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Search effort and imperfect detection: Influence on timed-search mussel (Bivalvia: Unionidae) surveys in Canadian rivers

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Abstract – Inventories and population monitoring are essential activities supporting the conservation of freshwater mussel diversity in Canadian rivers. Despite widespread use of timed-search methods to survey river mussels, the relationship between species detection and search effort has received limited study. In this study, repeat-sampling data from 54 Ontario river sites were used to estimate: (1) species detection probabilities; (2) the number of sampling events required to confidently detect species; and, (3) the power of timed-search surveys to detect future distribution declines. Mussels were collected using visual and tactile methods, and collection data were recorded separately for each 1.5 h of search time (up to 4.5 h). Thirteen species were collected; including two endangered species (Rainbow *Villosa iris* and Eastern Pondmussel *Ligumia nasuta*). In all cases, species detection was imperfect. However, detection probabilities (p) for most species were high (>0.69). Two repeat 4.5 h surveys are required to confidently assess whether most (83%) species are present at a site. Search effort had a positive effect on estimates of species richness, detection probability and site occupancy, and the power to detect future distribution declines. At all levels of sampling effort, detection probability and site occupancy estimates were positively correlated to mussel abundance.

Key-words: Unionids / endangered species / monitoring / sampling design

Résumé – Effort de prospection et détection imparfaite : Influence de la prospection chronométrée d'échantillonnage de moules (Bivalvia : Unionidae) dans les rivières canadiennes. Les inventaires et la surveillance de populations sont des activités essentielles pour soutenir la conservation de la diversité des moules d'eau douce dans les rivières canadiennes. Malgré l'utilisation généralisée de méthodes chronométrées pour étudier les moules de rivière, la relation entre la détection des espèces et l'effort de recherche a été peu étudiée. Dans cette étude, des données d'échantillonnage répété de 54 sites de rivière de l'Ontario ont été utilisées pour estimer : (1) les probabilités de détection de l'espèce ; (2) le nombre d'événements d'échantillonnage requis pour détecter de façon fiable toute les espèces ; et, (3) la puissance des échantillonnages chronométrés pour détecter de futures baisses de distribution. Les moules ont été recueillies à l'aide des méthodes visuelles et tactiles, et des données de collecte ont été enregistrées séparément pour chaque 1,5 h de temps de recherche (jusqu'à 4,5 h). On a récolté treize espèces ; y compris deux espèces en voie de disparition (Rainbow *Villosa iris* and Eastern Pondmussel *Ligumia nasuta*). Dans tous les cas, la détection des espèces était imparfaite. Cependant, les probabilités de détection (p) pour la plupart des espèces étaient élevées (>0,69). Deux répétitions de 4,5 h de prospection sont nécessaires pour évaluer en toute confiance que la plupart (83 %) des espèces sont présentes sur un site. L'effort de recherche a eu un effet positif sur les estimations de la richesse en espèces, la probabilité de détection et de l'occupation du site, et le pouvoir de détecter les futures baisses de distribution. À tous les niveaux de l'effort d'échantillonnage, les estimations de probabilité de détection et d'occupation du site étaient positivement corrélées à l'abondance des moules.

Mots-clés : Unionidés / espèces menacées / surveillance / conception d'échantillonnage

1 Introduction

North American water bodies contain the most diverse collection of freshwater mussels (family: Unionidae) in the world. However, freshwater mussels are also considered the most imperiled faunal group in North America (Williams *et al.*, 1992). Historical mussel fauna declines were related to the degradation of riverine habitats (*e.g.* the construction of dams and impoundments, loss of riparian zones, and water pollution) and commercial harvest (Metcalfe-Smith *et al.*, 1998; Haag, 2012). More recent and rapid declines have occurred since

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the introduction (and spread) of non-native dreissenid mussels to the Laurentian Great Lakes (Schloesser and Nalepa, 1994; Lucy *et al.*, 2013). In Canada, the greatest diversity of freshwater mussels is found in the province of Ontario. Fifteen of the 41 freshwater mussel species found in Ontario are listed as Endangered, Threatened or Special Concern under the federal *Species at Risk Act* and the provincial *Endangered Species Act* (COSEWIC, 2013; MNRF, 2014). Actions undertaken to protect and recover Ontario's mussels include surveys and the establishment of population index monitoring stations. Data collected through inventory and monitoring activities are essential for delineating areas of protected habitat, assessing population status and trends, and evaluating the effectiveness of recovery actions (Cudmore *et al.*, 2006; DFO, 2011).

