

Can Adaptive Cluster Sampling Improve Ontario Mussel Species at Risk Monitoring?

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TABLE OF CONTENTS

ABSTRACT.....	iv
RÉSUMÉ	iv
INTRODUCTION	1
METHODS.....	2
Simulation Data.....	2
Simulations.....	2
RESULTS	4
DISCUSSION.....	4
ACKNOWLEDGEMENTS	5
REFERENCES	5

ABSTRACT

Watershed inventories and population monitoring are essential components of efforts to conserve and recover freshwater mussel diversity. Since 2002, a quadrat-based sampling protocol has been used to monitor the status, distribution, and demographics of mussel species at risk in southern Ontario rivers. However, low population densities, typical of most mussel species at risk, limit the effectiveness of the current protocol to support monitoring objectives. In this study, simulation-based methods (computer program SAMPLE) were used to evaluate whether adaptive clustering sampling could improve single-species monitoring. Census data for eight mussel species (including three species at risk) collected from two sites (Rawdon Creek and Sydenham River) with contrasting mussel assemblages were used. Sampling design performance was assessed based on the accuracy of density and occupancy estimates and sampling efficiency, over a gradient of increasing sampling effort. In all cases, adaptive sampling was less accurate and efficient than simple random sampling or systematic sampling with random starts. Improvements to the monitoring program will only be achieved by increasing the spatial coverage of the existing systematic sampling design.

RÉSUMÉ

Les répertoires de bassins hydrographiques et la surveillance de la population sont des composantes essentielles des efforts visant la conservation et le rétablissement de la diversité des moules d'eau douce. Depuis 2002, un protocole d'échantillonnage fondé sur le quadrat a été utilisé pour surveiller l'état, la répartition et les données démographiques des espèces de moules en péril dans les rivières du sud de l'Ontario. Cependant, la faible densité de la population, phénomène représentatif de la plupart des espèces de moules en péril, limite l'efficacité du protocole actuel qui vise à appuyer les objectifs de surveillance. Dans le cadre de cette étude, des méthodes reposant sur la simulation (programme d'ordinateur 'SAMPLE') ont été utilisées pour évaluer si l'échantillonnage en grappes adaptatif améliorerait la surveillance d'une seule espèce. Les données proviennent du recensement de huit espèces de moules (y compris les trois espèces en péril) recueillies à partir de deux sites (ruisseau Rawdon et rivière Sydenham) et dont les communautés de moules contrastaient. Le rendement de la conception d'échantillon a été évalué en fonction de l'efficacité d'échantillonnage, et de l'exactitude de la densité et des estimations de l'occupation, selon un gradient d'effort d'échantillonnage croissant. Dans tous les cas, l'échantillonnage d'adaptation était moins précis et efficace que le simple échantillonnage aléatoire ou systématique comprenant des démarrages aléatoires. Les améliorations du programme de surveillance ne peuvent être réalisées qu'en augmentant la couverture spatiale de la conception existante de l'échantillonnage systématique.

INTRODUCTION

In Canada, there are 55 native freshwater mussel species with 41 species occurring in the province of Ontario (Metcalf-Smith et al. 2005). Almost a third of these species in Ontario are listed as Endangered, Threatened, or of Special Concern under the federal *Species at Risk Act* and the provincial *Endangered Species Act* (COSEWIC 2013, MNR 2014). Catastrophic declines to the mussel fauna occurred after the introduction and spread of non-native dreissenid mussels to the Laurentian Great Lakes (Schloesser and Nalepa 1994). In contrast to nearshore Great Lakes habitats, most Ontario rivers are not heavily infested by dreissenids and historical mussel diversity is largely intact (Clarke 1992, McNichols-O'Rourke et al. 2012). Actions undertaken to conserve remnant mussel diversity include the identification and protection of critical habitats, establishment of a network of permanent monitoring sites, implementation of best management practices to improve water quality and quantity (e.g. reduced loadings of chloride and nutrients), and evaluation of the feasibility of relocations, reintroductions and artificial propagation (Morris and Burridge 2006, DFO 2013).

Since 2002, the Ontario Freshwater Mussel Recovery Team (OFMRT) has implemented a quadrat-based protocol to assess the status, distribution, and demographics of mussel species at risk (SAR) in Ontario rivers, and to evaluate recovery actions. The protocol is based on a systematic design (with a fixed number of random starts within each sampling block) that samples 20% of the surface area of the monitoring site (Thompson 2002, Pooler and Smith 2005, Metcalf-Smith et al. 2007) (Figure 1). Each sampling block is comprised of 15 quadrats. The number of sampling blocks at each site varies based on the amount of suitable mussel habitat. Mussels are initially collected from the surface of 1 m² quadrats by visual and tactile methods. Afterwards, the substrate in each quadrat is excavated to a depth of 10-15 cm to improve detection of juveniles and small-bodied species. Systematic designs such as this protocol are considered efficient for sampling freshwater mussels when populations are expected to be clustered and rare (Christman 2000). The protocol has been implemented at more than 40 sites in five southwestern Ontario rivers. Information collected relevant to recovery efforts includes: locations of species and populations; descriptions of habitat attributes for different life-stages; population status (*i.e.* density, size and age structure, sex-ratio); and, the presence of invasive species (Cudmore et al. 2006, DFO 2011).

A recent assessment of data collected using the protocol (Reid and Morris 2017) identified that it will reliably detect most species at a site, and provide accurate and precise total mussel (*i.e.* all species combined) density estimates. Also, excavation is essential for detection of small individuals and to accurately estimate density. However, the protocol was not considered reliable for detecting most mussel species at risk. Further, imprecise estimates prevent future detection of all but large changes (>70%) to population size. Overall, low population densities (*i.e.* <0.5 m²) limit the effectiveness of the current design. Substantially greater sampling effort with the current design, or a fundamental revision of the approach is required to increase the likelihood of data collection achieving monitoring objectives.

For this study, we used a simulation-based approach to evaluate whether adaptive cluster sampling could improve mussel species at risk monitoring. Adaptive cluster sampling refers to a survey design in which an initial set of units is randomly selected, and, whenever the variable of interest of a selected unit satisfies a given criterion, additional units in the neighborhood of that unit are added to the sample (Thompson 2002). The final sample includes all clusters detected in the initial sample plus any sample units that were below the criterion threshold. The design was developed for sampling populations that are rare and clustered (Manly and Navarro Alberto 2015). A desirable property of adaptive cluster sampling

is that effort is targeted where the species is found, leading to assumed improvements in efficiency (Smith et al. 2004, Manly and Navarro Alberto 2015). However, adaptive cluster sampling of low density populations of riverine freshwater mussels has only had limited evaluation (Smith et al. 2003, Smith et al. 2004).

