Fisheries and Oceans Canada | Pêches et Océans Canada

Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2021/035

Central and Arctic Region

Estimating the Mortality of Fishes and Mussels of Conservation Concern Resulting from Bayluscide Applications within four rivers of the Huron-Erie Corridor

Eric R.B. Smyth and D. Andrew R. Drake

Great Lakes Laboratory for Fisheries and Aquatic Sciences
Fisheries and Oceans Canada,
867 Lakeshore Road,
Burlington, ON L7S 1A1 Canada

April 2021

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON KA 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-38486-3 Cat. No. Fs70-5/2021-035E-PDF

Correct citation for this publication:

Smyth, E.R.B., and Drake, D.A.R. 2021. Estimating the Mortality of Fishes and Mussels of Conservation Concern Resulting from Bayluscide Applications within four rivers of the Huron-Erie Corridor. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/035. xi + 198 p.

Aussi disponible en français :

Smyth, E.R.B., et Drake, D.A.R. 2021. Estimation de la mortalité des poissons et des moules dont la conservation est préoccupante résultant des applications de Bayluscide dans quatre rivières du corridor Huron-Érié. Secr. can. de consult. sci. du MPO. Doc. de rech. 2021/035. xi + 202 p.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Bayluscide, a chemical lampricide, is used by government agencies to assess and control invasive Sea Lamprey (*Petromyzon marinus*) in the Great Lakes basin. The toxicity of Bayluscide to non-target fishes has been previously investigated. However, the potential mortality of fishes and mussels assessed by COSEWIC or listed under Canada's *Species at Risk Act* as Endangered, Threatened, or Special Concern has not been evaluated based on the factors that dictate species responses in the wild. To assess the potential for mortality, the likelihood that fishes and mussels of conservation concern will be exposed to, and experience toxicity-induced mortality from, granular Bayluscide in the Detroit, St. Clair, Thames, and Sydenham rivers in southwestern Ontario were quantitatively estimated. Simulation models were based on: 1) habitat associations that predispose fishes and mussels species to occur in areas targeted for application; 2) population densities susceptible to exposure; and, 3) the toxicity of the compound, based on: i) assumed Bayluscide concentrations in the environment; ii) taxonomic or habitat match with surrogate species; and, iii) four dose-response relationships, using Sea Lamprey LC50 and LC99.9 concentrations as a benchmark (fishes) or a single point estimate (mussels). Population effects were evaluated by combining estimates of Bayluscide-induced mortality with age-structured models of Eastern Sand Darter (*Ammocrypta pellucida*; SARA Threatened), Northern Madtom (*Noturus stigmosus;* SARA Endangered), Channel Darter (*Percina copelandi;* Lake Erie DU, SARA Endangered), and *Ichthyomyzon* spp. In most cases, simulated applications resulted in no or low mortality of fishes and mussels. However, in some cases ($\leq$ 5%), mortality of ones to tens of fishes and potentially hundreds of freshwater mussels and Silver Lamprey (*Ichthyomyzon unicuspis*) or Northern Brook Lamprey (*I. fossor*) occurred following a single application cycle. Based on a model in which recovery from Bayluscide effects does not occur, the 50–100 year effect of repeated Bayluscide application could reduce abundance by as much as 100% from baseline for some species (Northern Madtom, *Ichthyomyzon* spp.) or up to 90% for others (Eastern Sand Darter) if populations are small and applications occurred annually but would be less severe if populations are large. Estimated mortality varied with the frequency and size of applications but relationships were non-linear. Several uncertainties were identified including: 1) environmentally relevant concentrations of Bayluscide; 2) species-specific Bayluscide toxicity and appropriateness of surrogates; 3) incidence and effect of avoidance behavior; 4) occurrence, density, and habitat associations based on a limited number of field studies; and, 5) unknown population abundance and trajectory for most species of conservation concern. Results indicate that in some cases, Bayluscide may result in mortality and population effects for species of conservation concern but mortality may be mitigated by several factors associated with the application cycle.

INTRODUCTION

Bayluscide, a chemical lampricide, is used by government agencies in the Great Lakes basin to assess and control Sea Lamprey (*Petromyzon marinus*) as part of the binational Sea Lamprey Control Program. This program is administered by the Great Lakes Fishery Commission (GLFC) with cooperation from Fisheries and Oceans Canada (DFO), the United States Fish and Wildlife Service (USFWS), and the Unites States Army Corps of Engineers (USACE). The granular form of Bayluscide, composed of 2', 5-dichloro-4'-nitrosalicylanilide or niclosamide ethanolamine salt (Dawson 2003), is applied within larval Sea Lamprey nursery habitat as one form of Sea Lamprey assessment when conventional assessment methods, such as backpack electrofishing, are unsuitable due to environmental conditions (e.g., high turbidity and/or non-wadeable assessment sites). Bayluscide is also used as a control tactic to reduce the population abundance of Sea Lamprey in systems where conventional lampricides (e.g., 3-trifluoromethyl-4-nitrophenol [TFM]) would be ineffective or overly costly such as connecting channels (e.g., St. Marys River) and other large flowing waters or portions of lakes themselves.

The use of Bayluscide for assessment and control of Sea Lamprey is a vital component of the binational Sea Lamprey Control Program and has been used in the Great Lakes basin since 1966 (Smith and Tibbles 1980). However, the potential effects of Bayluscide on fishes and mussels listed as Endangered, Threatened, or Special Concern under Canada's *Species at Risk Act* (SARA) as well as other species of conservation concern in the Canadian waters of the Great Lakes basin (e.g., Committee on the Status of Endangered Wildlife in Canada [COSEWIC] assessed species) is of concern for the management of those species. During the course of standard Sea Lamprey assessment activities, granular Bayluscide was recently applied in the Detroit, St. Clair, Sydenham, and Thames rivers in southwestern Ontario, Canada. As these rivers support significant fish and mussel species diversity, including many species listed under SARA, concerns were raised by conservation managers about potential non-target effects to SARA-listed species and other species of conservation concern. These concerns were supported by known sensitivities of certain fishes and mussels to Bayluscide exposure (Marking and Hogan 1967, Dawson 2003, Schreier et al. 2008, Ali 2012, Newton et al. 2017).

Andrews et al. (2021) identified several pathways and mechanisms by which Bayluscide applications could lead to individual or population effects for species of conservation concern. Effects can be direct such as changes in growth, mortality, or reproduction resulting from exposure and toxicity of Bayluscide. In addition, effects can be indirect, such as changes in growth, mortality, or reproduction of prey, competitors, predators, or host fishes (in the case of freshwater mussels), which may act individually or collectively on the abundance or viability of species of conservation concern. Physiological effects (e.g., toxicity), whether to target or non-target species, occur because niclosamide (the active ingredient of Bayluscide) uncouples the oxidative phosphorylation process and disrupts the mitochondrial membrane's proton gradient resulting in lower ATP production (Wilkie et al. 2019). Niclosamide may also interfere with pH regulation and glycolytic enzymes resulting in the inability to use glucose for anaerobic ATP production (Wilkie et al. 2019). In addition to physiological effects, Bayluscide in the aquatic environment may also promote non-physiological responses by species of conservation concern such as avoidance of the application site leading to increased predation risk. Notably, Bayluscide applications also result in the optimization of Sea Lamprey control efforts, which have the effect of reducing mortality and non-lethal effects (e.g., wounding) of Sea Lamprey on large-bodied species of conservation concern such as Lake Sturgeon (*Acipenser fulvescens*).

Although previous work has evaluated the potential effects of Bayluscide exposure to species of conservation concern, this work has been primarily focused on mortality as an endpoint with much of it qualitatively assessed or conducted in ways as to limit the assessment of population-level effects. Furthermore, most evidence of the potential mortality of non-target species is based on the sensitivity of common native species to Bayluscide exposure (e.g., Marking and Hogan 1967, Schreier et al. 2008, Newton et al. 2017). In a few cases, the effect of Bayluscide exposure on a common species has been used to inform potential effects on species of conservation concern (Boogaard et al. 2016) but such studies do not exist for the majority of SARA-listed species in Canada. Therefore, significant uncertainty exists about the potential for direct mortality to non-target species including the potential for population-level effects where relatively small increases in mortality could affect population viability. This research document focuses on estimating direct mortality associated with Bayluscide applications (i.e., toxicity-induced reductions in survival). Other direct and indirect mechanisms identified in Andrews et al. (2021) such as reductions in growth, behavioural effects leading to changes in vital rates (e.g., avoidance of spawning habitat near application sites), or linked food web effects resulting in population responses (Fleeger et al. 2003, Saaristo et al. 2018) are not assessed.

The goals of this study are fourfold. First, using simulation models, to quantify the potential for fishes and mussels of conservation concern to be exposed to granular Bayluscide resulting from applications in the Detroit, St. Clair, Sydenham, and Thames rivers. Second, given exposure, to quantify the resulting likelihood of individual and population-level mortality. Third, to evaluate the potential for altered population dynamics for select species of conservation concern. Fourth, to determine the sensitivity of mortality estimates to factors associated with the Bayluscide application cycle such as the frequency, number, and size of application sites.

METHODS

Evaluating the risk of acute direct mortality associated with Bayluscide applications was based on: 1) identifying fish and mussel species of conservation concern that occur in each of the four focal rivers (hereafter, ‘species composition’); 2) determining the likelihood that species of conservation concern occupy a Bayluscide application area (hereafter, ‘likelihood of occurrence’); and, 3) estimating the number of mortalities expected from a granular Bayluscide application, which was a function of 4) the estimated density of each species at an application site (hereafter, ‘density’); and, 5) species-specific dose-response relationships for the active ingredient of Bayluscide, niclosamide. Finally, estimated levels of mortality were related to changes in population dynamics. Due to data limitations, only a subset of species were chosen to estimate how Bayluscide-induced mortality may influence population dynamics. These were Eastern Sand Darter (*Ammocrypta pellucida*; SARA Threatened) in the Thames River, Channel Darter (*Percina copelandi*; Lake Erie Designatable Unit [DU], SARA Endangered) in the Detroit River, Northern Madtom (*Noturus stigmosus*); SARA Endangered) in the Detroit and Thames rivers, and *Ichthyomyzon* spp. in the Thames and St. Clair rivers, which includes Silver Lamprey *Ichthyomyzon unicuspis* (Great Lakes-Upper St. Lawrence River DU, SARA Special Concern) and Northern Brook Lamprey (*I. fossor*; Great Lakes-Upper Sr. Lawrence River DU, SARA Special Concern). Sensitivity analysis was used to understand the influence of model parameters including the effect of assumed values when deriving dose-response relationships.

DATA SOURCES

Field data were required to estimate: 1) the species composition; 2) the likelihood of occurrence for each species; and, 3) the density of each species. Given the widely different field programs and sampling approaches used to characterize species composition, the likelihood of occurrence, and density of fishes and mussels of conservation concern, databases that contained sampling data specific to different taxa were used (fishes excluding lampreys; non-target lampreys; and, mussels).

Data from DFO's Biodiversity Science Database (DFO unpublished data) were used to estimate the species composition, likelihood of occurrence, and density of non-lamprey fish species. This database contains species collection records following SARA-related research conducted by the Great Lakes Laboratory for Fisheries and Aquatic Sciences (DFO Science) from 2003 to 2017 (Figure 1). The database contains a mix of targeted, random, and exploratory sampling programs which involve a multitude of gear types (boat electrofishing, backpack electrofishing, boat seine, hoopnet, fyke net, trap net, trawls, and trammel nets) (Appendix A), each with different species-specific detection probabilities. The Biodiversity Science Database also contains historical and other museum records from 1936 to 2016 and collection records of species associated with SARA sampling permits by external partners or other agencies and is considered the most up to date source of the occurrence of SARA-listed and COSEWIC-assessed species in the Great Lakes basin. Most historical records contained in the database do not include sampling covariates such as habitat attributes or fished area and therefore were used only to inform the occurrence of a species in a focal river, rather than the likelihood of occurrence or density.