In contrast to Laurentian Great Lakes habitats, most rivers are not heavily infested by dreissenids and historical mussel diversity is largely intact (McNichols-O'Rourke *et al.*, 2012); providing important refugia for native mussels from dreissenid impacts (Clarke, 1992). Timed-search methods are commonly used for river mussel surveys (*e.g.* McNichols-O'Rourke *et al.*, 2012; Epp *et al.*, 2013; Randklev *et al.*, 2013); as some authors consider timed-search surveys to be more effective and efficient at rare species detection than quadrat sampling (Metcalfe-Smith *et al.*, 2000). A greater variety of habitats are targeted during visual and tactile sampling, and therefore, a greater number of species are expected to be collected. In addition to developing species lists for river reaches or watersheds, timed search data can be used to inform the design of more intensive (and costly) sampling efforts to estimate mussel population densities (Strayer and Smith, 2003). If the monitoring objective is to track changes in species distribution, the effort required to reliably detect a species may also be substantially less than tracking changes in abundance with quantitative sampling (*i.e.* quadrats) approaches (Gibbs *et al.*, 1998).

Freshwater mussel detection can be challenging as they often occur at low densities, are spatially clustered and imperfectly detected (Smith *et al.*, 2010). Past studies have found that detection is affected by a variety of factors, including: shell size and sculpture (Hornbach and Deneka, 1996); whether a mussel tends to be epibenthic or endobenthic (Amyot and Downing, 1991); river flow conditions and composition of bed material (Villella *et al.*, 2004; Wisniewski *et al.*, 2013a); and, experience of field staff (Wisniewski *et al.*, 2013a). Despite its widespread use, the relationship between species detection and search effort during qualitative river mussel sampling has received limited study (Strayer and Smith, 2003). To ensure that mussel surveys provide meaningful results, it is important to assess the effect of sampling design choices, such as search effort.

In this study, I used a repeat-sampling approach (MacKenzie *et al.*, 2002) to model the relationship between search effort and detection of freshwater mussel species. Species detection histories associated with discrete replicate surveys were used to jointly model occupancy (*i.e.* the proportion of sampled sites occupied) and detection probabilities. The approach has been broadly used for other aquatic and semi-aquatic taxa (Jeffress *et al.*, 2011; Schloesser *et al.*, 2012; Pearl *et al.*, 2013; Dextrase *et al.*, 2014; Smith *et al.*, 2014), but rarely for freshwater mussels (Wisniewski *et al.*, 2013a). Monitoring programs can use such repeat-sampling data to monitor changes in species distribution, while adjusting for the bias associated with imperfect detection. In this study, repeat-sampling data were used to estimate: (1) species-specific detection probabilities; (2) the number of sampling events required to confidently detect species; and, (3) the power of timed-search surveys to detect future distribution (or occupancy) declines. Mussel collection data were also used to characterize: (1) the influence of search effort on species richness estimates and detection of rare species; and (2) the level of agreement among collections by different field crew members.

2 Materials and methods

2.1 Mussel sampling

Fifty-four wadeable sites along the Trent ($n = 35$), Moira ($n = 12$) and Salmon ($n = 7$) rivers were sampled. The three southern Ontario rivers flow into the Bay of Quinte, eastern Lake Ontario (44°08′54″N; 77°22′46″W). Sites were selected based on historical records of the endangered Rainbow Mussel (*Villosa iris*),suitability of habitats for sampling (*i.e.* searches could be safely undertaken by foot) and permission to access private lands. Trent River sites were located along both the river mainstem and four tributaries (Cold, Percy, Rawdon and Salt creeks). As part of the Trent-Severn Waterway, the Trent River has been highly fragmented by navigational and run-of-the-river hydro-electric dams. The Moira and Salmon rivers are fragmented by old mill, low flow augmentation, and flood control dams. Surficial geology of the study area is a complex mix of Precambrian and Paleozoic (limestone) bedrock, glacial deposits and limestone till (Chapman and Putnam, 1984); resulting in a wide range of flow and bed material characteristics across sampling sites (Reid *et al.*, 2005). Reach lengths surveyed ranged from 31 to 362 m (mean = 134 m); channel widths ranged from 5 to 175 m (mean = 27 m). Mean water depths ranged from 0.14 to 0.71 m.

Live mussels and fresh shells were collected using a time-search method developed for the detection of rare species (Metcalfe-Smith *et al.*, 2000). A three person crew searched for mussels at each site with the aid of mussel viewers and polarized sunglasses. No fixed search pattern was used during visual and tactile sampling, and not all habitats were sampled in a site. Instead, crew members spread out and focused on suitable mussel habitats (*e.g.* riffles and margins of islands). Habitats too deep to be safely or effectively sampled were bypassed. The area searched by each person did not overlap. Mussel searches did not include the excavation of the riverbed.