The computer program SAMPLE (Smith and Nichols 2006) was used to simulate mussel quadrat-sampling associated with three designs: 1) simple random sampling (SRS), 2) grid-based systematic sampling (GSS), and, 3) adaptive grid-based systematic sampling (AGSS). GSS is the design currently used to sample mussels at Ontario river index monitoring sites. Simulations were run using census data (*i.e.* all quadrats in each block were sampled) collected at two 375 m² sites that represent contrasting mussel assemblages. Design performance was evaluated based on the accuracy of density and occupancy estimates and sampling efficiency.

METHODS

SIMULATION DATA

At the two census sites (Rawdon Creek and Sydenham River), we sampled all 15 quadrats in each of the 25 blocks using visual-tactile and excavation methods. Live mussels were identified to species (Metcalf-Smith et al. 2005), dimorphic species were sexed when possible, and shell lengths were measured. Sampling was completed at Rawdon Creek between August 27 and September 4, 2013, and at Sydenham River between August 7 and 14, 2012. Sampling Rawdon Creek took 5 days with a four to five-person crew, and sampling the Sydenham River took 6 days with a four to eleven-person crew (Reid and Morris 2017).

Rawdon Creek is a tributary of the Trent River which flows into Lake Ontario. The census site was located 17 km north of the city of Trenton (44°16'08" N; 77°33'12" W). Mean wetted channel width was 8.3 m and mean water depth was 0.29 m. The Sydenham River drains into Lake St. Clair, and the census site was located 11 km east of the town of Dresden (42°36'20" N; 82°02'40" W). Mean wetted channel width was 20.0 m and mean water depth was 0.34 m. River bed material (substrate) at both sites was a relatively even mix of sand, gravel and cobble. Site selection was informed by past quadrat (Sydenham River) and timed-search (Rawdon Creek) surveys (Metcalf-Smith et al. 2007, Reid 2016).

At the Rawdon Creek site, 866 individuals (representing 7 species) were collected. One mussel species-at-risk was detected, *Villosa iris*. Individual mussels were generally evenly-distributed across the site, with the exception of aggregations located at the bottom end of the site, and within the upper third of the site (Figure 2).

At the Sydenham River site, 6180 individuals (representing 25 mussel species) were collected (Table 1). Nine mussel species-at-risk were detected: *Epioblasma torulosa rangiana*, *Epioblasma triquetra*, *Obovaria subrotunda*, *Pleurobema sintoxia*, *Ptychobranchnus fascioloris*, *Quadrula quadrula*, *Simpsonaias ambigua*, *Truncilla donaciformis*, and *Villosa fabalis*. Individuals were distributed with slightly higher densities along the left side of the study area (facing upstream), and with the highest aggregation of mussels in the upper left region (Figure 3).

SIMULATIONS

We used the computer program SAMPLE (version: November 2006) to simulate mussel sampling using the counts and locations of each species at the census sites. SAMPLE was developed by the United States Geological Survey to support the design of population

monitoring programs by simulating adaptive and conventional survey designs. Software was downloaded from <ftp://ftpext.usgs.gov/pub/er/wv/leetown/Smith/SAMPLE/>.

The three designs simulated were:

- 1) **Simple random sampling (SRS)**. In this design, sampling units are randomly distributed throughout the site. The design served as a baseline for comparison with other sampling designs.
- 2) **Grid-based systematic sampling (GSS)**. It is the current river population monitoring design. Initial sampling units were selected randomly, then additional plots were selected at specified intervals in the x and y directions.
- 3) **Adaptive grid-based systematic sampling (AGSS)**. Initial sampling units were selected as in GSS, then sampling proceeded adaptively.

Density and variance estimators used in simulations are described in Thompson (2002).

Adaptive sampling is most efficient when populations are clustered, and when those clusters are rare (Smith et al. 2004). While some of the mussel species collected from Rawdon Creek and Sydenham River were rare, they tended to be dispersed rather than clustered. Moderately clustered populations tended to be less rare (Table 1). Therefore, AGSS simulations were only completed for species with densities ≤ 0.25 mussels per m^2 and characterized by a moderate degree of clustering (variance to mean ratios: 1.1 to 1.5). Species included in simulations were: *Ligumia recta*, *Alasmidonta marginata*, *Leptodea fragilis*, *Epioblasma torulosa rangiana*, *Quadrula pustulosa*, *Epioblasma triquetra*, and *Lasmigona complanata* from the Sydenham River, and *Villosa iris* from Rawdon Creek.

We simulated 112 SRS scenarios and 112 GSS scenarios (1 to 14 random starts for each of the eight species), and 64 AGSS scenarios (1 to 8 random starts for each of the eight species selected for this design). For the grid-based scenarios, the distances between rows and columns were 5 units and 3 units, respectively. The criterion to trigger adaptive sampling was set as ≥ 1 mussel in a quadrat. Due to the low densities of most species, more conservative criteria (*i.e.* > 2 mussel in a quadrat) were not simulated. A cross-shaped sampling neighbourhood (Smith et al. 2004) was used when the criterion was met. With this design, the four adjacent quadrats that share a boundary with the occupied quadrat are included. Design scenarios were replicated 1,000 times each and averages of these replications were given as output from the program. Sampling costs (*i.e.*, set-up time, rate of travel, and search time) were minimal and therefore not factored into this study. Detectability was assumed to be perfect as sampling units were excavated.

Prior to simulations, we modified datasets to be uniformly rectangular. Blocks that fell outside the uniform area were removed from the simulation dataset. The resultant dimensions were 6 m wide by 55 m long for the Sydenham River site, and 6 m wide by 60 m long for the Rawdon Creek site. Spatial distributions were mapped for each species within the mussel bed, and population files were created following an 'x, y, count' format at a 1 m^2 grain size.