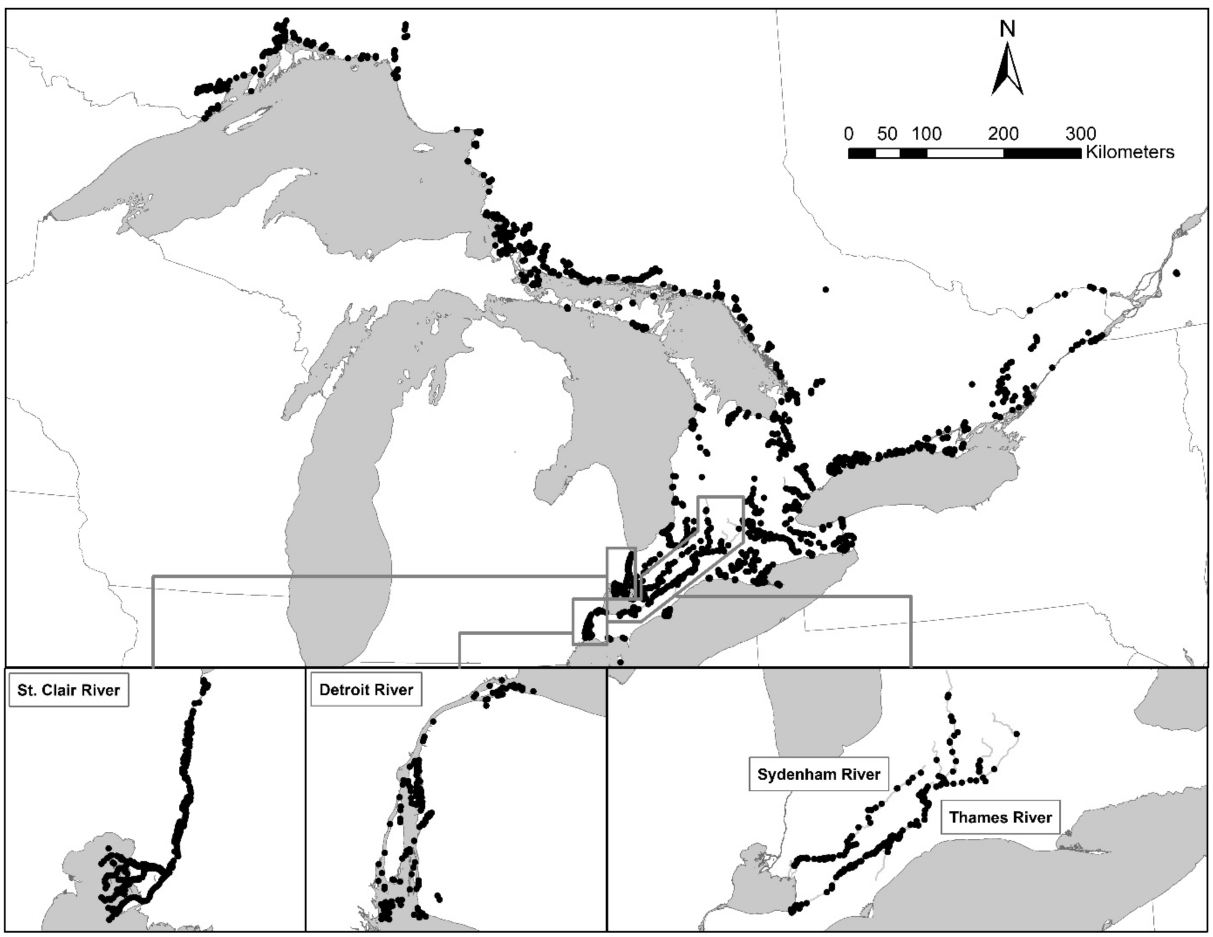

Figure 1. Locations of sampling sites contained in the DFO Biodiversity Science Database, DFO's Sea Lamprey Control Centre (SLCC) sampling database, and USFWS lamprey sampling database. Inset figures indicate the location of sampling sites used to evaluate fish (lamprey and non-lamprey) species composition in focal rivers.

Data from the DFO Mussel Database (DFO unpublished data) were used to estimate species composition, the likelihood of occurrence, and density of mussel species based on targeted mussel sampling from 1997 to 2017 by DFO Science and partners (Figure 2). The DFO Mussel Database includes timed search sampling from 1997 to 2017 where visual searching was completed to identify the presence of live organisms, fresh dead shells (whole shells or valves), or weathered shells (whole shells or valves) (Appendix A). Further details on timed search methods are available in Metcalfe-Smith et al. (2000). Given that search area and/or substrate data were not consistently measured following timed search sampling, these data were only used to evaluate mussel species composition within a focal river and the likelihood of occurrence at an application site. The DFO Mussel Database also included detailed quadrat sampling conducted in the Thames and Sydenham rivers from 1997 to 2017. Sampling sites and quadrats within these rivers were purposefully selected to sample habitat that would likely yield high densities of mussel species of conservation concern. Sampling sites were divided into sampling blocks which ranged from 20 to 28 blocks per site with each block divided into fifteen 1 m^2 quadrats. The sampling consisted of excavating 3 to 4 randomly selected 1 m^2 quadrats within each block (the same quadrats in each block were excavated) and quantifying substrate composition and species abundance within each quadrat. The number of sampling sites, blocks, and quadrats for each sampling year are provided in Appendix A. Further detail of quadrat sampling methods are provided in Metcalfe-Smith et al. (2007).

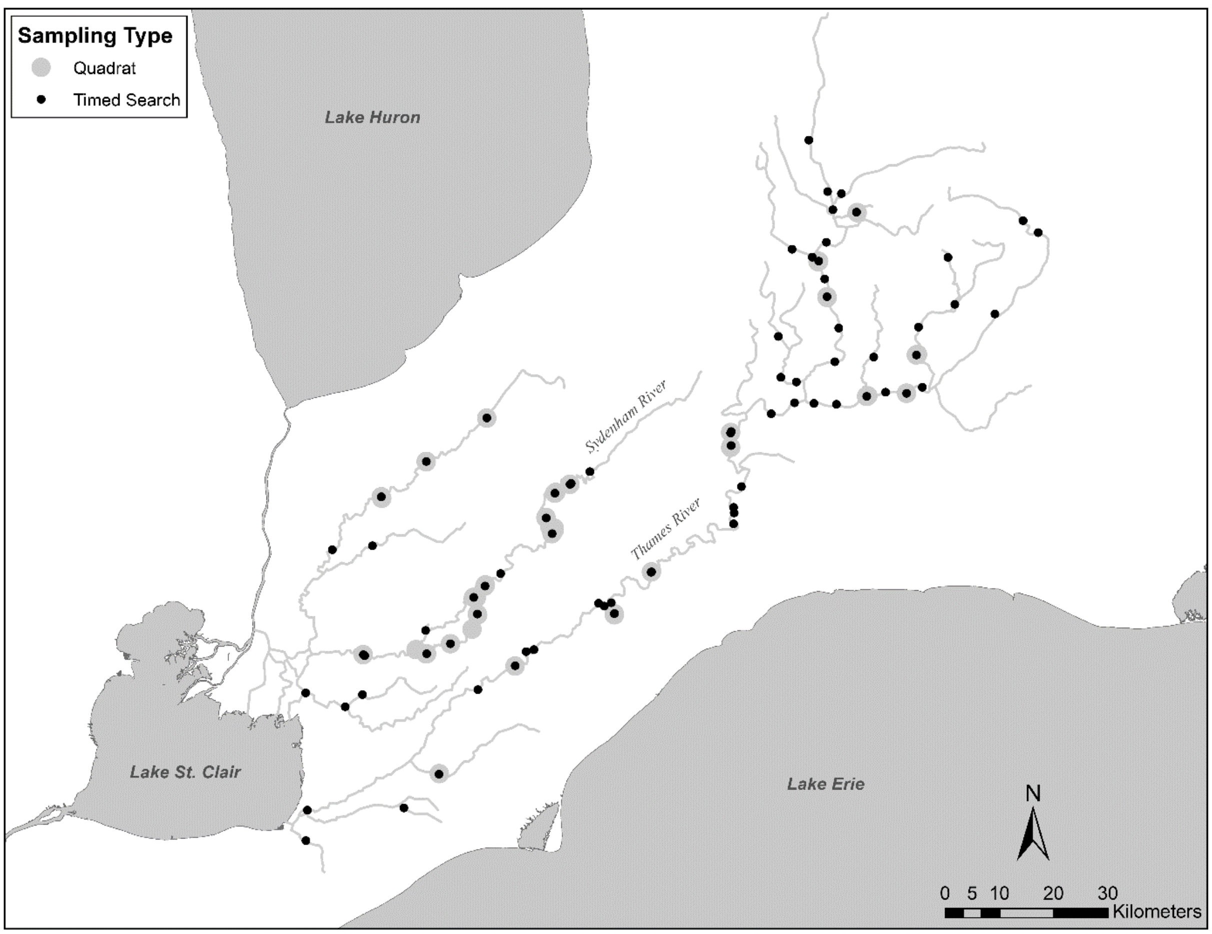

Figure 2. Locations of sampling sites (both timed searches and quadrats) contained in the DFO Mussel Database and used to evaluate mussel species composition in focal rivers.

Lamprey species composition, likelihood of occurrence, and density were informed with DFO's Sea Lamprey Control Centre (SLCC) sampling database, which contains invasive and native lamprey collection records from 2011 to 2017 in locations including the Detroit River and St. Clair River as a result of assessment and treatment activities plus other targeted research initiatives. Lamprey species composition, likelihood of occurrence, and density were also informed by the USFWS lamprey sampling database (USFWS unpublished data), which contained collection records from lamprey sampling in the Detroit River and St. Clair River from 2011 to 2015 and 2011 to 2017, respectively.

DETERMINING SPECIES COMPOSITION

A list of fish and mussel species assessed by COSEWIC as Endangered, Threatened, or Special Concern as well as those listed as Endangered, Threatened, or Special Concern under SARA was assembled. Only species fitting the above criteria and detected within at least one of four focal rivers (Sydenham, Thames, St. Clair, and Detroit) were included for quantitative analysis (see Tables 1 and 2). The composition of non-lamprey fish species in each river was primarily based on DFO's Biodiversity Science Database and included SARA-related research conducted by DFO Science, historical and other museum records, and collection records of species associated with SARA sampling permits by external partners or other agencies. Lake Sturgeon records were supplemented with monitoring data describing species presence in the Detroit River and the St. Clair River (Hayes and Caroffino 2012) and absence in Thames River (Quinlan and Maaskant 2017, Kessel et al. 2018) and Sydenham River (SCRCA 2017). Lamprey data were informed primarily by DFO's SLCC sampling database plus the USFWS lamprey sampling database and secondarily by the DFO Biodiversity Science Database to capitalize on instances where lampreys were detected during other sampling initiatives.

The species composition of mussels in each river was based on data from timed search and quadrat sampling (1997–2017). One limitation of mussel data sources was the absence of current data from the Detroit and St Clair rivers during the study period (1997–2017). Schloesser et al. (2006) sampled the Detroit River and deemed native unionids as extirpated from the main channels. The little sampling that has been conducted in the Canadian waters of St. Clair River where extant populations exist in an isolated stronghold (Walpole Island) is not reflective of the status of unionids in the rivers as a whole. For these reasons, the Detroit and St Clair rivers were excluded from the analyses. However, recent sampling in 2019 by Allred et al. (2020) demonstrated that populations of several species at risk (Threehorn Wartyback [*Obliquaria reflexa*], Round Pigtoe [*Pleurobema sintoxia*], and Mapleleaf [*Quadrula quadrula*] mussels) still occur within the Canadian waters of the Detroit River.

In total, 19 fish species (treating *Ichthyomyzon* ammocoetes as a single species) and 13 mussel species of conservation concern exist in the four focal systems (Tables 1 and 2). Quantitative estimates of exposure and mortality in this study are limited to these species.

Table 1. Composition of fish species of conservation concern in focal rivers as of March 2019.

Species Common Name	Species Scientific Name	Detroit River	St. Clair River	Sydenham River	Thames River	Combined Species Presence
Black Redhorse	*Moxostoma duquesnei*	-	-	-	X	X
Blackstripe Topminnow	*Fundulus notatus*	-	X	X	-	X
Channel Darter	*Percina copelandi*	X	X	X	-	X
Eastern Sand Darter	*Ammocrypta pellucida*	X	-	X	X	X
Grass Pickerel	*Esox americanus vermiculatus*	X	X	X	X	X
Lake Chubsucker	*Erimyzon sucetta*	-	X	-	-	X
Lake Sturgeon	*Acipenser fulvescens*	X	X	-	-	X
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	X[1]	-	X	X
Northern Madtom	*Noturus stigmosus*	X	X	-	X	X
Northern Sunfish	*Lepomis peltastes*	X	X	X	X	X
Pugnose Minnow	*Opsopoeodus emiliae*	X	X	X	-	X
Pugnose Shiner	*Notropis anogenus*	X	X	X	-	X
River Darter	*Percina shumardi*	-	-	X	X	X
River Redhorse	*Moxostoma carinatum*	-	X	-	X	X
Silver Chub	*Macrhybopsis storeriana*	-	-	-	X	X
Silver Lamprey[2]	*Ichthyomyzon unicuspis*	X	X	X	X	X
Silver Shiner	*Notropis photogenis*	-	-	-	X	X
Spotted Sucker	*Minytrema melanops*	X	X	X	X	X
Unidentified Northern Brook or Silver Lamprey ammocoetes	*Ichthyomyzon sp.*	-	X	-	X	X
	Count	**10**	**14**	**10**	**13**	**19**

[1] Northern Brook Lamprey occurrence in the St. Clair River was based on records from the Royal Ontario Museum (Accessed through the Fishnet 2 Portal).

