with the Missouri bladderpod. *Popul. Ecol.* **2008**, *50*, 417–425. [CrossRef]
34. Krebs, C.J. *Ecological Methodology*; Harper and Row Publishers: New York, NY, USA, 1989.
35. Rosenberg, M.S.; Anderson, C.D. PASSaGE: Pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods Ecol. Evol.* **2011**, *2*, 229–232. [CrossRef]
36. Smith, D.R.; Villella, R.F.; Lemarie, D.P. Application of adaptive cluster sampling to low-density populations of freshwater mussels. *Environ. Ecol. Stat.* **2003**, *10*, 7–15. [CrossRef]
37. Villella, R.F.; Smith, D.R. Two-phase sampling to estimate river-wide populations of freshwater mussels. *J. N. Am. Benthol. Soc.* **2005**, *24*, 357–368. [CrossRef]

38. Thompson, W.L. Future directions in estimating abundance of rare or elusive species. In *Sampling Rare or Elusive Species. Concepts, Designs, and Techniques for Estimating Population Parameters*; Thompson, W.L., Ed.; Island Press: Washington, DC, USA, 2004; pp. 389–399.
39. Witmer, G.W. Wildlife population monitoring: Some practical considerations. *Wildl. Res.* **2005**, *32*, 259–263. [CrossRef]
40. Smith, D.R.; Villella, R.F.; Lemarie, D.P.; von Oettingen, S. How much excavation is needed to monitor freshwater mussels? In Proceedings of the First Mollusk Conservation Society Symposium, Chattanooga, TN, USA, 16–20 March 1999; Ohio Biological Society: Columbus, OH, USA, 2000; pp. 203–218.
41. Wisniewski, J.M.; Rankin, N.M.; Weiler, D.A.; Strickland, B.A.; Chandler, H.C. Occupancy and detection of benthic invertebrates: A case study of unionids in the lower Flint River, Georgia, USA. *Freshw. Sci.* **2013**, *32*, 1122–1135. [CrossRef]
42. Metcalfe-Smith, J.L.; Maio, J.; Staton, S.; Mackie, G. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. *J. N. Am. Benthol. Soc.* **2000**, *19*, 725–732. [CrossRef]
43. Epp, J.M.; Morris, T.J.; McNichols-O'Rourke, K.A. *A Preliminary Search for Epioblasma torulosa rangiana (Northern Riffleshell) in the Maitland River Watershed*; Canadian Manuscript Report Fisheries and Aquatic Sciences 3025; Fisheries and Oceans Canada: Ottawa, ON, Canada, 2013.
44. Randklev, C.R.; Johnson, M.S.; Tsakiris, E.T.; Groce, J.; Wilkins, N. Status of the freshwater mussel (Unionidae) communities of the Leon River, Texas. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2013**, *23*, 390–404. [CrossRef]
45. Smith, T.A.; Crabtree, D. Freshwater mussel (Unionidae: Bivalvia) distributions and densities in French Creek, Pennsylvania. *Northeast. Nat.* **2010**, *17*, 387–414. [CrossRef]
46. Guillera-Arroita, G.; Lahoz-Monfort, J.J. Designing studies to detect differences in species occupancy: Power analysis under imperfect detection. *Methods Ecol. Evol.* **2012**, *3*, 860–869. [CrossRef]
47. Salehi, M.M.; Smith, D.R. Two-stage sequential sampling: A neighbourhood-free adaptive sampling procedure. *J. Agric. Biol. Ecol. Stat.* **2005**, *10*, 84–103. [CrossRef]
48. Committee on the Status of Endangered Wildlife in Canada (COSEWIC). *COSEWIC Assessment and Status Report on the Wavy-Rayed Lampmussel Lampsilis fasciola in Canada*; Committee on the Status of Endangered Wildlife in Canada: Ottawa, ON, Canada, 2010.