Sampling effort was broken down into three 1.5 hour (h) intervals for a total of 4.5 h of sampling. Live individuals and shells were identified to species (Metcalfe-Smith *et al.*, 2005). Live individuals were enumerated by each 1.5 h sampling interval, and crew member. Species detections associated with shells were recorded for each sampling event. Each site was sampled on three separate dates. First and last sampling visits were separated, on average, by 45 days (range: 18–97). Across site sampling visits, areas searched were rotated among crew members so that habitats were not resampled by the same person. Sampling was completed between 4 June and 25 September 2013 along the Trent River, and 27 June and 26 September 2014 along the Moira and Salmon rivers. Within each year, the membership of field crews was the same. Mussel fieldwork experience ranged from 0 to seven years in 2013, and from one to two years in 2014.

2.2 Data analysis

For each year, the similarity of mussel collections among field crew members was assessed by comparing the number of individuals collected and the number of species detected and, calculating an extension of Cohen's Kappa statistic for multiple observers (Fleiss *et al.*, 2003, Agresti, 2013). The Kappa statistic is an index of agreement between different classification methods; with +1 indicating perfect agreement and 0 indicating a level of agreement that would be expected by chance alone. For each species, Fleiss's Kappa was calculated using site detection histories (0 for absent, 1 for present) associated with the three sampling events completed by each field crew member.

To evaluate the effect of search effort (1.5, 3.0 and 4.5 h) on the number of species detected (richness) during surveys, sample-based rarefaction curves were computed using Mao's τ (Colwell *et al.*, 2004). Species richness was estimated based on the frequency of species that occurred only once (singletons) or twice (doubletons) in a sample. Curves were computed using data from the first sampling visit and the software PAST version 1.94 (Hammer *et al.*, 2001). To determine the effect of increasing sampling effort on the detection of locally rare species, the species were classified as rare (1–5 individuals per 4.5 h sampling event), uncommon (6–10 individuals) or common (>10 individuals) for each site (Metcalfe-Smith *et al.,* 2000). For each abundance category, the level of effort required to first detect species at a sampling site was identified and summarized.

For 12 species, the probability of at least one individual being detected (p) from a site and the probability of a site being occupied by a species (ψ) were estimated using PRESENCE 9.7 (2015) software (http://www.mbr-pwrc.usgs.gov/software/presence.html). Using repeat sampling data, site occupancy and detection probability are jointly estimated using a multinomial likelihood occupancy model (MacKenzie *et al.*, 2006). The method parallels a closed-population, Capture-Mark-Recapture model, with an additional parameter representing probability of species presence (MacKenzie *et al.*, 2002). Occupancy models use detection probabilities to adjust estimates of naïve occupancy (the proportion of sites observed to be occupied by a species); thereby reducing bias associated with imperfect species detection. Probabilities were estimated using detection histories from three successive sampling events, and the constant probability, single-season model (MacKenzie *et al.*, 2002). Single-season models assume that sites are closed to immigration or emigration at the species level. Probabilities were estimated using detection histories for: (1) live individuals associated with 1.5 h, 3.0 h and 4.5 h of cumulative search effort, and (2) live individuals plus fresh shells collected during 4.5 h of cumulative search effort. Detection probabilities were not estimated for Eastern Pondmussel (*Ligumia nasuta*),because it was collected from only one site. The Spearman rank correlation was used to test for positive relationships between number of individuals collected at a site and estimates of p and ψ.

Detection probabilities were used to estimate the minimum number of repeat surveys ($N_{\min}$) required to confidently detect a species at a sampling site. For the analysis, a repeat survey refers to multiple sampling events that are undertaken on different dates. $N_{\min}$ was calculated with the formula:

$$\log(1 - CL)/\log(1 - p) \quad \text{(Pellet and Schmidt, 2005).}$$

The confidence level (CL) was 0.95 (*i.e.* 95% confident that a species is absent from a site).