[2] Silver Lamprey presence records were based on unidentified transformer/adult records (Detroit, Sydenham, and Thames rivers) from the DFO Biodiversity Science Database and confirmed transformer records (St. Clair River) from the USFWS lamprey sampling database.

Table 2. Composition of mussel species of conservation within each focal river as of March 2019. The Detroit and St. Clair rivers are not included because of limitations with available current/recent data.

Species Common Name	Species Scientific Name	Sydenham River	Thames River	Combined Species Presence
Fawnsfoot	*Truncilla donaciformis*	X	X	X
Kidneyshell	*Ptychobranchus fasciolaris*	X	X	X
Lilliput[1]	*Toxolasma parvum*	X	-	X
Mapleleaf	*Quadrula quadrula*	X	X	X
Northern Riffleshell	*Epioblasma rangiana*	X	-	X
Rainbow	*Villosa iris*[2]	X	X	X
Rayed Bean	*Villosa fabalis*[3]	X	X	X
Round Hickorynut[4]	*Obovaria subrotunda*	X	-	X
Round Pigtoe	*Pleurobema sintoxia*	X	X	X
Salamander Mussel[5]	*Simpsonaias ambigua*	X	-	X
Snuffbox[4]	*Epioblasma triquetra*	X	-	X
Threehorn Wartyback	*Obliquaria reflexa*	X	X	X
Wavyrayed Lampmussel	*Lampsilis fasciola*	X	X	X
	Count	**13**	**8**	**13**

[1] Only one Lilliput record in Thames River from 2010. Adequate sampling has not been conducted in the preferred habitat of Lilliput so the species was excluded from further analysis.

[2] The species' scientific name was recently revised and is now *Cambarunio iris*; however, *Villosa iris* will be used throughout the report to remain consistent with the scientific species name as it is listed under SARA.

[3] The species' scientific name was recently revised and is now *Paetulunio fabalis*; however, *Villosa fabalis* will be used throughout the report to remain consistent with the scientific species name as it is listed under SARA.

[4] A single fresh valve was found in Thames River in 2005. Not considered evidence of a reproducing population so Round Hickorynut was excluded from further analysis.

[5] A single fresh valve was found in the Thames River in 1998. Since no live or additional fresh shells have been observed in the Thames River since 1998, it was determined there was insufficient information available and the species was excluded from further analysis.

[6] A single fresh valve was found in the Thames River in 1998. Since no live or additional fresh shells have been observed in the Thames River since 1998,it was determined there was insufficient information available and the species was excluded from further analysis.

ESTIMATING SPECIES EXPOSURE TO BAYLUSCIDE

The number of organisms present at a Bayluscide application site was based on the probability that each species of conservation concern would occur within an application site (likelihood of occurrence) and the corresponding density of each species when present. In this document, the term application site is used to describe a single 500 m^2 site that is the target of granular Bayluscide applications while the term application cycle describes the six 500 m^2 sites that constitute a standard granular Bayluscide application in a given river and time period. Likelihood of occurrence and density were estimated for each species within a focal river using methods described below. However, data limitations required different approaches for Lake Sturgeon and native lampreys (also described below).

Species-specific Likelihood of Occurrence within an Application Site

Because larval Sea Lamprey display an affinity for the depositional zones of rivers or lakes, granular Bayluscide is applied in a non-random targeted manner within tributary streams focusing on areas dominated by soft substrates. Therefore, only certain habitats within a focal river will be targeted for application driven by operator decisions about which river features constitute preferred or acceptable sites for larval Sea Lamprey production. Standard Sea Lamprey habitat classifications exist to guide application decisions, which are based on the suitability of different substrates for Sea Lamprey production. Habitat classifications derived by the Sea Lamprey Control Program are defined as follows, with granular Bayluscide applications focused in Type I and Type II habitats:

1. Type I habitat is defined as nursery habitat preferred by Sea Lamprey, composed of fine particle substrate, usually dominated by silt, but may also contain some fine sand and detritus;
2. Type II habitat is defined as nursery habitat acceptable to Sea Lamprey, composed of coarser substrates relative to Type I, including coarse sand, some silt and detritus, and little gravel; and,
3. Type III is defined as habitat not conducive to Sea Lamprey production, composed of hard and very coarse substrate that prevents or deters burrowing.

Because Bayluscide applications are focused in Type I and II habitats, calculating the likelihood of species occurrence within an application site involved determining the probability that each species of conservation concern would occur in Type I or Type II habitat features. DFO fish and mussel sampling sites and quadrats were classified as being composed of either Type I, Type II, or Type III habitat based on a habitat classification decision tree. The classification tree was developed in consultation with the Sea Lamprey Control Program based on the substrate composition used to differentiate among habitat types (Figure 3). For example, a site that is 60% sand, 30% detritus, and 10% boulder would be classified as Type II habitat while a site that is 50% clay, 30% cobble, 15% silt, and 5% boulder would be classified as Type III. Once each site and quadrat was assigned a habitat classification, the likelihood of species occurrence was calculated using:

$$Likelihood\ of\ Occurrence = \frac{Number\ of\ Type\ X\ habitat\ sites\ where\ species\ Y\ was\ detected}{Total\ number\ of\ Type\ X\ habitat\ sites\ sampled} \qquad (1)$$

where X is the habitat class of interest (Type I or Type II) and Y is the species of interest. Sampling sites used to derive equation 1 included all DFO fish sampling sites with suitable substrate data (n = 4,024), mussel sampling quadrats with suitable substrate data (n = 3,374), and a subset of timed search records (n = 59) having suitable substrate data. The likelihood of occurrence equation incorporates the habitat preference of a species as well as its corresponding rarity within a system (i.e., not all sand substrates will contain a species of interest even if sand is the preferred substrate). Therefore, a low likelihood of occurrence may represent a species that does not prefer Type I or Type II habitat, exists at very low abundance in the system and is rarely encountered, or a combination of both factors. Although the data used to calculate the likelihood of occurrence are subject to inherent biases given that they resulted from a mix of targeted, random, and exploratory sampling programs, the high number of sampling sites in the database suggest that the likelihood of occurrence values approximate the true probability of each species occurring in a given habitat class. For mussel species, the quadrat and subset of timed searched datasets from the Thames and Sydenham rivers were used.

Given the spatial and temporal breadth of the field collection records, there were multiple ways to estimate the likelihood of occurrence for Type I and Type II habitat based on available data. Likelihood values were generated based on field data from the focal rivers where the target species has been detected.

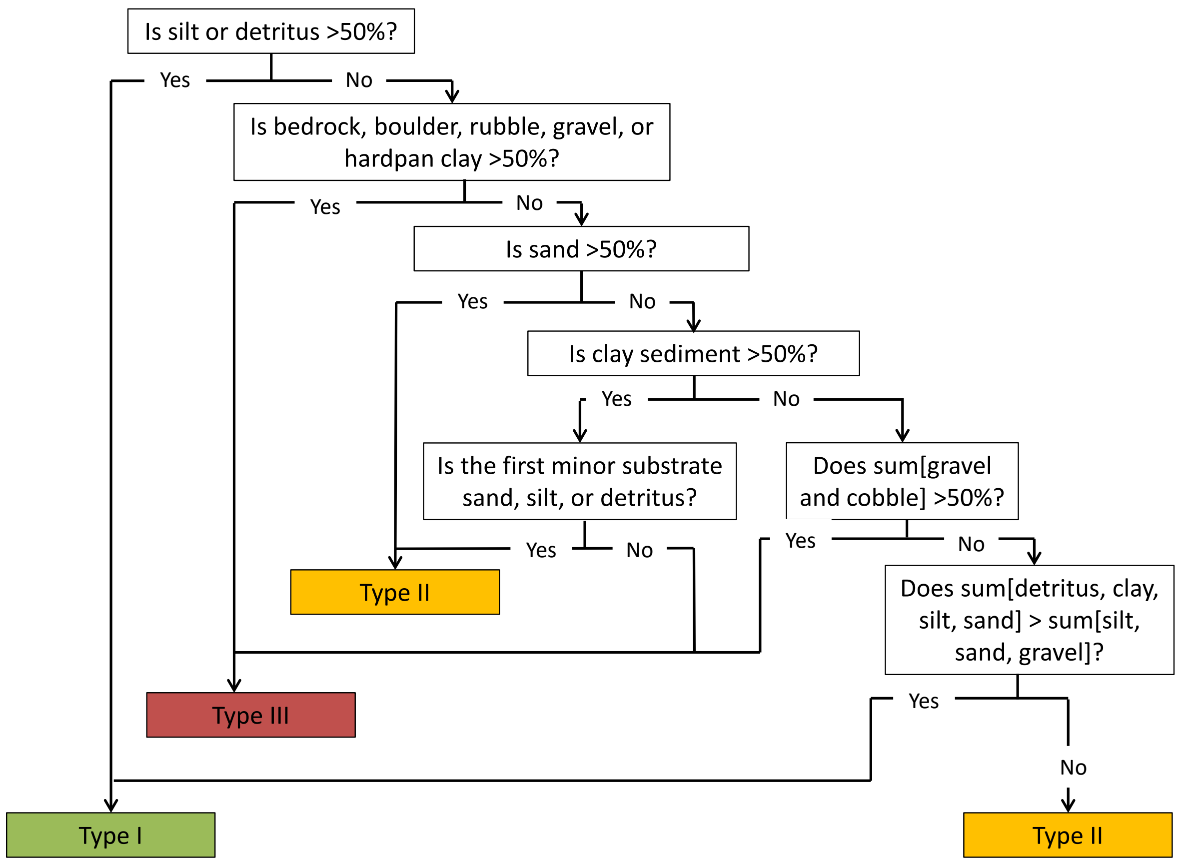

Figure 3. Decision tree used to assign Sea Lamprey habitat classes (i.e., Type I, Type II, or Type III) to fish and mussel sampling sites based on substrate composition.

Species-specific Density

Density estimates for each species were generated using capture records from a subset of sampling programs in the DFO Biodiversity Science Database and the DFO Mussel Database. To calculate species-specific fish densities, sampling sites in the DFO Biodiversity Science

Database were selected where active gears were used (e.g., trawling, boat or backpack electrofishing, boat or shore seining), where an estimate of the sampled area was taken, and where the target species was detected. Mussel species-specific densities were based on quadrat sampling in the DFO Mussel Database and included all quadrats where the target species was detected. These criteria were used to derive density estimates in locations where the species occurs, as densities of zero (i.e., species absence) were accounted for in the likelihood of occurrence calculations. In later stages of the model, the approach calculates the expected density of a species in each habitat class, as the likelihood of occurrence values are multiplied by the density of the species across all habitats in which it has been found.

In most cases, a relatively low number of data points resulted in the inability to separate density data by habitat type or, for a few cases, river system. River-specific density estimates were used when > 5 data points were available. Otherwise, density estimates were derived using data aggregated across multiple rivers.

To describe variability of the density of fishes and mussels of conservation concern, statistical distributions were fit to the empirical density data. A normal distribution was fit to each species for each system with $\geq$ 3 density values. The mean and standard deviation was calculated using the fitdistrplus package in R (Delignette-Muller et al. 2020) and rescaled as the number of organisms per 100 m^2. The mean and standard deviation were manually calculated if a species had two to three density values. If a species had a single density value, then the single value was used to estimate density and no underlying statistical distribution was assumed.

Lake Sturgeon

Since the Lake Sturgeon species composition data was based on external capture records (Hayes and Caroffino 2012, Hutton 2012, Kessel et al. 2018), many of which did not contain substrate information, sufficient habitat data was unavailable to relate the likelihood of occurrence of Lake Sturgeon to Sea Lamprey habitat classes. Therefore, it was assumed that Type I, Type II, or Type III habitat was utilized equally as juvenile and young-of-year (YOY) habitat (Hutton 2012) as well as adult habitat in the Detroit River. Although Lake Sturgeon was detected in various substrates including sand, gravel, and clay, the proportion of Lake Sturgeon found in these substrates matched the proportional availability of these substrates, suggesting little to no substrate preference (Hutton 2012).