We evaluated each sampling design individually for each species. Performance of the designs was evaluated based on: 1) accuracy of density estimates and occupancy estimates, and 2) efficiency. Accuracy of density estimates was based on percent-difference from true density. The observed population of each species (sum of counts from all quadrats divided by the total sample area) will be referred to as the *true density*. Efficiency was defined as the ratio between the variance of density estimate from the SRS design and the variance of the sampling scenario in question (Brown et al. 2008). Ratios were calculated using estimates from scenarios with equivalent levels of sampling effort. Occupancy was defined as the proportion

of sampling units occupied by at least one individual of a species: estimates of occupancy were evaluated against the occupancy values of the true populations using percent relative bias. Relative risk was also calculated, representing the likelihood of sampling occupied units: it was calculated as the ratio of the probability of sampling an occupied unit under a specified design to the probability of sampling an occupied unit under SRS (Smith et al. 2011).

RESULTS

The accuracy of AGSS-based density estimates was poor compared to GSS and SRS-based sampling and generally underestimated population density (Figure 4). For most species, this bias increased with greater sampling effort. In the worst cases, densities were underestimated by 9 to 18%. GSS and SRS-based density estimates were very similar across all levels of sampling effort and within 5% of true density.

For all species, AGSS was not more efficient than the GSS sampling design (Figure 5). In most cases, the efficiency of GSS declined as more of the site was sampled. For *Alasmidonta marginata*, *Epioblasma torulosa rangiana*, *Epioblasma triquetra*, and *Lasmigona complanata*, GSS was more efficient than SRS when less than 40% of the site was sampled.

In comparison to the known numbers of occupied quadrats, AGSS underestimated the proportion of quadrats occupied by each species, and the difference between AGSS and the other sampling designs increased with greater spatial coverage of the site (Figure 6). Relative risk values indicate that AGSS was more likely to sample quadrats occupied by *Villosa iris* or *Liguma recta* than SRS when sampling included less than 60% of the site (Figure 7). For *Alasmidonta marginata*, *Epioblasma torulosa rangiana*, *Epioblasma triquetra*, and *Quadrula pustulosa*, AGSS was less likely to sampled occupied quadrats as sample coverage increased from 20-40% of the site. There was little difference between GSS and SRS sampling designs across all levels of sampling effort.

DISCUSSION

Obtaining accurate estimates of the population parameters for aquatic species at risk that demonstrate high spatial variability in distribution and abundance is difficult. When many sampling units do not contain individuals of the species of interest, conventional sampling designs either provide inaccurate and imprecise data or require a relatively high cost to obtain necessary information (Morrison et al. 2008). Freshwater mussel detection and monitoring can be challenging as they often occur at low densities, are spatially clustered and imperfectly detected (Smith et al. 2010). Adaptive sampling designs allow effort to be increased in areas where the species of interest are being detected during sampling (Thompson 2002). Intuitively, adaptive cluster sampling is, therefore, well suited for sampling mussel species at risk and its application to monitoring freshwater mussels has been evaluated for some riverine populations (Smith et al. 2003, 2004, 2011). For example, Smith et al. (2003) found (relative to SRS) adaptive cluster sampling improved uncommon species detection as well as the total number of individuals collected (*i.e.* yield) from sites along the Cacapon River, West Virginia.

However, results from our simulation study indicate that adaptive cluster sampling is not expected to improve site-level efforts to monitor freshwater mussel populations in southern Ontario rivers. In all cases, AGSS represented the least effective design, with efficiency consistently lower than SRS and GSS, and biases in estimates of density and occupancy that increased with sample size. Additionally, based on the degree of clustering, only 8 of 28 species collected from Rawdon Creek and Sydenham River were considered suitable for

simulating sampling with AGSS. Improvements to data collection at individual monitoring sites will therefore only be achieved by increasing the spatial coverage of sampling with the existing systematic design (Reid and Morris 2017). However, our interpretation does not preclude the testing of adaptive sampling methods for characterizing the distribution and abundance of mussel species at risk along much larger sampling units such as rivers (e.g. Villella and Smith 2005, Brown et al. 2008). Adopting the river (or reach), rather than the mussel bed, as the sampling frame would also be a better match for population-level inference (Reid and Morris 2017), as it avoids biases associated with preferential selection of high density sites.

Census datasets, in combination with simulation approaches, can help to inform the design of freshwater mussel population monitoring programs. We recognize that in this study adaptive cluster sampling was only evaluated using a rather limited dataset (8 species, each from only 1 site). The effectiveness of adaptive cluster sampling can be expected to vary across populations (Smith et al. 2004) and therefore our results may not be broadly applicable. Factors such as mussel density and the degree of spatial clustering influence the performance of this sampling design (Smith et al. 2003, 2010). For Ontario rivers, census data from additional sites with low density (<0.2 m²) species that exhibit a greater degree of clustering would increase the breadth of our results. However, the very large amount of effort that was required to fully sample the Rawdon Creek and Sydenham River sites renders the future collection of a large census dataset unlikely.

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REFERENCES

- Brown, J.A., M. Mohammad Salehi, M. Moradi, G. Bell, and D.R. Smith. 2008. An adaptive two-stage sequential design for sampling rare and clustered populations. *Population Ecology* 50:239-245.
- Christman, M.C. 2000. A review of quadrat-based sampling of rare, geographically clustered populations. *Journal of Agricultural, Biological, and Environmental Statistics* 5: 168-201.
- Clarke, A.H. 1992. Ontario's Sydenham River, an important refugium for native freshwater mussels against competition from the zebra mussel, *Dreissena polymorpha*. *Malacology Data Net* 3:43-55.
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2013. Wildlife Species Search. Committee on the Status of Endangered Wildlife in Canada. Available from http://www.cosewic.gc.ca/eng/sct1/searchform_e.cfm [accessed September 17 2013].
- Cudmore, B., N.E. Mandrak, T.J. Morris and A. Edwards. 2006. Allowable harm analysis workshops for freshwater species at risk in central and Arctic region. Fisheries and Oceans Canada, Canadian Science Advisory Secretariat Proceedings Series 2006/026.
- DFO (Department of Fisheries and Oceans Canada). 2011. *Assessment of methods for the identification of critical habitat for freshwater mussels*. Fisheries and Oceans Canada,