A prospective power analysis was undertaken to evaluate whether timed search sampling could reliably detect future declines in species distributions. Using p and ψ, power estimates were calculated for each species using an Excel-based sample size/power calculator (OccPower.xlsx). The power to detect differences in species occupancy between two independent, single-season surveys is calculated using a closed-form estimator (equation (3) in Guillera-Arroita and Lahoz-Monfort, 2012). Power (based on the critical region of a two-tailed test) is a function of the initial occupancy estimate (ψ), proportional change in occupancy between surveys (R), species detection probability (p), number of replicate surveys (K), and number of sites surveyed (S). The likelihood of detecting a change in occupancy between surveys improves as parameter values increase. Rates of declines (R = 30, 50 and 70%) were based on the quantitative criteria applied during Canadian (Committee on the Status of Endangered Wildlife in Canada, www.cosewic.gc.ca) and international (IUCN Red List of Threatened Species, www.iucnredlist.org) assessments of species status. A Type I error rate of 0.05 was adopted. Estimates of power $\geqslant$80 are considered high (Cohen, 1988).

3 Results

3.1 Summary of collections

Thirteen species were collected from the Trent (n = 12 species), Moira (n = 10) and Salmon rivers (n = 11). Species diversity ranged from 1 to 10 species per site (median = 7 species). Mussel assemblages were less diverse than found in southwestern Ontario rivers (Lake Erie and Lake St. Clair watersheds) (Metcalfe-Smith *et al.*, 1998). Seventy-two percent of species detections were from the collection of live individuals and 28% from the collection of fresh shells. Mean catch per unit effort (CPUE) of live mussels was similar among rivers: 48.2 mussels.h^{-1} along the Trent River, 44.2 mussels.h^{-1} along the Salmon River and 42.0 mussels.h^{-1} along the Moira River. The most abundant species was Eastern Elliptio (*Elliptio complanata*), comprising 72% of all live individuals collected. Individually, other species represented less than 9% of collections. The most widespread species were Elktoe (*Alasmidonta marginata*), Eastern Elliptio, Fatmucket (*Lampsilis siliquoidea*), Creek Heelsplitter (*Lasmigona compressa*), and Flutedshell (*Lasmigona costata*) (Table 1). Two mussel species-at-risk were detected (Rainbow Mussel from 24 sites, and Eastern Pondmussel from one Trent River site).

In 2013, there was little variation among crew members in the number of individuals collected and species detected

Table 1. Summary of freshwater mussel collection data based on repeated timed-searches (4.5 h) at 54 southern Ontario river sites.

Species	Total Number	Rank Abundance[1]	Naive Occupancy[2]	Mean (range) Shell Length
Alasmidonta marginata	17	11.7	5	57 (34–74)
Alasmidonta undulata	37	10.7	4	43 (24–54)
Anodontoides ferussacianus	540	3.3	18	49 (18–95)
Elliptio complanata	8244	1	41	68 (11–131)
Lampsilis cardium	91	9	14	94 (51–141)
Lampsilis siliquoidea	228	7	18	54 (14–110)
Lasmigona compressa	162	8	16	64 (24–92)
Lasmigona costata	952	2	29	100 (17–191)
Ligumia nasuta	1	13	1	79
Ligumia recta	29	10.7	10	123 (65–157)
Pyganodon grandis	344	5	11	83 (36–132)
Strophitus undulatus	331	5.3	22	61 (26–106)
Villosa iris	401	4.3	21	70 (17–115)

(1)mean value over three sampling events; (2)percentage of sites species collected from.

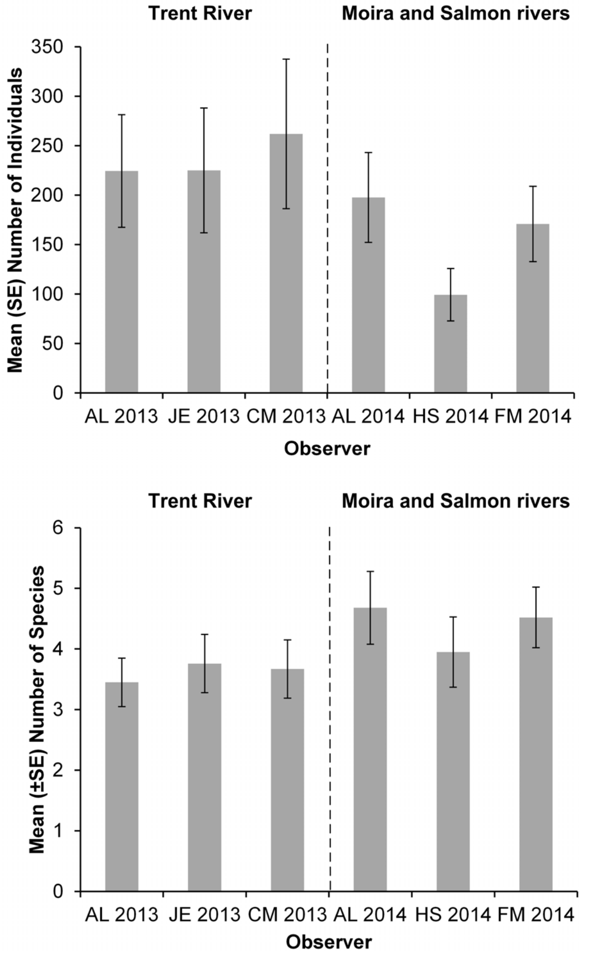

Fig. 1. Among field crew member (searcher) variation in number of individuals and mussel species collected during timed-search (4.5 h) surveys of 54 southern Ontario river sites.