The likelihood of occurrence of Lake Sturgeon was calculated by estimating the likelihood that a site will have a Lake Sturgeon density of 1 fish/100 m^2, which was informed by the estimated density of the species in the St. Clair River and Detroit River. Due to the lack of existing density data, population abundance was used to estimate system-wide densities. Lake Sturgeon population abundance was estimated at 4,422 fish in the Detroit River (Justin Chiotti, USFWS, pers. comm.) and 15,882 fish in the St. Clair River and Lake St. Clair (Hayes and Caroffino 2012). River area estimates used to calculate density were based on multiplying river length by the average of 20 river width measurements obtained from Google Earth®. Since the St. Clair Lake Sturgeon population includes fish in both Lake St. Clair and St. Clair River, fish that do not move into the St. Clair River and solely reside in Lake St. Clair were removed from the density estimate. Based on the work by Kessel et al. (2018), the proportion of fish within the entire St. Clair system that would be present at some point in the St. Clair River was calculated. The population estimate of 15,882 fish in the St. Clair system was multiplied by this proportion to estimate the potential population size of Lake Sturgeon within the St. Clair River. Using the updated population sizes for the Detroit and St. Clair rivers, the density was calculated for each river based on a site size of 100 m^2. The river-specific density estimates were then used as estimates of the corresponding likelihood of occurrence values, indicating whether a site would have a fixed Lake Sturgeon density of 1.0 fish/100 m^2. This approach to calculating harm to

Lake Sturgeon allows a simulation of the situation in which the majority of Bayluscide application sites will not have Lake Sturgeon present rather than having all sites with <1 sturgeon present.

Ichthyomyzon spp.

The SLCC sampling database (SLCC unpublished data) and USFWS lamprey sampling database contained records of native lamprey that were definitively identified to species as Silver Lamprey, Northern Brook Lamprey, and Chestnut Lamprey (*I. castaneus*). However, both databases also contained records of *Ichthyomyzon* sp., indicating that positive identification to species, primarily for ammocoetes, had not been made. To develop likelihood of occurrence estimates for Silver Lamprey and Northern Brook Lamprey while accounting for unidentified specimens, two approaches were pursued. The first approach involved only using data that were definitively identified to species for Silver Lamprey or Northern Brook Lamprey at the likely expense of underestimating the likelihood of occurrence and density of each species. The second approach involved combining the species-level data (e.g., Silver Lamprey) with all unidentified *Ichthyomyzon* records, given that unidentified *Ichthyomyzon* could be Silver Lamprey or Northern Brook Lamprey. The latter approach likely over-estimated the likelihood of occurrence and density for each species but both approaches (1: definitive species; 2: definitive species + all unidentified species) allowed the potential bounds of Bayluscide exposure and mortality to be estimated for native lamprey species of conservation concern.

Lamprey collection records contained in SLCC and USFWS databases did not include site-specific substrate data so the classification of substrate associated with *Ichthyomyzon* occurrences could not be completed using the technique that was applied to most fishes. To resolve this issue, it was assumed that all habitat sampled in these databases were composites of Type I or Type II habitat since the purpose of sampling programs was to evaluate the presence and abundance of Sea Lamprey. Therefore, field crews would be unlikely to sample for Sea Lamprey in poor ammocoete habitat (Type III). With this approach, it was assumed that habitat preferred by Sea Lamprey ammocoetes would be the same for *Ichthyomyzon* spp.

Given the large amount of native lamprey data for each focal river, river-specific likelihood of occurrence and density values were estimated for: Northern Brook Lamprey, Northern Brook Lamprey + *Ichthyomyzon* sp., Silver Lamprey, Silver Lamprey + *Ichthyomyzon* sp., and a final category that included only *Ichthyomyzon* sp. In cases where a species was not detected in the SLCC sampling database or the USFWS lamprey sampling database but a record existed in the DFO Biodiversity Science Database (i.e., Northern Brook Lamprey in the St. Clair River and Silver Lamprey in the Detroit, Sydenham, and Thames rivers) (Table 1), the likelihood of occurrence was estimated using the following equation:

$$Likelihood\ of\ Occurrence = \frac{1}{(n_{sites} + 1)} \tag{2}$$

where n_{sites} is the number of sampled sites for a system. This equation calculates the maximum likelihood of occurrence for a species that is present within a system but has yet to be detected by a sampling program.

The density estimates for Silver Lamprey and Northern Brook Lamprey were estimated using the approach described above for other fishes of conservation concern using the SLCC sampling database and USFWS lamprey sampling database.

CALCULATING SPECIES-SPECIFIC BAYLUSCIDE TOXICITY

The potential toxicity of Bayluscide to each species of conservation concern was based on first estimating the concentration of Bayluscide in the aquatic environment and second, estimating the toxicity (i.e., Bayluscide-induced mortality) of the compound to fishes and mussels at the estimated concentrations.

The protocol to apply granular Bayluscide is based on weight of compound per unit area (175 kg of Bayluscide per hectare; USFWS and DFO 2016). Given a fixed weight-per-area application, it is likely that different environmental conditions at an application site (e.g., water depth and flow, density of aquatic vegetation) can lead to different in-water concentrations of Bayluscide. However, the influence of these factors has not been evaluated in sufficient detail to generalize the concentration of Bayluscide across application locations. Therefore, the concentration and duration of Bayluscide in the aquatic environment following an application is not known with certainty. For the fish species assessments, two likely target concentrations were estimated based on benchmarks involving the toxicity of Bayluscide to Sea Lamprey. The rationale behind this approach was that the realized concentration in the aquatic environment must be sufficient to impose at least some mortality to Sea Lamprey given the goals of assessment and control with the compound. Therefore, the Bayluscide concentrations that resulted in 9-h LC50 (0.035 mg/L) and 9-h LC99.9 (0.057 mg/L) of Sea Lamprey (Scholefield and Seelye 1992) were used. Two concentrations were chosen to understand the sensitivity of mortality estimates to different assumed concentrations. For the mussel species assessments, the same target concentrations used by Newton et al. (2017; 8-h 11 mg/L) was assumed. The 11 mg/L concentration is substantially higher than 8-h LC50 values derived for numerous fishes (Marking and Hogan 1967, Bills and Marking 1976, Dawson 2003) and is well beyond the benchmark concentrations used in the fish analysis (0.035 mg/L and 0.056 mg/L over nine hours). However, incorporating the 11 mg/L concentration was necessary to incorporate mussel mortality data from Newton et al. (2017). Given the different assumed environmental concentrations, the mussel and fish results are not directly comparable.

The potential mortality from a Bayluscide application was estimated using two different methods: one for fish species based on two generated dose-response curves (i.e., a steep slope and a gentle slope), which were derived by compiling surrogate 8-h LC50 values and relating these to the two Sea Lamprey mortality benchmarks, and one for mussel species based on the eight hour results from Newton et al. (2017). Each method is described further below.

Fish Species Sensitivity

Toxicity data were unavailable for most fish species of conservation concern, so surrogate species were used to estimate mortality. Surrogate species were assumed to be reasonable proxies for species of conservation concern following a taxonomic and habitat-based surrogate assignment (see below). However, even for well-studied surrogate species, toxicity data provided only a snapshot of species sensitivity to Bayluscide (i.e., LC50 values), often based on exposure lengths well beyond the estimated duration benchmark of eight or nine hours. To address these limitations, the 8-h LC50 value for each surrogate species was estimated, with the exception of *Ichthyomyzon* spp., by building a logarithmic line of best fit using LC50 values for a given surrogate species and the same temperature across different exposure lengths (Table 3). A logarithmic relationship was selected based on Peterson et al. (2001) and Van Ginneken et al. (2017). Relationships were standardized across temperature to remove variability imposed by temperature-specific responses.

Using the 8-h LC50 value, two dose-response curves for each surrogate species were generated that captured the potential extremes of Bayluscide sensitivities to different concentrations. Regardless of the slope shape or steepness, the dose-response curves were designed to intersect the estimated 8-h LC50 value. The first dose-response curve (gentle slope) was built assuming that a mortality rate of 0.001 would occur at a dose of 0.001 mg/L. The second dose-response curve (steep slope) was built assuming that a mortality rate of 0.01 would occur at a dose that is 80% of the LC50 value (Figure 4).

Surrogates were assigned based on species of shared genus or, if no genus-level match was available, shared family of the species of conservation concern. If multiple species met the surrogate match for a given species of conservation concern, the species with the greatest sensitivity to Bayluscide (i.e., lowest LC50) was selected as the surrogate. Exceptions to the hierarchal approach were used for Lake Sturgeon, Blackstripe Topminnow (*Fundulus notatus*), and Grass Pickerel (*Esox americanus vermiculatus*). The taxonomic match would have resulted in Rainbow Trout (*Oncorhynchus mykiss*) as a surrogate for Lake Sturgeon and Grass Pickerel, and White Sucker (*Catostomus commersonii*) as a surrogate for Blackstripe Topminnow. Given the different life history and habitat preferences between the surrogate and species of interest, an alternative approach was used where species with similar life history and habitat preferences were selected. Channel Catfish (*Ictalurus punctatus*) was selected as a surrogate for Lake Sturgeon given large body size and shared affinity for benthic habitat and Yellow Perch (*Perca flavescens*) was selected as a surrogate for Grass Pickerel given similar habitat preferences. Fathead Minnow (*Pimephales promelas*) was selected as a surrogate for Blackstripe Topminnow given similar body size and lifespan.

The difference in exposure duration between lamprey species (nine hours) and the other fish species (eight hours) is due to differences in toxicity data where lamprey species only had 9-h LC50 and 9-h LC99.9 values (the remaining fish species had LC50 values across multiple exposure durations, allowing for the development of a logarithmic relationship to estimate LC50 values across multiple exposure durations). Although the lamprey species LC50 values were based on a different exposure duration than the remaining species of conservation concern, this difference was expected to yield little difference in estimated mortality given the relatively similar LC50 values for the surrogate species across different durations (Table 3). Based on the two dose-response curves generated for each species and the two derived Bayluscide benchmark concentrations, each fish species had four different potential mortality rates, considered equally likely in this assessment. Mortality rates are displayed as the intersection between the dose-response curves and the grey and black vertical benchmark concentrations in Figure 4.

Mussel Species Sensitivity

Similar to fish species, toxicity was unavailable for most mussel species of conservation concern so surrogate species were used to estimate mortality. Surrogate species were selected using a hierarchal process based on shared genus, tribe, or if unavailable, family of the focal species. In scenarios where multiple species or life stages (i.e., juvenile and adult) met the surrogate criteria for a single species of conservation concern, the species and life stage with the greatest sensitivity to Bayluscide (i.e., highest mortality value) was selected as the surrogate.

Unlike the approach used for fishes, mussel mortality values were estimated directly from Newton et al. (2017) where the number of dead mussels were measured 21 days after an eight hour exposure to Bayluscide. As only a single benchmark concentration taken directly from Newton et al. (2017) was used in the mussel analysis, single mortality estimates were derived for each mussel species of conservation concern.

Table 3. Logarithmic line of best fit for species- and temperature-specific LC50 values across exposure durations. Estimated LC50 values for 1, 3, 6, 9, 12, 18, and 24 hour exposure durations based on the corresponding line of best fit.