- Canadian Science Advisory Secretariat Science Advisory Report 2011/047: Ottawa, ON, Canada.
- DFO (Department of Fisheries and Oceans Canada). 2013. 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.
- Manly, B.J., and J.A. Navarro Alberto. 2015. Introduction to ecological sampling. CRC Press, Boca Raton, FL. 212 p.
- McNichols-O'Rourke, K.A., A. Robinson, and T.J. Morris. 2012. Summary of freshwater mussel timed search surveys in southwestern Ontario in 2010 and 2011. Fisheries and Oceans Canada, Canadian Manuscript Report Fisheries and Aquatic Sciences 3009: Ottawa, ON.
- Metcalfe-Smith, J.L, MacKenzie, A., Carmichael, I., and D. McGoldrick. 2005. Photo field guide to the freshwater mussels of Ontario. St. Thomas Field Naturalist Club Inc., St. Thomas, ON, Canada.
- Metcalfe-Smith, J.L., D.J. McGoldrick, D.T. Zanatta, and L.C. Grapentine. 2007. Development of a monitoring program for tracking the recovery of endangered mussels in the Sydenham River, Ontario. Environment Canada, Water Science and Technology Directorate Contribution 07-510: Burlington, ON, Canada.
- MNRF [Ontario Ministry of Natural Resources and Forestry]. 2014. Species at Risk in Ontario (SARO) List. Available from <http://www.ontario.ca/environment-and-energy/species-risk-ontario-list> [accessed September 16 2014].
- Morris, T.J., M. Burrige. 2006. 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.
- Morrison, L.W., D.R. Smith., C.C. Young, and D.W. Nichols. 2008. Evaluating sampling designs by computer simulation: a case study with the Missouri bladderpod. *Population Ecology* 50:417-425.
- Pooler, P.S., and D.R. Smith. 2005. Optimal sampling design for estimating spatial distribution and abundance of a freshwater mussel population. *Journal of the North American Benthological Society* 24: 525-537.
- Reid, S.M. 2016. Search effort and imperfect detection: Influence on timed-search mussel (*Bivalvia: Unionidae*) surveys in Canadian rivers. *Knowledge and Management of Aquatic Ecosystems* 417: 17.
- Reid, S.M., and T. Morris. 2017. Tracking Freshwater Mussel Recovery in Ontario Rivers: Evaluation of a Quadrat-based Monitoring Protocol. *Diversity* 9: 5.
- Schloesser, D.W. and T.F. Nalepa. 1994. Dramatic decline of unionid bivalves in the offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha*. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 2234-2242.
- Smith, D.R., R.F. Vilella, and D.P. Lemarié. 2003. Application of adaptive cluster sampling to low-density populations of freshwater mussels. *Environmental and Ecological Statistics* 10:7-15.
- Smith, D.R., J.A. Brown, and N.C.H. Lo. 2004. Application of adaptive sampling to biological populations. Thompson, W.L. editor. In: *Sampling rare or elusive species. Concepts, designs, and techniques for estimating population parameters*. Island Press, Washington, D.C. 429 p.

- Smith, D.R., and D. Nichols 2006. SAMPLE. User's manual (Revised version, November 2006). United States Geological Survey, Leetown Science Center. Kearneysville, West Virginia. 29 p.
- Smith, D.R., B.R. Gray, T.J. Newton, and D. Nichols. 2010. Effect of imperfect detectability on adaptive and conventional sampling: simulated sampling of freshwater mussels in the upper Mississippi River. *Environmental Monitoring and Assessment* 170: 499-507.
- Smith, D.R., B.R. Gray, and T.J. Newton 2011. Evaluation of single and two-stage adaptive sampling designs for estimation of density and abundance of freshwater mussels in a large river. *River Research and Application* 27:122-133.
- Thompson, S.K. 2002. *Sampling*. 2nd ed., John Wiley and Sons, New York, NY, USA.
- Villella, R.F., and D. R. Smith. 2005. Two-phase sampling to estimate river-wide populations of freshwater mussels. *Journal of the North American Benthological Society* 24:357-368.

Table 1. Density (x), variance to mean ratio (s^2/x), and frequency of occurrence (FO) of freshwater mussels collected from two census quadrat-sampling sites (adapted from Reid and Morris 2017). FO is the percentage of all quadrats sampled where the species was collected. Species-at-risk are identified by asterisk.

Species	Common Name	Rawdon Cr. (Total = 866 mussels)			Sydenham R. (Total = 6180 mussels)		
		X (# m ⁻²)	s ² /x	FO (%)	X (# m ⁻²)	s ² /x	FO (%)
<i>Actinonaias ligamentina</i>					0.54	1.8	32.0
<i>Alasmidonta marginata</i>					0.17	1.1	0.5
<i>Amblema plicata</i>					1.78	2.1	70.7
<i>Cyclonaias tuberculata</i>					6.98	5	96.0
<i>Elliptio complanata</i>		1.97	2.49	68.3			
<i>Elliptio dilatata</i>					1.4	1.5	68.3
<i>Epioblasma torulosa rangiana</i> *					0.16	1.1	14.1
<i>Epioblasma triquetra</i> *					0.19	1.1	16.8
<i>Fusconaia flava</i>					0.43	1.2	31.2
<i>Lampsilis cardium</i>		0.06	1.04	5.6	0.02	1	1.6
<i>Lampsilis siliquoidea</i>		0.02	0.98	1.9			
<i>Lasmigona complanata</i>					0.12	1.1	10.7
<i>Lasmigona costata</i>		0.01	0.99	1.1	0.99	2.8	68.5
<i>Leptodea fragilis</i>					0.18	1.1	16.3
<i>Ligumia recta</i>		0.003	1.0	0.3	0.24	1.2	19.5
<i>Obovaria subrotunda</i> *					0.003	1	0.3
<i>Pleurobema sintoxia</i> *					0.08	1.1	6.9
<i>Potamilus alatus</i>					0.08	1.1	6.9
<i>Ptychobranchnus fascioloris</i> *					0.46	1.1	36.0
<i>Pyganodon grandis</i>					0.01	1	1.1
<i>Quadrula pustulosa</i>					0.17	1.2	14.4
<i>Quadrula quadrula</i> *					0.74	1.7	42.1
<i>Simpsonaias ambigua</i> *					0.02	1	1.3
<i>Strophitus undulatus</i>		0.01	0.99	0.8	0.04	1	4.0
<i>Truncilla donaciformis</i> *					0.03	4.3	1.1