from the Trent River (Figure 1). There was at least moderate agreement among field crew members (Kappa > 0.45, Landis and Koch, 1977) regarding the presence of each species at sampling sites. For eight of the 11 species detected, agreement ranged from substantial to almost perfect (Kappa: 0.61 to 0.96). There were more noticeable differences among crew members in 2014; with one member collecting 45 to 50% fewer individuals and detecting 13 to 16% fewer species from the Moira and Salmon rivers. Regarding the presence of eight of the 11 species (73%) detected, there was moderate to substantial agreement among field crew members (Kappa: 0.48 to 0.79). For Eastern Elliptio, Flutedshell and Giant Floater (*Pyganodon grandis*), agreement was only fair (Kappa: 0.21 to 0.30).

3.2 Impact of sampling effort on species detection

In comparison to 1.5 h of search effort, species detection occurred at a greater rate when search effort was 3.0 or 4.5 h (Figure 2). Rarefaction curves also indicate that a small number of species will only be detected by searching longer than 1.5 h. Fifty-seven percent of mussel species collected from each site were classified as locally rare, 10% uncommon and 33% common. For uncommon and common species, detection typically occurred during the first 1.5 h of searching (72 and 94% of detections, respectively) (Figure 3). However, only 31% of detections of locally rare species were in the first 1.5 h. For rare species, detection improved with greater search effort (48% of detections in the second 1.5 h period, and 21% of detections in the third 1.5 h period).

In all cases, species detection was imperfect. While 76% of species detections occurred during the first sampling event, 20% of detections required a second sampling event and 4% required a third sampling event. Based on 4.5 h of search effort, detection probabilities (p) for all species except Triangle Floater (*Alasmidonta undulata*) were high (>0.69) (Table 2). Only two repeat surveys are required to confidently determine whether most (83%) mussel species are present at a site (based on $N_{\min}$). It is predicted that four surveys are required to reliably detect Triangle Floater. While the percentage of sites occupied was higher for more abundant species, 4.5 h based detection probabilities were generally independent of species abundance.

Increasing search effort from 1.5 to 4.5 h resulted in a mean increase (calculated across species) of 25% to p and 16%

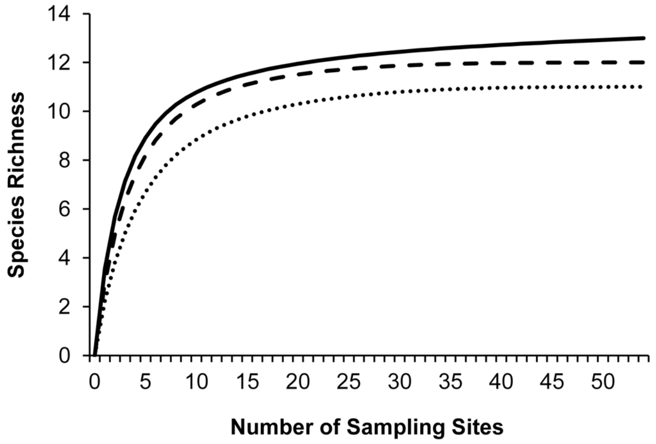

Fig. 2. Sample-based rarefaction curves associated with freshwater mussel collection data from 1.5 h (**...**), 3.0 h (**- - -**) and 4.5 (**—**) h of search effort at river sampling sites. Species richness estimates were computed based on Mao's τ (Colwell *et al.*, 2004).