Species Common Name	Species Scientific Name	Temp (°C)	Slope of Best Fit	Intercept of Best Fit	r^2	LC_{50} at 1 hr	LC_{50} at 3 hrs	LC_{50} at 6 hrs	LC_{50} at 9 hrs	LC_{50} at 12 hrs	LC_{50} at 18 hrs	LC_{50} at 24 hrs
Black Bullhead	*Ameiurus melas*	12	-0.037	0.235	0.860	0.23	0.19	0.17	0.15	0.14	0.13	0.12
Bigmouth Buffalo	*Ictiobus cyprinellus*	17	-0.023	0.153	1.000	0.15	0.13	0.11	0.10	0.10	0.09	0.08
Bluegill	*Lepomis macrochirus*	12	-	-	-	-	-	-	-	-	-	-
		17	-0.013	0.127	0.901	0.13	0.11	0.10	0.10	0.09	0.09	0.09
		22	-0.013	0.116	0.956	0.12	0.10	0.09	0.09	0.08	0.08	0.07
Brook Trout	*Salvelinus fontinalis*	12	-0.003	0.072	0.694	0.07	0.07	0.07	0.06	0.06	0.06	0.06
Brown Bullhead	*Ameiurus nebulosus*	17	-0.011	0.108	0.750	0.11	0.10	0.09	0.08	0.08	0.08	0.07
Channel Catfish	*Ictalurus punctatus*	12	-0.004	0.067	0.887	0.07	0.06	0.06	0.06	0.06	0.06	0.05
		17	-0.001	0.089	0.750	0.09	0.09	0.09	0.09	0.09	0.09	0.09
		22	-0.004	0.064	0.629	0.06	0.06	0.06	0.06	0.05	0.05	0.05
Common Carp	*Cyprinus carpio*	12	-0.033	0.266	0.856	0.27	0.23	0.21	0.19	0.18	0.17	0.16
		17	-0.014	0.291	1.000	0.29	0.28	0.27	0.26	0.26	0.25	0.25
Fathead Minnow	*Pimephales promelas*	17	-0.003	0.115	0.923	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Flathead Catfish	*Pylodictis olivaris*	17	-	-	-	-	-	-	-	-	-	-
Goldfish	*Carassius auratus*	12	-0.03	0.346	0.781	0.35	0.31	0.29	0.28	0.27	0.26	0.25
		17	-0.026	0.359	0.756	0.36	0.33	0.31	0.30	0.29	0.28	0.28
		22	-0.022	0.311	0.850	0.31	0.29	0.27	0.26	0.26	0.25	0.24
Green Sunfish	*Lepomis cyanellus*	17	-0.042	0.286	0.928	0.29	0.24	0.21	0.19	0.18	0.16	0.15
Largemouth Bass	*Micropterus salmoides*	17	-0.035	0.227	0.942	0.23	0.19	0.16	0.15	0.14	0.13	0.12
Rainbow Trout	*Oncorhynchus mykiss*	7	-	-	-	-	-	-	-	-	-	-
		12	-	-	-	-	-	-	-	-	-	-
		17	-0.001	0.055	0.889	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Redear Sunfish	*Lepomis microlophus*	17	-0.05	0.325	0.793	0.33	0.27	0.24	0.22	0.20	0.18	0.17
Smallmouth Bass	*Micropterus dolomieu*	17	-0.021	0.160	0.750	0.16	0.14	0.12	0.11	0.11	0.10	0.09
Tilapia	*Tilapia mossambica*	17	-0.051	0.345	0.992	0.34	0.29	0.25	0.23	0.22	0.20	0.18
White Sucker	*Catostomus commersonii*	12	-0.039	0.158	0.831	0.16	0.12	0.09	0.07	0.06	0.05	0.03

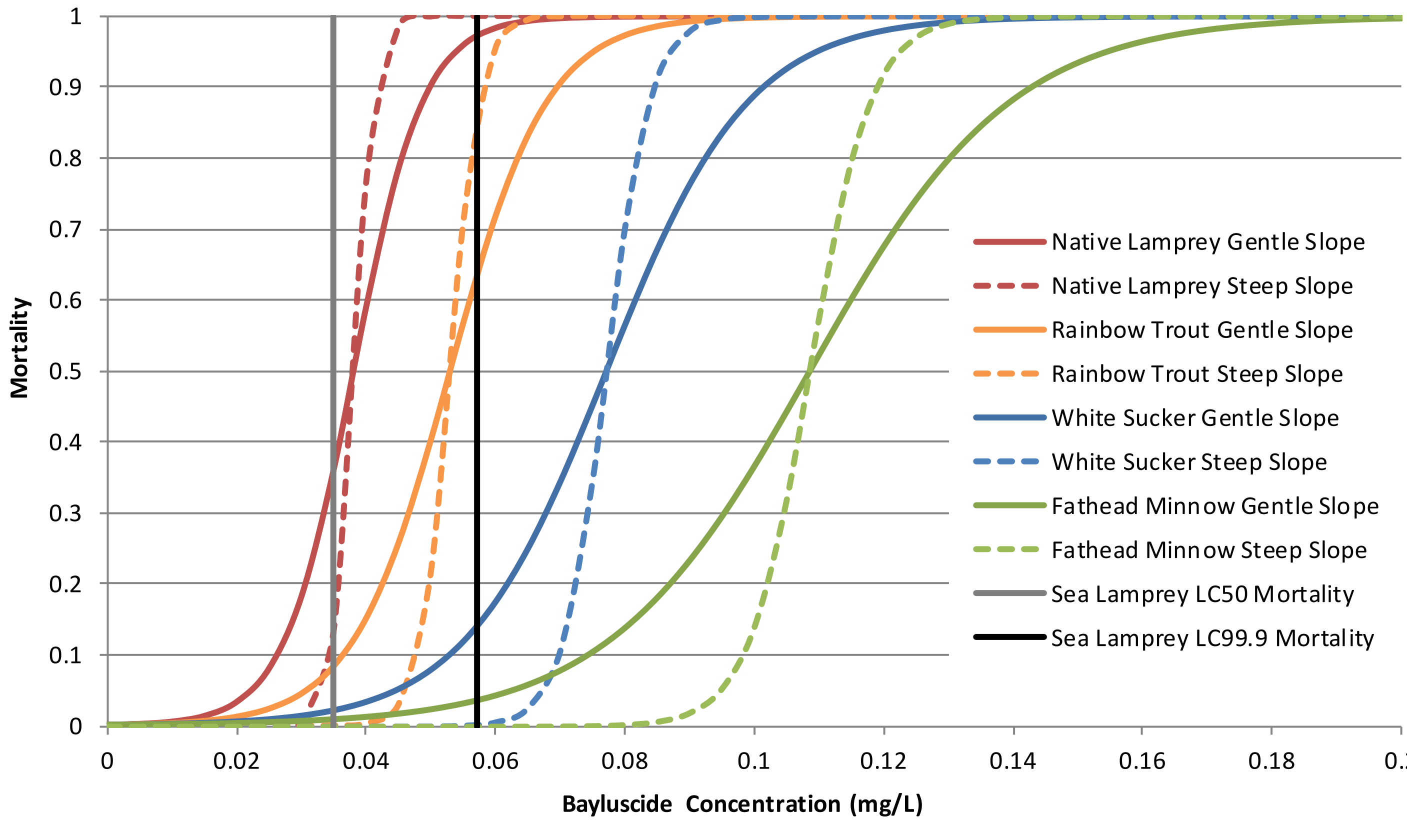

*Figure 4. Steep and gentle dose-response curves for native lampreys (*Ichthymyzon spp.*), Rainbow Trout, White Sucker, and Fathead Minnow in relation to Bayluscide benchmark concentrations (e.g., Sea Lamprey 9-hr LC99.9 and LC50 mortality). Non-lamprey fish relationships are based on an eight hour exposure time.*

ESTIMATING MORTALITY FROM BAYLUSCIDE APPLICATIONS

The number of individuals experiencing mortality from a single Bayluscide application cycle (i.e., six 500 m^2 application sites) was estimated based on the likelihood of a species of conservation concern occurring within a single application site, species density at a site, and estimated Bayluscide-induced mortality. These components were combined within a decision tree framework where the output of a single path through the tree represented the estimated mortality of a species of conservation concern in a focal river following the application of Bayluscide at a single 500 m^2 site (Figure 5). The total mortality of a species within a focal river due to a single Bayluscide application cycle was based on the sum of the results of six paths through the decision tree, representing applications at six sites.

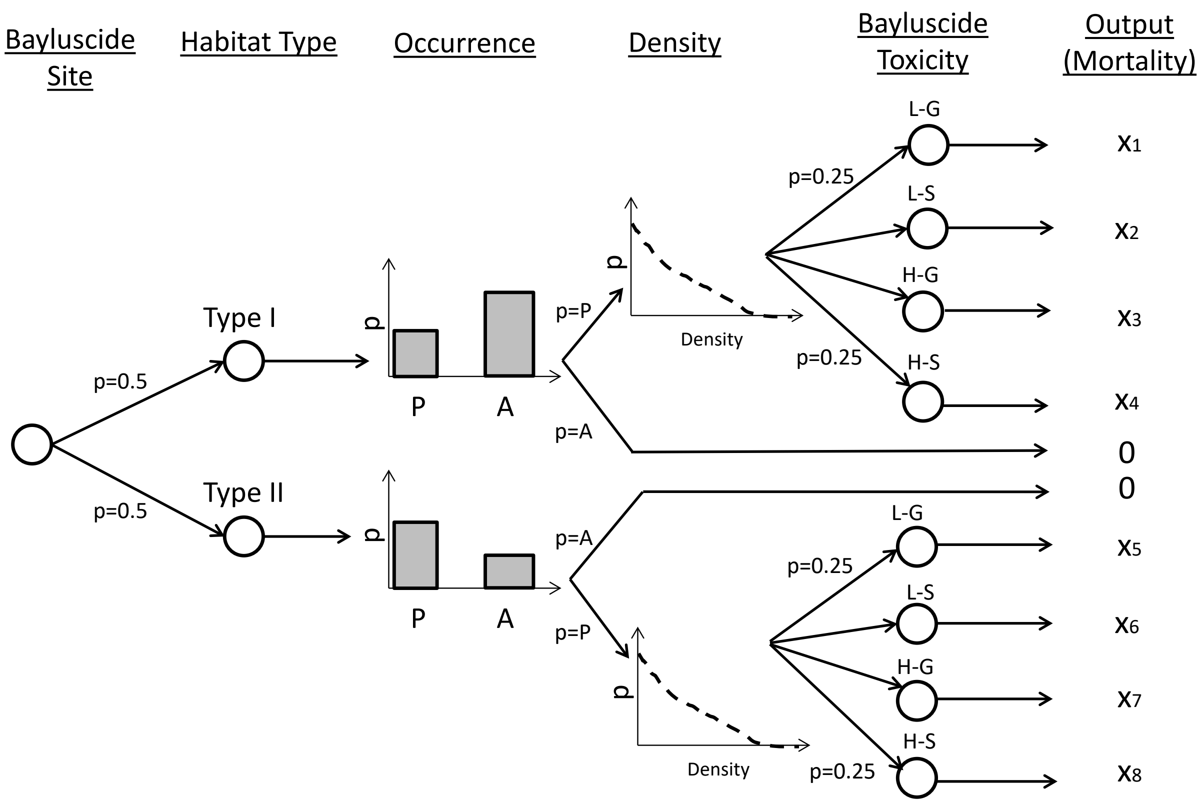

Figure 5. Decision tree used to calculate the mortality of fish species of conservation concern during Bayluscide applications. The diagram displays the outputs (X_n) of all potential pathways surrounding habitat type (Type I or Type II), the likelihood of occurrence (P – present or A – absent), density, and Bayluscide toxicity (L-G – low concentration, gentle slope; L-S – low concentration, steep slope; H-G – high concentration, gentle slope; H-S – high concentration, steep slope). The corresponding probabilities for each uncertainty state (p) provide information regarding the probability of each output occurring.

For each Bayluscide application site, an equal, 50%, chance of being homogenous Type I or Type II habitat was assumed to determine the corresponding likelihood of occurrence values for each fish and mussel species. Although Bayluscide application sites within focal rivers are typically composites of Type I and Type II habitat (Mike Steeves, SLCC, pers. comm.), the proportion of habitat that is Type I or Type II within each application site is typically unknown due to the imprecision of field methods so the model was simplified to consider either 100% Type I or 100% Type II habitat. Once the habitat class was randomly assigned to a site, the occurrence of each species was randomly determined based on the likelihood of occurrence value for the corresponding habitat class and species. If a species was absent for the site then the simulation was stopped and no mortality occurred. If a species was present within a site then the corresponding species density was calculated based on a random draw of the density distribution of the species in that system. Finally, the number of species-specific mortalities for the site was calculated by multiplying the species-specific Bayluscide toxicity by the number of individuals present within the site (in this final step, for fishes, the simulation was run for the two concentration benchmarks and the two dose-response curves). The simulation was repeated across the five remaining application sites using the same random draws to reflect a single Bayluscide application cycle. A total of 5,000 iterations (i.e., 5,000 Bayluscide application cycles) for each species and tributary were used to generate a distribution of species-specific mortality values which were plotted as probability distributions. Calculations were completed in R v.3.5.0. (R Core Team 2014).

This general simulation was utilized for all species. However, the number of parameter values and the approach for incorporating uncertainty varied across species. The mortality calculations for all fish species of conservation concern, except Lake Sturgeon and lampreys, utilized the standard simulation (Figure 5). Lampreys did not have habitat-specific likelihood of occurrence values so the probability of Type I or Type II habitat was not incorporated into the decision tree (Figure 6). Lake Sturgeon did not have habitat specific likelihood of occurrence values or a distribution of density values as likelihood of occurrence was based on the probability of yielding a density of 1 fish/100 m^2 (Figure 7). The decision tree was also adjusted for mussels as there was no variability in the estimated sensitivity to Bayluscide (Figure 8). Therefore, for mussel calculations, only a single value was used for Bayluscide sensitivity. In cases where species density data was unavailable (e.g., Lilliput [*Toxolasma parvum*]), mortality estimates were not generated.