Species	Common Name	Rawdon Cr. (Total = 866 mussels)			Sydenham R. (Total = 6180 mussels)		
		X (# m ⁻²)	s ² /x	FO (%)	X (# m ⁻²)	s ² /x	FO (%)
<i>Truncilla truncata</i>					0.01	1	0.5
<i>Villosa fabalis</i> *					0.69	1.6	41.9
<i>Villosa iris</i> *		0.24	1.29	19.5			
All Mussels		2.31	2.7		16.48	11.5	

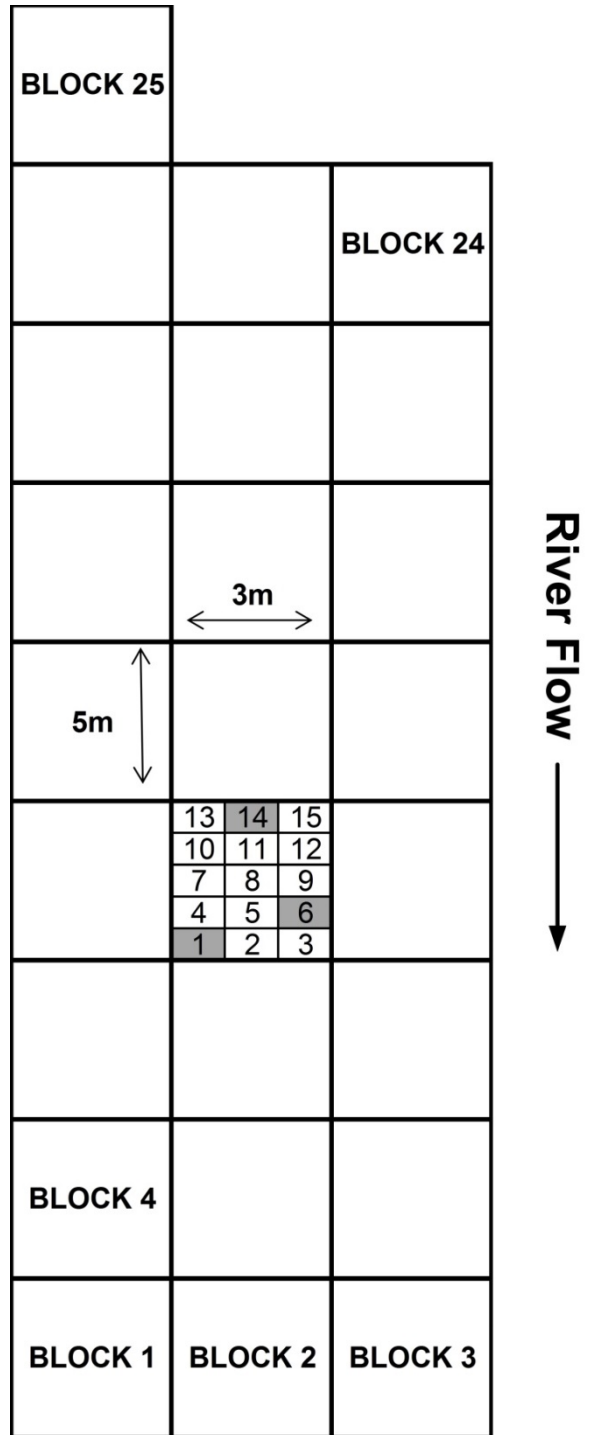


Figure 1. Representative diagram of the systematic, quadrat-sampling design used to monitor mussel populations in Ontario rivers (Reid and Morris 2017). Locations of 3 random starts within each block are highlighted in grey.

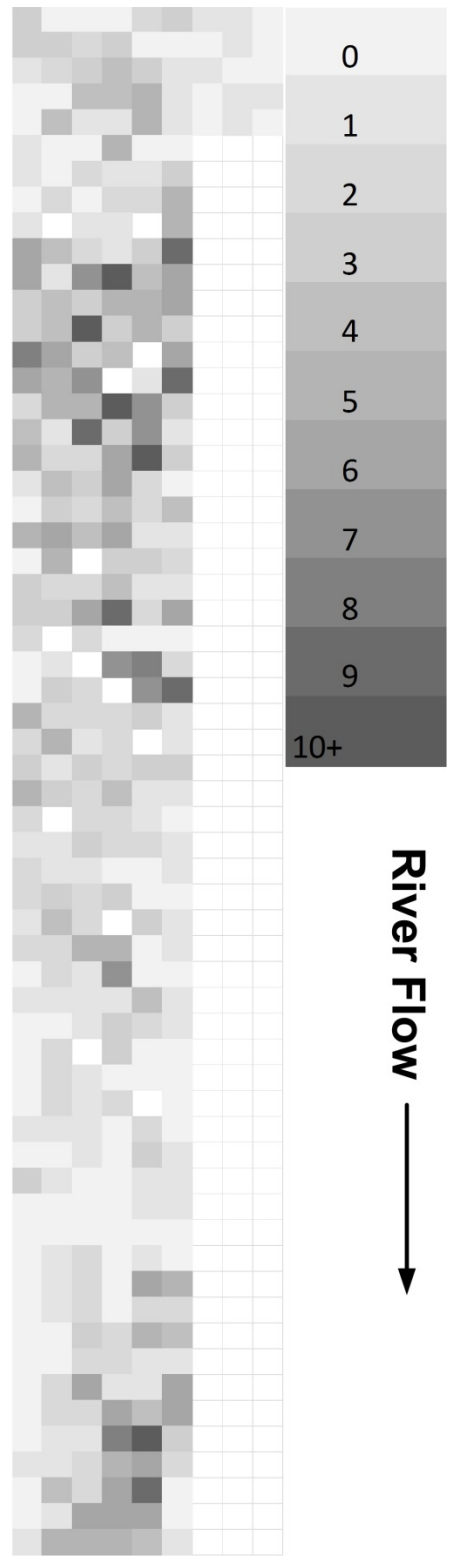


Figure 2. Distribution of mussel counts (all species pooled) across all quadrats sampled at the Rawdon Creek census site (August 27 to September 4, 2013).