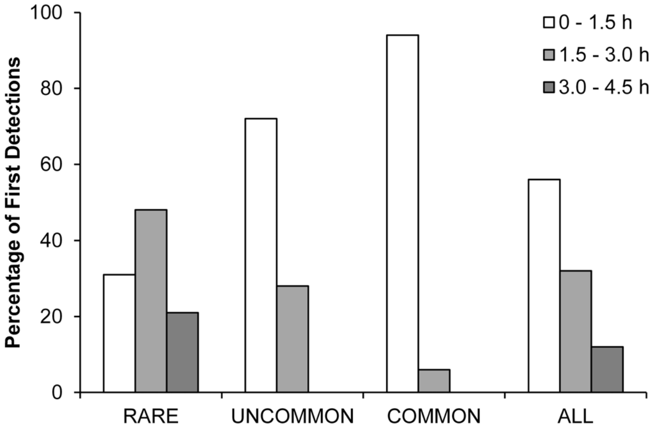

Fig. 3. Comparison of the percentage of first detections of rare, uncommon and common mussels associated with each sampling time interval. Species classification: rare = 1 to 5 individuals per 4.5 h; uncommon = 6 to 10 individuals; common >10 individuals.

to ψ (Table 2). The difference between detection probabilities calculated for 1.5 h and 4.5 h of search effort was largest for Elktoe (+0.39), Plain Pocketbook (*Lampsilis cardium*) (+0.23) and Triangle Floater (+0.48). Based on non-overlapping CIs, significant improvements to mean p (1.5 h search effort) were limited to Elktoe and Triangle Floater. Eastern Elliptio, Flutedshell and Triangular Floater occupancy estimates were strongly affected by search effort. Overall, the collection of fresh shells had little influence on detection probabilities (increased mean p for all species from 0.82 to 0.83). Alternately, there was a large effect on occupancy estimates; mean ψ for all species increased from 0.47 to 0.64. Changes to occupancy estimates were greatest (>0.20) for Creeper (*Strophitus undulatus*), Elktoe, Fatmucket and Triangle Floater. For all levels of search effort, p and ψ were positively correlated to the total number of individuals collected over the three sampling events p: $r_s = 0.58–0.77$, $p < 0.05$; ψ: $r_s = 0.65–0.78$, $p \leqslant 0.03$).

3.3 Impact of sampling effort on long-term monitoring

For all species, power to detect future changes in mussel species distribution improved with greater search effort (Figure 4). In comparison to 1.5 h, the power of 4.5 h timed-search surveys was 20 to 54% greater. The detection of 70% declines in future site occupancy is likely for most species (including Rainbow). However, detecting declines in occupancy of 50% is only predicted for four species (Creek Heelsplitter, Cylindrical Papershell (*Anodontoides ferussacianus*), Eastern Elliptio, and Flutedshell). The inclusion of shells increased the species list to include Creeper, Elktoe, Fatmucket and Rainbow (power = 79 for Rainbow). In almost all cases, power to detect small changes (30%) is not predicted to be high. Power to detect 30% and 50% occupancy declines was strongly affected by search effort and fresh shell collection (Figure 4).

4 Discussion

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 useful to detect species and track trends in distribution. The currently applied timed-search method used to survey Ontario rivers is based on 4.5 h of search effort (Metcalfe *et al.*, 2000). The search effort recommendation was based on comparing frequencies of rare species detection and species richness estimates associated with 1.5, 3.0 and 4.5 h of cumulative search effort at 28 Ontario river sites. At the time of the study, 4.5 h was at least twice the effort typical of timed-search mussel surveys. More recent research has found that timed-searches of <8 h severely underestimated mussel species richness at Illinois stream sites (Huang *et al.*, 2011). At the sites sampled along the Moira, Salmon and Trent rivers, only 75% of species detections occurred during the first visit; evidence of substantial underestimation of species richness. While greater search effort (or number of sampling visits) may further improve detection, some species may only be collected by excavating river bed material. During recent quadrat-based sampling of Ontario river sites, between 11 and 36% of species detections required excavation (Reid and Morris, unpublished data). As an alternative to surveying a site for a constant length of time, other researchers have proposed spatially-based survey designs where search area is based on search efficiency (Smith, 2006) or hydromorphologically significant features (Lamand and Beisel, 2014).

Imperfect detection of freshwater mussels has been found to bias estimates of site occupancy (Wisniewski *et al.*, 2013a), and population parameters such as density and survival (Smith, *et al.*, 2010, Wisniewski *et al.*, 2013b). In this study, detection of mussels using the timed-search method was imperfect. Species may have been missed by field staff on a given sampling date, or were unavailable for collection due to the burrowing behaviour of mussels (Haag, 2012). For most species, detection probabilities are still considered high; well above the minimum value ($p > 0.3$) recommended by Mackenzie *et al.*, (2006) for occupancy-based studies. Based on these probabilities, two 4.5 h repeat surveys (with fresh shell collection) are recommended in order to confidently assess

Table 2. Comparison of detection probability (p) and occupancy (ψ) estimates for 12 freshwater mussel species across four different levels of sampling effort. Estimates were calculated using a constant probability, single-season occupancy model (PRESENCE 9.7 software). For detection probability, 95% confidence intervals are provided in parentheses.