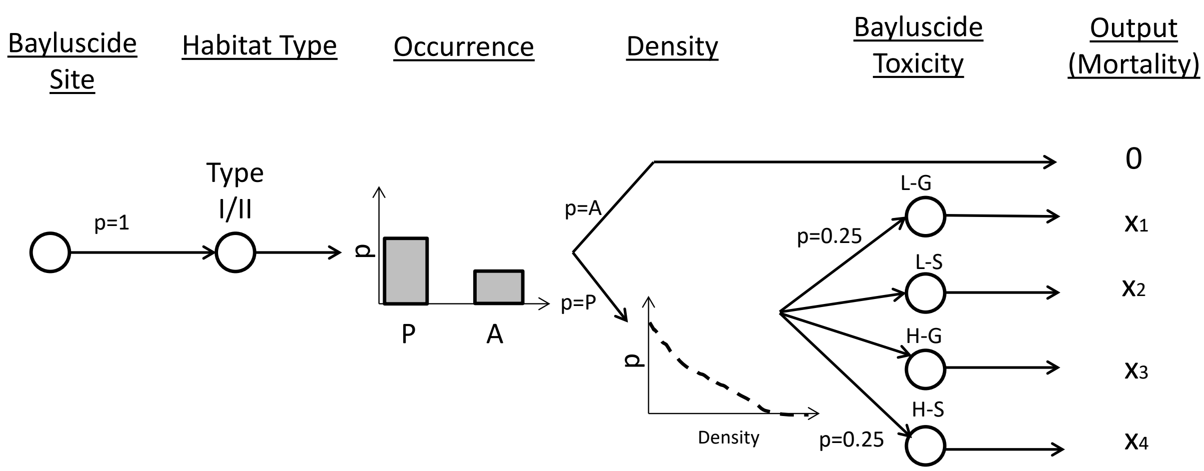

Figure 6. Decision tree used to calculate the mortality of Ichthyomyzon *species during Bayluscide applications. The diagram displays the outputs (X_n) of all potential pathways surrounding the likelihood of occurrence (P – present or A – absent), density, and Bayluscide toxicity (L-G – low concentration, gentle slope; L-S – low concentration, steep slope; H-G – high concentration, gentle slope; H-S – high concentration, steep slope). Unlike Figure 5, variability in habitat type was not considered and a single habitat value was considered (Type I/II). The corresponding probabilities for each uncertainty state (p) provide information regarding the probability of each output occurring.*

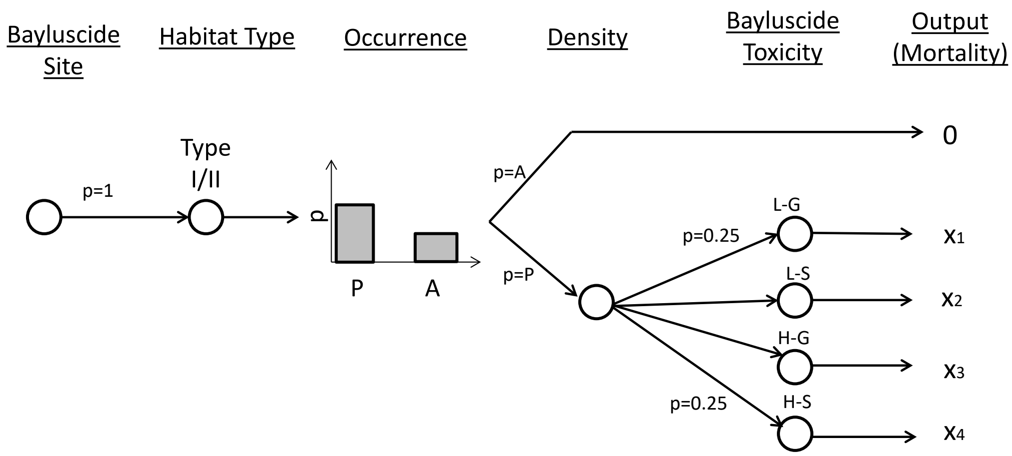

Figure 7. Decision tree used to calculate the mortality of Lake Sturgeon during Bayluscide applications. The diagram displays the outputs (X_n) of all potential pathways surrounding the likelihood of occurrence (P – present or A – absent), density, and Bayluscide toxicity (L-G – low concentration, gentle slope; L-S – low concentration, steep slope; H-G – high concentration, gentle slope; H-S – high concentration, steep slope). Unlike Figure 5, variability in habitat type or density was not considered and a single value was considered for each uncertainty. The corresponding probabilities for each uncertainty state (p) provide information regarding the probability of each output occurring.

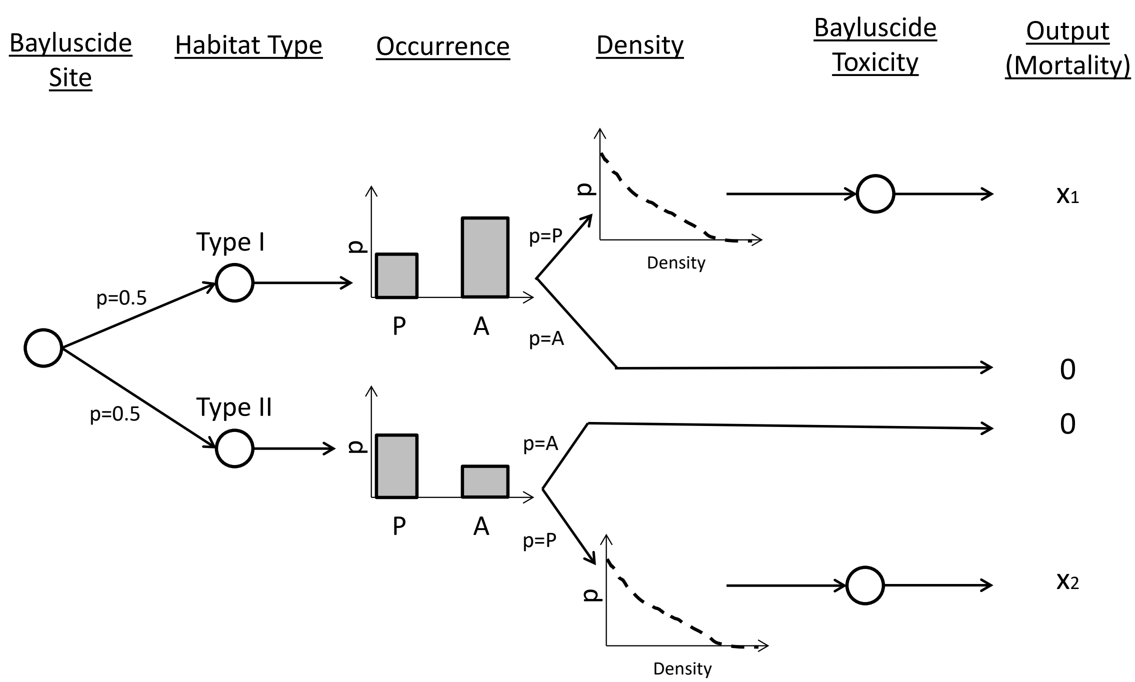

Figure 8. Decision tree used to calculate the mortality of mussel species of conservation concern during Bayluscide applications. The diagram displays the outputs (X_n) of all potential pathways surrounding habitat type (Type I or Type II), the likelihood of occurrence (P – present or A – absent), density, and Bayluscide toxicity. Unlike Figure 5, variability in Bayluscide toxicity was not considered and a single value was considered. The corresponding probabilities for each uncertainty state (p) provide information regarding the probability of each output occurring.

SENSITIVITY ANALYSES

To understand how changes to the Bayluscide application cycle would lead to different mortality estimates, the number and size of Bayluscide applications were modified relative to the standard application cycle (i.e., six 500 m^2 application sites) during sensitivity analysis. In addition, an alternative approach to estimate the density of species was examined to determine if underestimates of species density, as may be expected with imperfectly-detected field collection records, had bearing on the results. These methods are elaborated on below.

Adjustments to Bayluscide Application Cycle

Typically, a Bayluscide application cycle involves six 500 m^2 sites within a tributary during an assessment year (Mike Steeves, SLCC, pers. comm.). The sensitivity of fish and mussel mortality estimates to the number and size of sites used in a given application cycle was examined. Alternative numbers of application sites, ranging from three 500 m^2 sites/cycle to ten 500 m^2 sites/cycle, as well as the various site sizes ranging from six 250 m^2 sites/cycle to six 5,000 m^2 sites/cycle were considered. Reductions from baseline were selected to evaluate the effect of measures that could be pursued to mitigate the mortality of fish and mussel species of conservation concern. Alternatively, increases from baseline were incorporated to understand potential changes in mortality associated with multi-hectare control operations.

Area-per-Individual Calculations

Field collection records are inherently biased by imperfect detection and the inefficiency of capture gear leading to density estimates that likely underestimate true fish and mussel density in the wild. Therefore, the sensitivity of mortality estimates to assumed field densities was explored by replacing the density estimates with those generated using the Area-per-Individual (API) framework developed by Minns (2003). The API value describes the average habitat area (m^2) required by an individual fish of given total length. Using the maximum length (cm) of each of species in this study based on data from Coker et al. (2001), the area needed for each species was estimated and converted to density of fish per 100 m^2 (Table 4). Empirical density estimates for all fish species excluding *Ichthyomyzon* spp. and Lake Sturgeon were replaced with API values and mortality from Bayluscide applications was recalculated. The API approach was not used for *Ichthyomyzon* spp. or mussels as the API is suitable only for free swimming fishes. In addition, the API approach was not completed for Lake Sturgeon as the likelihood of occurrence was based on density estimates as opposed to field-based measures.

Table 4. Density estimates (fish/100 m²) for each focal fish species of conservation concern based the maximum species total length from Coker et al. (2001) and the corresponding Area Per Individual (API) estimate from Minns (2003). Density estimates could not be calculated for Lake Sturgeon or lamprey species.

Species Common Name	Species Scientific Name	Maximum Total Length (mm)	Density (fish/100 m²)
Black Redhorse	*Moxostoma duquesnei*	658	0.17
Blackstripe Topminnow	*Fundulus notatus*	97	23.87
Channel Darter	*Percina copelandi*	61	78.97
Eastern Sand Darter	*Ammocrypta pellucida*	81	38.00
Grass Pickerel	*Esox americanus vermiculatus*	328	1.03
Lake Chubsucker	*Erimyzon sucetta*	292	1.39
Lake Sturgeon[1]	*Acipenser fulvescens*	-	-
Northern Brook Lamprey[1]	*Ichthyomyzon fossor*	-	-
Northern Madtom	*Noturus stigmosus*	130	11.21
Northern Sunfish[2]	*Lepomis peltastes*	250	2.07
Pugnose Minnow	*Opsopoeodus emiliae*	64	69.77
Pugnose Shiner	*Notropis anogenus*	60	82.41
River Darter	*Percina shumardi*	80	39.23
River Redhorse	*Moxostoma carinatum*	617	0.20
Silver Chub	*Macrhybopsis storeriana*	231	2.54
Silver Lamprey[1]	*Ichthyomyzon unicuspis*	-	-
Silver Shiner	*Notropis photogenis*	130	11.21
Spotted Sucker	*Minytrema melanops*	449	0.46
Unidentified Northern Brook or Silver Lamprey ammocoetes[1]	*Ichthyomyzon* sp.	-	-

[1] API calculations were not calculated for these species.

[2] Species data unavailable. Bluegill data used as a surrogate.

CALCULATING POPULATION-LEVEL EFFECTS FROM BAYLUSCIDE

A primary goal of this research document was to understand how Bayluscide-induced mortality of individual fish may lead to population-level effects. Population-level effects were assessed for select species including Eastern Sand Darter in the Thames River, Northern Madtom in the Thames and Detroit rivers, Channel Darter in the Detroit River, and *Ichthyomyzon* spp. in the Thames and St. Clair rivers. Population-level effects were only assessed for fishes as population models for mussels are limited in scope.

To convert the species- and tributary-specific mortality estimates from a Bayluscide application cycle (i.e., a site-specific mortality rate given *n* fishes present and *n* fishes killed) to population-level mortality rates, the population abundance of each focal species (i.e., total population size in a given focal river) was calculated using the species' system-specific density and either the area of recognized or proposed critical habitat if available for a SARA Threatened or Endangered species or the area bounding the species' recorded distribution within a study system if critical habitat had not been defined. Since the suitability of the critical habitat polygon or bound area is unknown for most species (i.e., not all areas within the habitat polygon may support individuals of the species), a habitat correction factor was incorporated that estimated the resulting population size and concordant effect of Bayluscide applications if 0.01% (very little suitable habitat) to 100% (maximum suitable habitat) of the habitat polygon supported the species. The formulas used to calculate population level mortality rates are outlined below:

$$M = \frac{m}{A_x} \tag{3}$$

$$A_x = D_x \times (CH \times Hab) \tag{4}$$

where M is the mortality rate from a Bayluscide application cycle, m is the number of mortalities caused by a Bayluscide application cycle (i.e., six 500 m^2 sites), A is the total abundance of species *x*, D_x is the density of species *x*, CH is the area of critical habitat or bounded distribution in m^2, and Hab is the habitat correction factor (habitat occupancy) ranging from 0.0001 to 1.0.