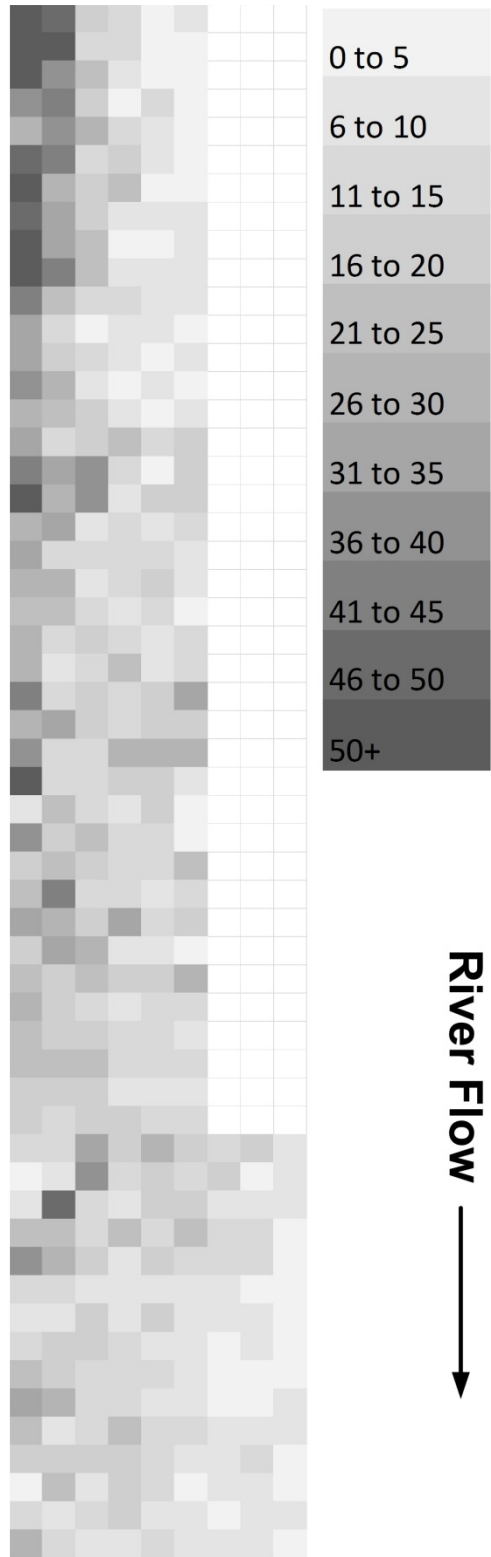


Figure 3. Distribution of mussel counts (all species pooled) across all quadrats sampled at the Sydenham River census site (August 7 and 14, 2012).

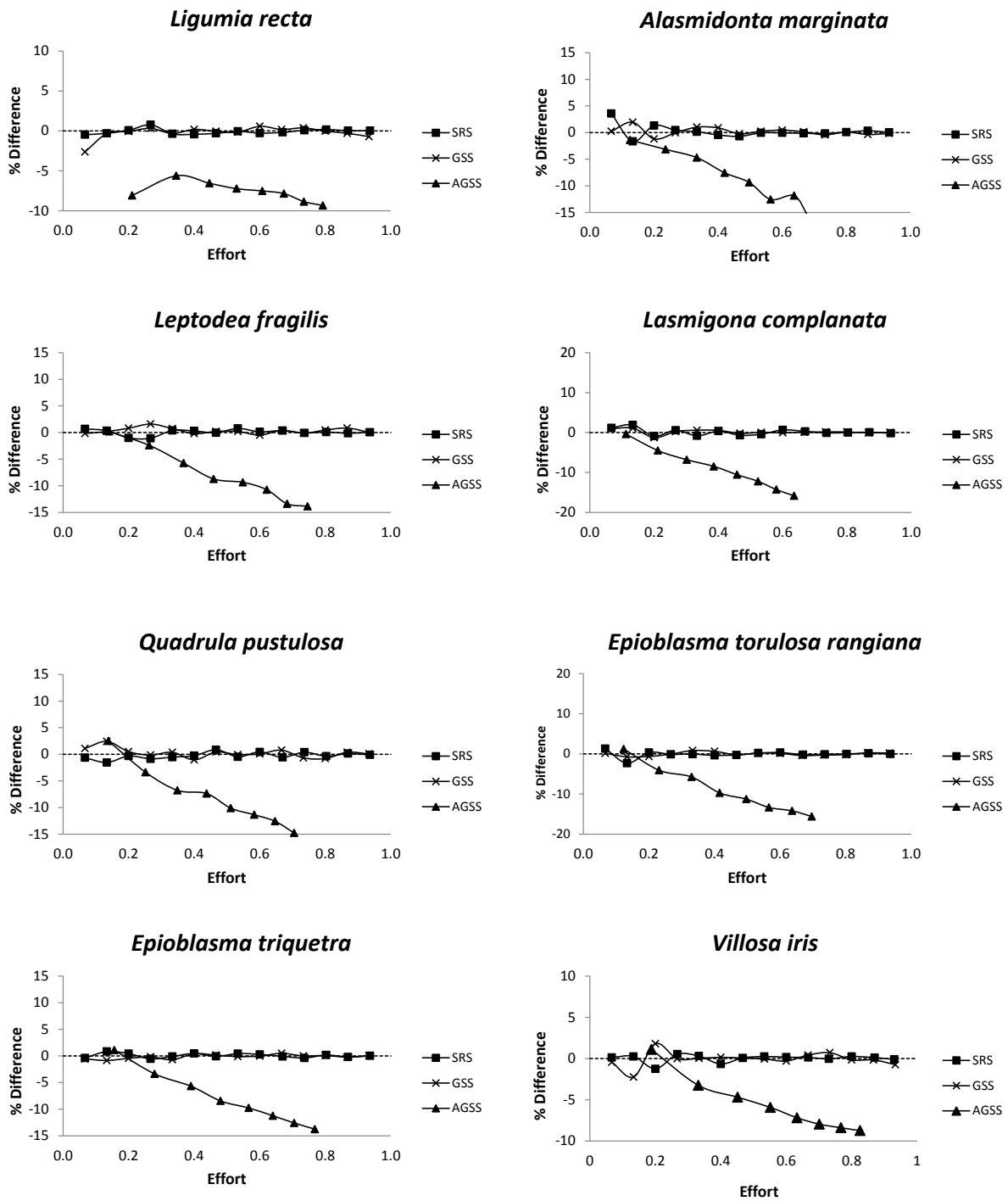


Figure 4. Comparison of the accuracy of density estimates for 8 mussel species using three sampling designs (SRS: simple random sampling; GSS: grid-based systematic sampling; AGSS: adaptive grid-based systematic sampling). Accuracy was calculated as the percent difference from census data (all quadrats sampled). Effort is the proportion of the site sampled by each design.