Species	1.5 h		3 h		4.5 h		4.5 h plus shells	
	p	ψ	p	ψ	p	ψ	p	ψ
Alasmidonta marginata	0.38 (0.12, 0.75)	0.28	0.77 (0.51, 0.91)	0.32	0.77 (0.51, 0.91)	0.32	0.89 (0.76, 0.95)	0.79
Alasmidonta undulata	0.09 (0.05, 0.14)	*	0.63 (0.34, 0.85)	0.10	0.57 (0.32, 0.79)	0.14	0.74 (0.60, 0.84)	0.38
Anodontoides ferussacianus	0.78 (0.63, 0.87)	0.40	0.80 (0.68, 0.89)	0.49	0.84 (0.73, 0.91)	0.54	0.80 (0.70, 0.88)	0.67
Elliptio complanata	0.90 (0.83, 0.95)	0.69	0.87 (0.80, 0.92)	0.80	0.85 (0.78, 0.90)	0.88	0.92 (0.87, 0.96)	0.89
Lampsilis cardium	0.59 (0.38, 0.77)	0.20	0.86 (0.70, 0.94)	0.22	0.82 (0.68, 0.90)	0.32	0.80 (0.68, 0.88)	0.45
Lampsilis siliquoidea	0.70 (0.56, 0.81)	0.36	0.77 (0.64, 0.86)	0.39	0.82 (0.72, 0.89)	0.49	0.77 (0.68, 0.84)	0.84
Lasmigona compressa	0.69 (0.53, 0.81)	0.46	0.81 (0.68, 0.90)	0.50	0.83 (0.72, 0.91)	0.57	0.82 (0.71, 0.89)	0.75
Lasmigona costata	0.65 (0.53, 0.76)	0.51	0.69 (0.59, 0.78)	0.66	0.79 (0.70, 0.86)	0.69	0.82 (0.74, 0.88)	0.76
Ligumia recta	0.64 (0.40, 0.82)	0.15	0.67 (0.49, 0.81)	0.25	0.69 (0.53, 0.82)	0.27	0.76 (0.62, 0.85)	0.38
Pyganodon grandis	0.69 (0.46, 0.85)	0.15	0.67 (0.49, 0.81)	0.25	0.80 (0.65, 0.90)	0.26	0.81 (0.66, 0.90)	0.30
Strophitus undulatus	0.82 (0.69, 0.90)	0.35	0.84 (0.74, 0.91)	0.44	0.92 (0.82, 0.96)	0.44	0.80 (0.71, 0.87)	0.65
Villosa iris	0.78 (0.65, 0.87)	0.35	0.78 (0.67, 0.87)	0.44	0.88 (0.78, 0.94)	0.45	0.93 (0.85, 0.97)	0.52
Mean	**0.69**	**0.35**	**0.78**	**0.43**	**0.82**	**0.47**	**0.83**	**0.64**

*: data insufficient to model occupancy.

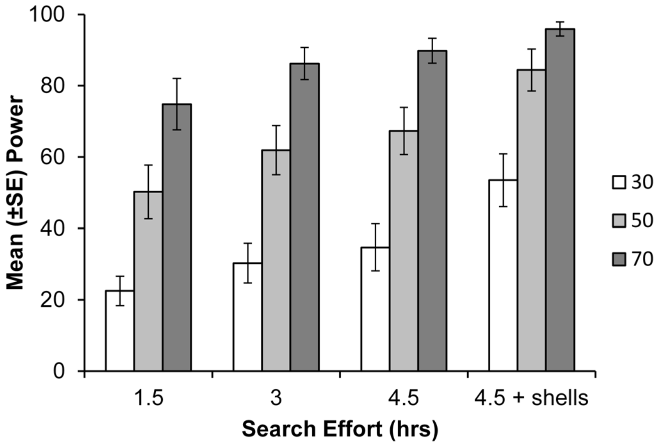

Fig. 4. Influence of search effort on the power of visual-tactile surveys to detect changes in riverine mussel distribution. Power was calculated for declines in site occupancy of 30%, 50% and 70%.

species occurrence at an individual site (Pellet and Schmidt, 2005), and three repeat surveys are recommended for larger scale occupancy-based monitoring programs (MacKenzie and Royle, 2005). While detection probabilities estimated for 1.5 and 3.0 h of search effort were similar, results indicate that rare species detection and occupancy estimates are greatly improved at the recommended search effort.