Stage- or age-structured models (Figure 9) were used to estimate population responses by adjusting baseline population mortality rates by the additional estimated mortality imposed by Bayluscide applications. Stage-structured models were obtained from the literature, if available, and YOY survival was adjusted to allow for a population growth rate of $\lambda = 1.0$. By fixing the growth rate at 1.0, the population abundance was not allowed to recover following Bayluscide applications, thereby identifying the most extreme potential effect of Bayluscide on population abundance. To simulate the effect of Bayluscide applications, every year that an application occurred, the survival rates for each life stage were reduced to account for the increased mortality imposed by the application:

$$SB_{ix} = S_{ix} \times (1 - M_x) \tag{5}$$

where SB_{ix} is the stage-specific survival rate following Bayluscide application for stage *i* and species *x*, where S_{ix} is the natural stage specific survival for stage *i* and species *x* and M_x is the mortality rate from Bayluscide for species *x*.

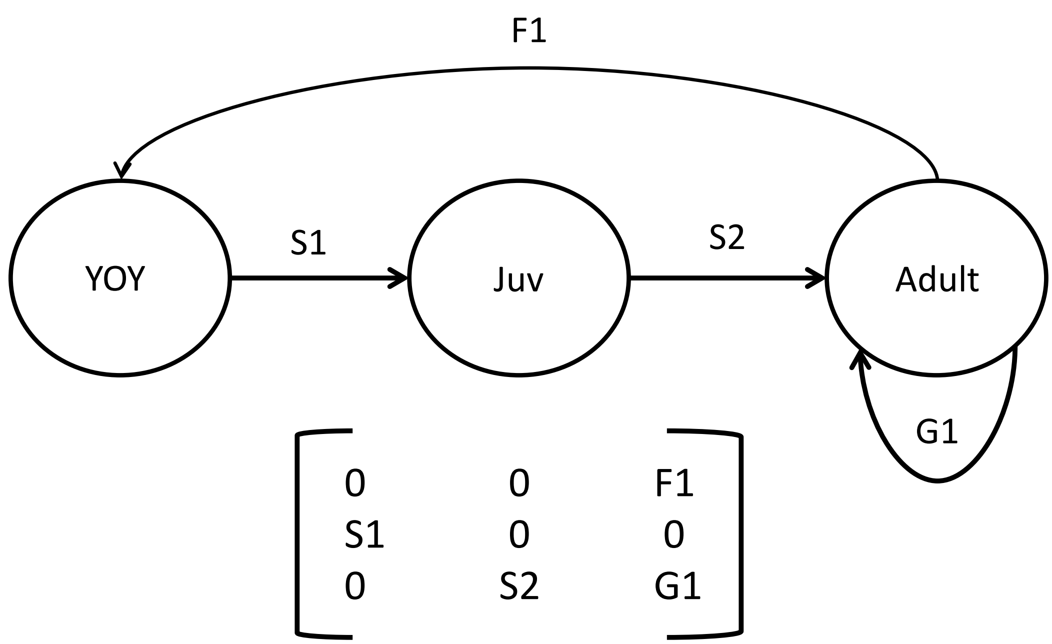

Figure 9. Example of a three-stage life history model (young-of-the-year [YOY], juvenile [Juv], and adult). The model is based on three parameters: surviving a given year and progressing to the next life stage (S), surviving in a given year and remaining in the current life stage (G), and the fecundity of the life stage (F). The corresponding matrix structure is also provided.

Simulations were conducted to account for the high variability of estimated mortality values for each species. For each year that a Bayluscide application occurred, a mortality value was generated based on random selection of the Bayluscide-induced mortality rates for a species within the appropriate system. Each population iteration was run for 100 years to describe the effect of Bayluscide applications following 50 and 100 years of an ongoing application cycle. The influence of Bayluscide application frequency on population abundance were also examined. For this simulation, the application cycle was adjusted to range from a Bayluscide application occurring every year (i.e., cycle of one year) to once every ten years (i.e., cycle of ten years). In total, 250 different simulations were run that encompassed different habitat correction factor values and Bayluscide application frequencies. For each simulation, a total of 5,000 iterations were completed in R v. 3.5.0 (R Core Team 2014) and the mean output value was used for comparisons among species and parameter sets.

Species-specific information used to derive population mortality estimates for the four focal species (Eastern Sand Darter, Northern Madtom, Channel Darter, and *Ichthyomyzon* spp.) is outlined below.

Eastern Sand Darter

The population estimate of Eastern Sand Darter in the Thames River was based on multiple density estimates and bounding of the species' distribution within the Thames River. Finch et al. (2018) estimated that Eastern Sand Darter density was 3,602 fish/10,000 m^2 which was substantially greater than the densities generated in this analysis (2.28 fish/100 m^2 or 228 fish/10,000 m^2). Given the discrepancy between these values, both were retained to generate separate population abundance estimates for comparison. To measure the bound range in the Thames River, the river distance between the downstream- and upstream-most observations of Eastern Sand Darter was measured in Google Earth® and the average stream width was calculated based on 20 random stream width measurements.

The model for Eastern Sand Darter was based on an age-structured model from Finch et al. (2018). This model incorporated four ages of Eastern Sand Darter (age 0, 1, 2, and 3), each with corresponding survival and fecundity values.

Northern Madtom

The population estimate of Northern Madtom in the Detroit River and Thames River was based on tributary-specific densities (0.57 fish/100 m^2 and 0.60 fish/100 m^2; respectively) and defined critical habitat areas. The critical habitat areas were based on those used in Andrews et al. (2021) to ensure comparability between studies.

The model used for both river systems was based on a stage-structured model from Vélez-Espino et al. (2009). This model had two stages (YOY and adult); each with their own corresponding survival and fecundity values. For this model, the YOY survival rate was modified to reflect the YOY survival needed to maintain $\lambda = 1.0$.

Channel Darter

The population estimate for Channel Darter in the Detroit River was based on the Detroit-specific density estimate (0.5 fish/100 m^2). The potential range of the species was estimated by bounding Channel Darter detections within the Detroit River resulting in four spatially distinct polygons.

The model used for the Detroit River was based on an age-structured model from Venturelli et al. (2010). This model had seven specified ages (age 0, 1+, 2+, 3+, 4+, 5+, and 6+); each with their own corresponding survival and fecundity values. For this model, the YOY survival rate was modified to reflect the YOY survival needed to maintain λ = 1.0.

Ichthyomyzon spp.

Given the presence of definitively identified Northern Brook Lamprey and Silver Lamprey as well as *Ichthyomyzon* records that could not be definitively identified in the St. Clair River and Thames River, the potential population-level consequences of Bayluscide applications to Northern Brook Lamprey and Silver Lamprey were examined while including and excluding unidentified *Ichthyomyzon* ammocoetes. The population estimates for each of the four species scenarios (i.e., Northern Brook Lamprey, Northern Brook Lamprey and unidentified *Ichthyomyzon*, Silver Lamprey, Silver Lamprey and unidentified *Ichthyomyzon*) for each river were based on the tributary-specific density estimates and the bounded area of *Ichthyomyzon* spp. detections within both rivers. For these models, it was assumed that only ammocoetes would be affected by Bayluscide applications given that these are the targeted Sea Lamprey life stage.

The Northern Brook Lamprey model used for both river systems was based on the Northern Brook Lamprey model developed in Smyth (2011). The model was a stage structured model (YOY, ammocoete, and adult stages). Based on the available data in Smyth (2011), it was assumed that ammocoete duration was 7 years based on the maximum estimate. The survival rate for ammocoetes was based on the mean of the range of survival estimates presented in Smyth (2011). Since an estimate of adult survival was not available, it was assumed that adult survival was equal to ammocoete annual survival. For this model, the YOY survival rate was modified to reflect the YOY survival needed to maintain λ = 1.0.

The Silver Lamprey model was based on the Northern Brook Lamprey model described above with two minor adjustments. First, a parasitic stage survival rate of 0.65 was used based on the survival rate of Sea Lamprey during the parasite stage (Bergstedt et al. 2003). Second, the fecundity estimate was adjusted to 19,012 eggs, which was an average fecundity estimate from Silver Lamprey in Quebec and is within the range of average Silver Lamprey fecundities from Lake Michigan tributaries (COSEWIC 2011). The Northern Brook Lamprey model was adopted for Silver Lamprey because of the similarity between these two species. Recent genetic research has suggested that these two species may represent two ecotypes rather than distinct species (Docker et al. 2012, Ren et al. 2014). As with the Northern Brook Lamprey model, the YOY survival rate was modified to reflect the YOY survival needed to maintain λ = 1.0.

RESULTS

SPECIES EXPOSURE TO BAYLUSCIDE

The likelihood of occurrence of fishes varied across Sea Lamprey habitat classes (Table 5). Species with the greatest likelihood of occurrence in Type I or Type II habitat were Spotted Sucker (*Minytrema melanops*) (p = 0.402; Type I habitat), Eastern Sand Darter (p = 0.308; Type II habitat), and Blackstripe Topminnow (p = 0.288; Type II habitat). However, almost all fish species of conservation concern had non-zero probabilities of occurring within Type I or Type II habitat with the exception of Black Redhorse (*Moxostoma duquesnei*) and River Redhorse (*Moxostoma carinatum*). These results indicate that the majority of species of conservation concern in this analysis may be susceptible to Bayluscide exposure during application based on habitat factors alone. The likelihood of occurrence values for Lake Sturgeon and the lamprey species varied little between systems except for the St. Clair and Thames rivers where Northern Brook and Silver Lamprey were combined with unidentified *Ichthyomyzon* (Table 6). Although the likelihood of occurrence for these species was not split across habitat types, the overall values were substantially lower than many of the fish species except for unidentified *Ichthyomyzon*. Lake Sturgeon likelihood of occurrence values were based on system-wide density estimates as opposed to empirical data, resulting in substantially lower likelihood of occurrence values.

The estimated density of species also varied (Tables 7 and 8) with mean density values ranging from 0.08 fish/100 m^2 (River Redhorse) to 76.7 fish/100 m^2 (Northern Brook Lamprey + unidentified *Ichthyomyzon*). Species with high occurrence values such as Blackstripe Topminnow and Eastern Sand Darter had relatively high densities while River Darter (*Percina shumardi*), which had a low occurrence value, also had a relatively low density value. Beyond these examples, there was no general relationship between density and the likelihood of occurrence.

Table 5. Likelihood of occurrence of fish species of conservation concern within Sea Lamprey habitat classes.

Species Common Name	Species Scientific Name	Type I Habitat Likelihood of Occurrence	Type I n	Type II Habitat Likelihood of Occurrence	Type II n
Black Redhorse	*Moxostoma duquesnei*	0.000	75	0.000	205
Blackstripe Topminnow	*Fundulus notatus*	0.206	126	0.288	111
Channel Darter	*Percina copelandi*	0.086	152	0.192	151
Eastern Sand Darter	*Ammocrypta pellucida*	0.141	199	0.308	331
Grass Pickerel	*Esox americanus vermiculatus*	0.100	201	0.003	316
Lake Chubsucker	*Erimyzon sucetta*	0.065	77	0.029	68
Northern Madtom	*Noturus stigmosus*	0.026	227	0.101	356
Northern Sunfish	*Lepomis peltastes*	0.081	124	0.079	126
Pugnose Minnow	*Opsopoeodus emiliae*	0.100	201	0.041	194
Pugnose Shiner	*Notropis anogenus*	0.164	201	0.088	194
River Darter	*Percina shumardi*	0.000	124	0.008	248
River Redhorse	*Moxostoma carinatum*	0.000	75	0.000	205
Silver Chub	*Macrhybopsis storeriana*	0.000	75	0.005	205
Silver Shiner	*Notropis photogenis*	0.000	75	0.141	205
Spotted Sucker	*Minytrema melanops*	0.402	276	0.198	399

Table 6. Likelihood of occurrence of Lake Sturgeon and native lamprey species based on available data for each study system. Although Silver Lamprey have been observed in all four focal rivers, there were no instances within the SLCC database where Silver Lamprey were captured in the Detroit or Sydenham River so Equation 2 was used to estimate likelihood of occurrence.