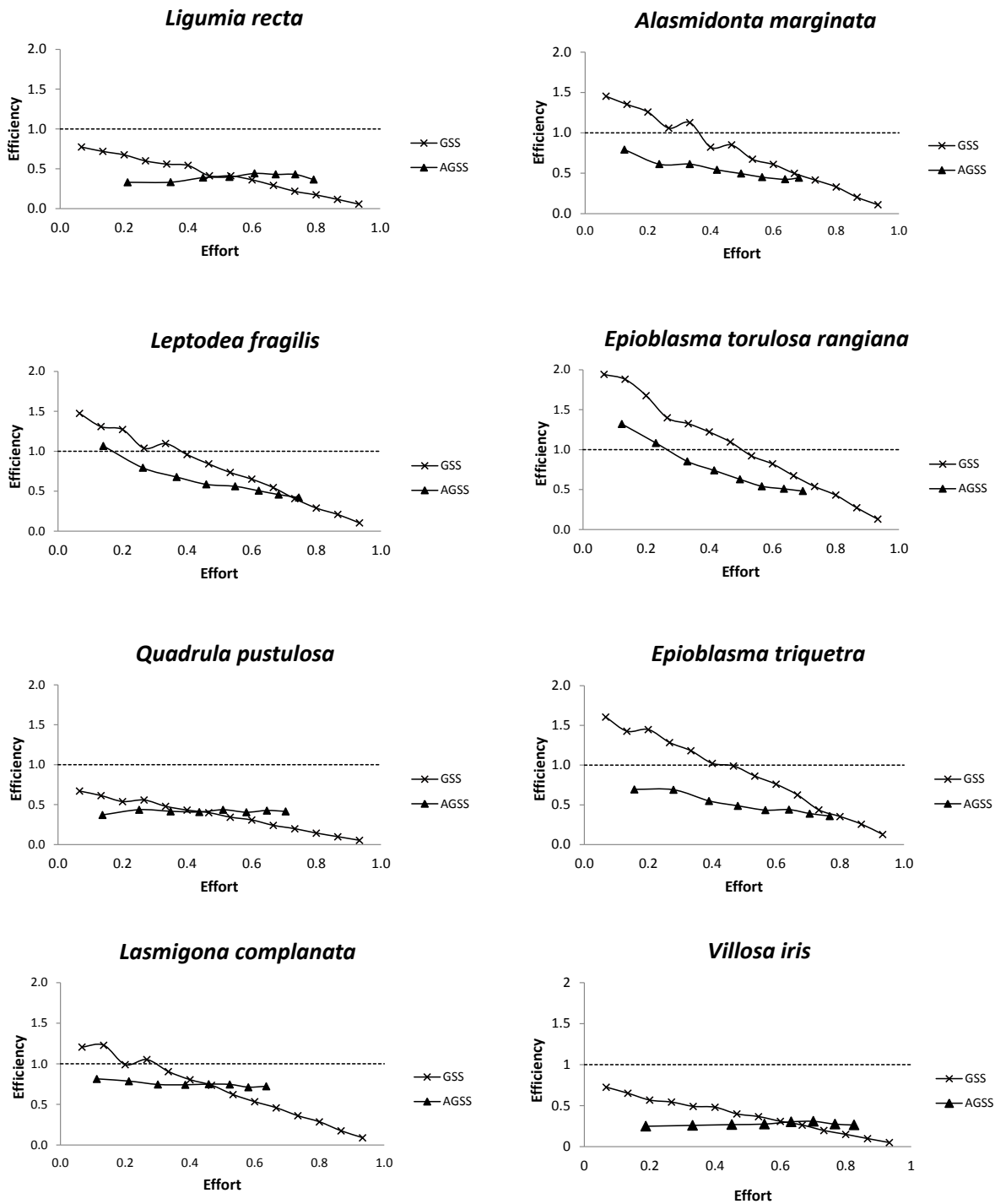


Figure 5. Comparison of the efficiency of two sampling designs (GSS: grid-based systematic sampling; AGSS: adaptive grid-based systematic sampling) for mussel sampling. Efficiency was assessed based on the variance of density estimates from each design relative to that of random sampling. Effort is the proportion of the site sampled by each design.