For a third of the species encountered, fresh shell collection increased estimates of site occupancy by 48 to 171%. Muskrats and raccoons can consume large quantities of mussels, and leave behind easily detected fresh shells and shell middens (*i.e.* piles). These animals selectively prey on certain sizes and species present at mussel beds. Muskrat have been found to preferentially select medium-sized, round and inflated shells; although large-thin shelled species are also taken (Watters, 1994; Haag, 2012). Therefore, shell data will be biased and have an unequal impact on species occupancy estimates. The largest increases to occupancy estimates were associated with species with smaller and inflated shells. In this study, it was assumed that: (1) fresh shells were of local origin (not washed into the site from an upstream location); and (2) any movement of shells did not change the occupancy status of a species between repeat sampling events. Considering the substantial influence of shells on occupancy estimates, it is important that these assumptions are tested by future field study. If not validated, fresh shells would only be useful for developing river-specific species lists.

Mussel surveys are affected by the abundance and biological characteristics of species in mussel beds, the physical habitat conditions of survey sites, and the capabilities of field staff (Strayer and Smith, 2003; Wisniewski *et al.*, 2013a). Despite the use of a standardized sampling method, these factors can result in heterogeneous detection probabilities for populations found in different rivers. When sampling conditions are worse (*e.g.* poor water clarity) or populations are at lower densities than encountered during the development of sampling protocols, search effort may not be adequate to reliably detect species at risk (Dextrase *et al.*, 2014). While such factors were not included as covariates in occupancy-models, some comments can be made regarding the influence of abundance, shell size, and field staff experience on timed-search surveys results.

Elktoe and Triangle Floater were two of the smallest species collected in this study and comprised only a small percentage of all mussels collected (Table 1). Detection probabilities for these two species were substantially less than other species when only 1.5 h was spent searching. The potential effect of low abundance and small shell size was less evident when more time was spent searching for mussels. Positive correlations between abundance and detection probabilities are consistent with theoretical expectations for sampling populations of rare mussels (Green and Young, 1993). Surface detection of mussels can be affected by searcher experience; with more experienced staff being less affected by sampling fatigue and better able to negotiate challenging sampling conditions (Wisniewski *et al.*, 2013a). There was strong agreement in mussel collections among crew members in 2013, but agreement was only moderate in 2014. Interestingly, there was much less variation in sampling experience among 2014 staff (one year) than in 2013 (seven years).

Occupancy modelling has been applied to investigate factors influencing species detection and distribution, and the effect of drought on freshwater mussels in the lower Flint River basin, Georgia (USA) (Shea *et al.*, 2013; Wisniewski *et al.*, 2013a). However, in comparison to other aquatic taxa such as fishes and amphibians, the use of replicate survey data and occupancy models to estimate freshwater mussel detection probabilities is rare. To date, survey design has been informed by empirical relationships between search effort and species richness (Metcalfe *et al.*, 2000; Huang *et al.*, 2011) and theoretical relationships between species abundance and detection probability (Strayer and Smith, 2003). Given that mussel species detection is imperfect, occupancy models are well suited for designing timed-search species inventories and monitoring. In this study, detection probabilities were used to: (1) estimate the number of repeat surveys required to confidently detect species; and (2) jointly with occupancy estimates, evaluate the power of timed-search surveys to detect distribution declines of magnitudes relevant to species status assessments. Despite large differences in relative abundance, among-species differences in detectability were relatively limited at 4.5 h; therefore, sampling recommendations will be broadly sufficient. Larger differences in the detectability of mussel species have been reported in more diverse rivers (Wisniewski *et al.*, 2013a). The mussel fauna present in the Moira, Salmon and Trent rivers is less diverse (and has fewer imperiled species) than southwestern Ontario rivers. Although the current timed-search protocol was originally developed in southwestern Ontario rivers, species detection probabilities should be estimated for these rivers in order to ensure sampling sufficiency across species of interest.

Incorporating detection estimates is recommended for future mussel monitoring programs as: (1) it provides a measure of sampling confidence that can be used to adjust detection/non-detection data (Wisniewski *et al.*, 2013a); and (2) the influence of uncontrollable factors (*e.g.* weather or flow conditions) during surveys on species detection can be assessed through occupancy-based models (Tingley and Beissinger, 2009). Over time, such an approach would address concerns related to “true absences” or the comparability between surveys when historical data are used for species status assessment. The collection of replicate survey data can be done on separate dates within a season, or by recording and treating collections made by multiple observers as discrete sampling events (MacKenzie *et al.*, 2006). The second option would not require additional resources to complete field sampling, and would reduce the risk of violating the closure assumption of occupancy models.

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