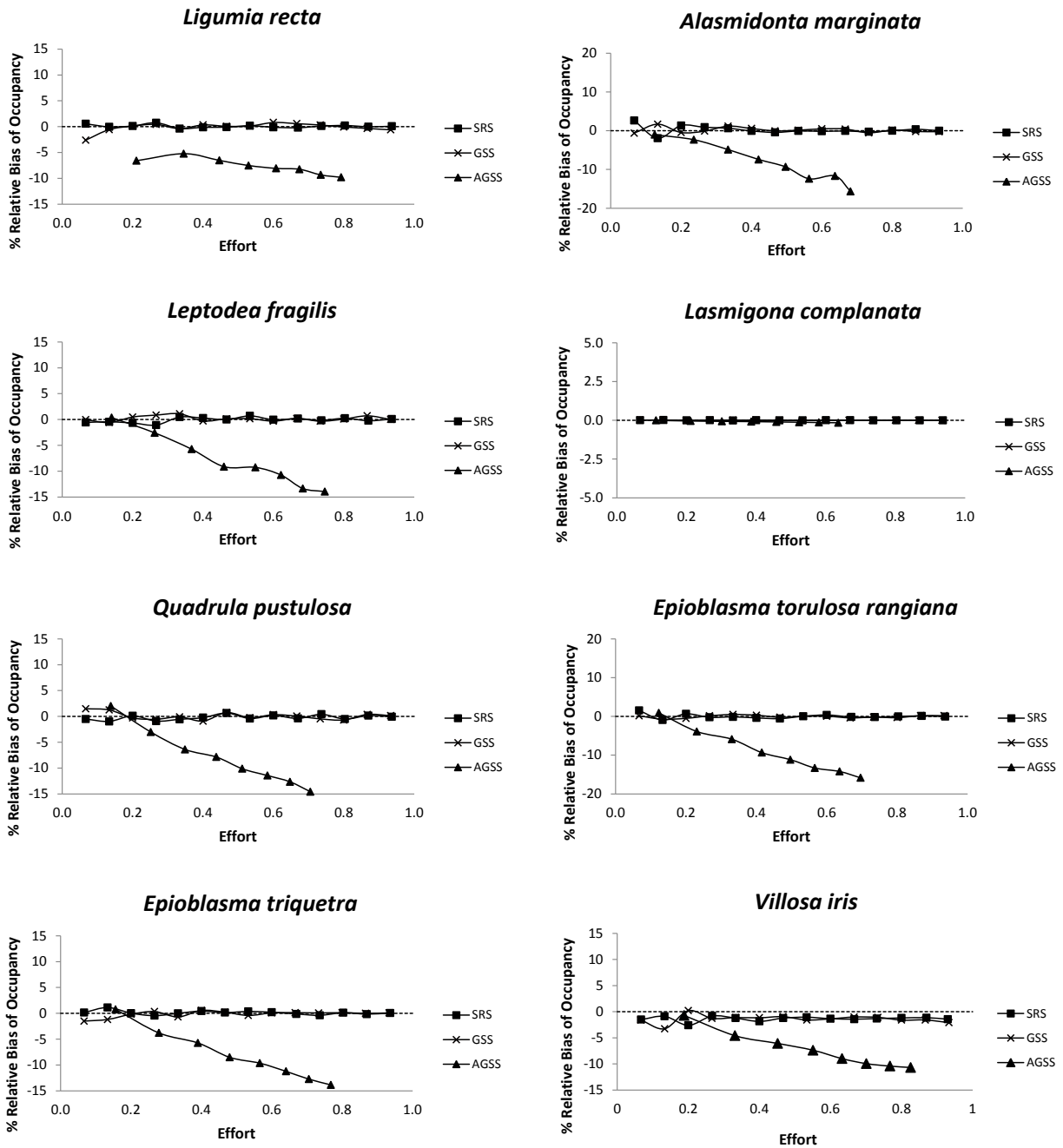


Figure 6. Comparison of occupancy estimates for 8 mussel species using three sampling designs (SRS: simple random sampling; GSS: grid-based systematic sampling; AGSS: adaptive grid-based systematic sampling). Relative bias was calculated as the percent difference of the number of quadrats occupied by a species relative to the census data (all quadrats sampled). Effort is the proportion of the site sampled by each design.

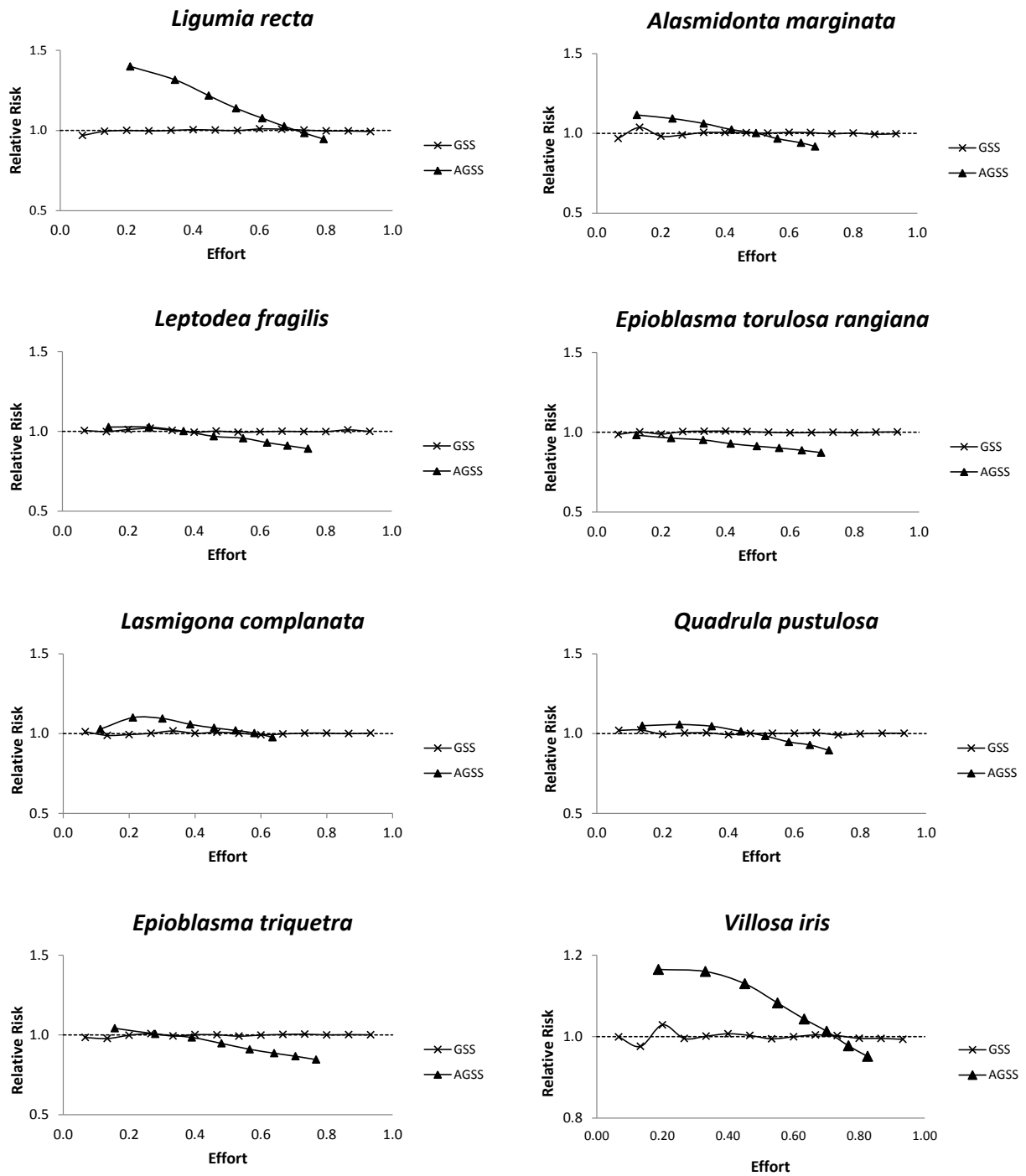


Figure 7. Comparison of the likelihoods of detecting 8 mussel species using grid-based systematic sampling (GSS) and adaptive grid-based systematic sampling (AGSS). Relative risk is the probability of sampling an occupied unit under a specified design relative to that of random sampling. Effort is the proportion of the site sampled by each design.