Species Common Name	Species Scientific Name	Detroit River Type I/II Habitat	Detroit River n	St. Clair River Type I/II Habitat	St. Clair River n	Sydenham River Type I/II Habitat	Sydenham River n	Thames River Type I/II Habitat	Thames River n
Lake Sturgeon[1]	*Acipenser fulvescens*	0.00001	-	0.00003	-	-	-	-	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	-	0.002	512	-	-	0.015	66
Northern Brook Lamprey + unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-	-	0.252	129	-	-	0.167	11
Silver Lamprey	*Ichthyomyzon unicuspis*	0.01	77	0.01	511	0.05	19	0.02	66
Silver Lamprey + unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	0.01	77	0.26	511	0.05	19	0.17	66
Unidentified *Ichthyomyzon*	*Ichthyomyzon* sp.	-	-	0.25	129	-	-	0.17	11

[1] Lake Sturgeon likelihood of occurrence was based on a single system-wide density and therefore no *n* values are provided.

Table 7. Mean and standard deviation of the density (# of fish/100 m²) of fish species of conservation concern.

Species Common Name	Species Scientific Name	Detroit River Mean	Detroit River St. Dev.	St. Clair River Mean	St. Clair River St. Dev.	Sydenham River Mean	Sydenham River St. Dev.	Thames River Mean	Thames River St. Dev.
Black Redhorse	*Moxostoma duquesnei*	-	-	-	-	-	-	0.14[2]	0.21
Blackstripe Topminnow	*Fundulus notatus*	-	-	1.67	0.59	3.87	6.73	-	-
Channel Darter	*Percina copelandi*	0.50[4]	0.24	0.50[3]	0.24	0.50[4]	0.24	-	-
Eastern Sand Darter	*Ammocrypta pellucida*	2.26[1]	3.34	-	-	2.26[1]	3.34	2.28	3.35
Grass Pickerel	*Esox americanus vermiculatus*	1.99[1]	1.86	1.99	1.86	1.99[1]	1.86	1.99[1]	1.86
Lake Chubsucker	*Erimyzon sucetta*	-	-	8.52[2]	17.72	-	-	-	-
Northern Madtom	*Noturus stigmosus*	0.57[1]	0.30	0.51	0.29	-	-	0.60	0.29
Northern Sunfish	*Lepomis peltastes*	0.25[1]	0.29	0.25[1]	0.29	0.22	0.27	0.25[1]	0.29
Pugnose Minnow	*Opsopoeodus emiliae*	0.97	2.29	0.97[1]	2.29	0.97[1]	2.29	-	-
Pugnose Shiner	*Notropis anogenus*	2.84[1]	4.68	2.82	4.87	2.84[1]	4.68	-	-
River Darter	*Percina shumardi*	-	-	-	-	0.48[1]	0.15	0.43	0.12
River Redhorse	*Moxostoma carinatum*	-	-	0.08	0.02	-	-	0.08[1]	0.02
Silver Chub	*Macrhybopsis storeriana*	-	-	-	-	-	-	0.50[3]	-
Silver Shiner	*Notropis photogenis*	-	-	-	-	-	-	3.00	2.14
Spotted Sucker	*Minytrema melanops*	0.10	0.19	0.18	0.26	0.15	0.23	0.08	0.05

Species Common Name	Species Scientific Name	Detroit River Mean	Detroit River St. Dev.	St. Clair River Mean	St. Clair River St. Dev.	Sydenham River Mean	Sydenham River St. Dev.	Thames River Mean	Thames River St. Dev.
Silver Shiner	*Notropis photogenis*	-	-	-	-	-	-	3.00	2.14
Spotted Sucker	*Minytrema melanops*	0.10	0.19	0.18	0.26	0.15	0.23	0.08	0.05

[1] Mean and standard deviation calculated from grouped data from the Detroit, St. Clair, Sydenham, and Thames rivers.

[2] Mean and standard deviation calculated from grouped data from tributaries where species is present.

[3] Mean and standard deviation manually calculated from river-specific data.

[4] Mean and standard deviation manually calculated from grouped data from the Detroit, St. Clair, Sydenham, and Thames rivers.

Table 8. Mean and standard deviation of the density (# of fish/100 m^2) of Lake Sturgeon and native lamprey species.

Species Common Name	Species Scientific Name	Detroit River Mean	Detroit River St. Dev.	St. Clair River Mean	St. Clair River St. Dev.	Sydenham River Mean	Sydenham River St. Dev.	Thames River Mean	Thames River St. Dev.
Lake Sturgeon[1]	*Acipenser fulvescens*	1.0	-	1.0	-	-	-	-	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	-	13.9[2]	-[3]	-	-	13.9	-[3]
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-	-	0.7	1.7	-	-	76.7	125.6
Silver Lamprey	*Ichthyomyzon unicuspis*	0.2[2]	0.0	0.2	0.0	0.2[2]	0.0	0.2[2]	0.0
Silver Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	6.5[2]	39.8	0.7	1.7	6.5[2]	39.8	75.5	122.8
Unidentified *Ichthyomyzon*	*Ichthyomyzon* sp.	-	-	0.7	1.7	-	-	75.5	122.8

[1] Lake Sturgeon densities were based on 1 fish/100 m^2 as explained in the Lake Sturgeon exposure section of the methods.

[2] Mean and standard deviation based on grouped densities from all focal rivers.

[3] Only a single density value was available so no standard deviation is provided.

The likelihood of occurrence for mussel species (Table 9) was generally lower than for fishes. However, the estimated density of mussel species (Table 10) was substantially greater than for fishes. All mussel species of conservation concern had non-zero values of occurring within Type I or Type II habitat. Likelihood of occurrence was generally higher in Type II than Type I habitat and, in some cases, moderately high values were found for certain species such as Kidneyshell (*Ptychobranchus fasciolaris*) (p = 0.189, Type II habitat), Northern Riffleshell (*Epioblasma rangiana*) (p = 0.165, Type II habitat), and Mapleleaf (p = 0.141, Type II habitat). Density values varied greatly between systems and ranged from 100.0 mussels/100 m^2 (e.g., Round Hickorynut [*Obovaria subrotunda*] in the Sydenham River) to 283.6 mussels/100 m^2 (e.g., Mapleleaf in the Thames River). Similar to the results for fishes, there was no discernable pattern between the likelihood of occurrence and density for mussels.

Table 9. Likelihood of occurrence of mussel species of conservation concern based on rivers where the focal species was detected using quadrat and timed search data.

Species Common Name	Species Scientific Name	Type I Habitat Likelihood of Occurrence	Type I Habitat n	Type II Habitat Likelihood of Occurrence	Type II Habitat n
Fawnsfoot	*Truncilla donaciformis*	0.000	208	0.029	2,509
Kidneyshell[1]	*Ptychobranchus fasciolaris*	0.026	195	0.189	1,416
Lilliput	*Toxolasma parvum*	0.000	195	0.002	1,416
Mapleleaf	*Quadrula quadrula*	0.077	208	0.141	2,509
Northern Riffleshell	*Epioblasma rangiana*	0.026	195	0.165	1,416
Rainbow	*Villosa iris*	0.005	208	0.035	2,509
Rayed Bean	*Villosa fabalis*	0.019	208	0.130	2,509
Round Hickorynut	*Obovaria subrotunda*	0.000	195	0.001	1,416
Round Pigtoe	*Pleurobema sintoxia*	0.014	208	0.039	2,509
Salamander Mussel	*Simpsonaias ambigua*	0.000	195	0.006	1,416
Snuffbox	*Epioblasma triquetra*	0.005	195	0.107	1,416
Threehorn Wartyback	*Obliquaria reflexa*	0.000	208	0.007	2,509
Wavyrayed Lampmussel[2]	*Lampsilis fasciola*	0.000	13	0.126	1,093

[1] Only data from the Sydenham River were used to estimate likelihood of occurrence even though Kidneyshell was observed in both systems.

[2] Only data from the Thames River were used to estimate likelihood of occurrence even though Wavyrayed Lampmussel was observed in both systems.

Table 10. Mean and standard deviation of the density (# of mussels/100 m^2) of mussel species of conservation concern present in the Sydenham or Thames rivers using quadrat data.

Species Common Name	Species Scientific Name	Presence in Sydenham River	Sydenham River Mean Density	Sydenham River St. Dev.	Presence in Thames River	Thames River Mean Density	Thames River St. Dev.
Fawnsfoot	*Truncilla donaciformis*	Y[1]	100.0	0.0	Y	192.5	145.3
Kidneyshell	*Ptychobranchus fasciolaris*	Y	161.2	105.4	Y[2]	-	-
Lilliput	*Toxolasma parvum*	Y[2]	-	-	N	0.0	0.0
Mapleleaf	*Quadrula quadrula*	Y	149.4	85.9	Y	283.6	256.8
Northern Riffleshell	*Epioblasma rangiana*	Y	189.7	141.5	N	0.0	0.0
Rainbow	*Villosa iris*	Y	100.0	0.0	Y	297.9	288.4
Rayed Bean	*Villosa fabalis*	Y	217.0	195.8	Y	170.0	110.0
Round Hickorynut[1]	*Obovaria subrotunda*	Y[1]	100.0	-	N	0.0	0.0
Round Pigtoe	*Pleurobema sintoxia*	Y	112.5	41.5	Y	127.6	63.8
Salamander Mussel	*Simpsonaias ambigua*	Y	107.1	25.8	N	0.0	0.0
Snuffbox	*Epioblasma triquetra*	Y	150.3	93.0	N	0.0	0.0
Threehorn Wartyback	*Obliquaria reflexa*	Y[1]	100.0	0.0	Y[1]	100.0	0.0
Wavyrayed Lampmussel	*Lampsilis fasciola*	Y[2]	-	-	Y	132.4	66.0

[1] Mean and standard deviation calculated manually.

[2] Individuals were not captured during quadrat sampling, so densities could not be estimated.

SPECIES-SPECIFIC BAYLUSCIDE TOXICITY

Surrogate values of Bayluscide toxicity varied across fishes (Table 11) and mussels (Table 12). However, toxicity to fish species exhibited greater variability than toxicity to mussel species likely due to the greater variety of surrogate species used to estimate toxicity. Overall, Bayluscide toxicity was greatest for *Ichthyomyzon* spp. followed by species with Channel Catfish assigned as a surrogate species. Toxicity was greatest for the mussel species Kidneyshell and Wavyrayed Lampmussel (*Lampsilis fasciola*) and species that used those species as a surrogate for toxicity estimates. Across all species, Bayluscide was the least toxic to *Percidae* and *Lepomis* species.

Table 11. Estimated mortality rates for fish species of conservation concern based on surrogate species responses to high (0.057 mg/L) and low (0.035 mg/L) Bayluscide concentrations and the gentle and steep dose-response curves following an exposure duration of eight hours for all species except for lamprey, which were based on an exposure duration of nine hours.

Species Common Name	Species Scientific Name	Surrogate Species	Mortality Rates from High Concentrations with Gentle Slope	Mortality Rates from High Concentrations with Steep Slope	Mortality Rates from Low Concentrations with Gentle Slope	Mortality Rates from High Concentrations with Steep Slope
Black Redhorse	*Moxostoma duquesnei*	White Sucker	0.139	0.002	0.021	0.000
Blackstripe Topminnow	*Fundulus notatus*	Fathead Minnow	0.035	0.000	0.009	0.000
Channel Darter	*Percina copelandi*	Yellow Perch	0.046	0.000	0.010	0.000
Eastern Sand Darter	*Ammocrypta pellucida*	Yellow Perch	0.046	0.000	0.010	0.000
Grass Pickerel	*Esox americanus vermiculatus*	Yellow Perch	0.046	0.000	0.010	0.000
Lake Chubsucker	*Erimyzon sucetta*	White Sucker	0.139	0.002	0.021	0.000
Lake Sturgeon	*Acipenser fulvescens*	Channel Catfish	0.532	0.603	0.067	0.000
Northern Brook Lamprey	*Ichthyomyzon fossor*	*Ichthyomyzon* spp.	0.972	1.000	0.364	0.140
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	*Ichthyomyzon* spp.	0.972	1.000	0.364	0.140
Northern Madtom	*Noturus stigmosus*	Channel Catfish	0.532	0.603	0.067	0.000
Northern Sunfish	*Lepomis peltastes*	Bluegill	0.076	0.000	0.014	0.000
Pugnose Minnow	*Opsopoeodus emiliae*	Fathead Minnow	0.035	0.000	0.009	0.000
Pugnose Shiner	*Notropis anogenus*	Fathead Minnow	0.035	0.000	0.009	0.000
River Darter	*Percina shumardi*	Yellow Perch	0.046	0.000	0.010	0.000
River Redhorse	*Moxostoma carinatum*	White Sucker	0.139	0.002	0.021	0.000
Silver Chub	*Macrhybopsis storeriana*	Fathead Minnow	0.035	0.000	0.009	0.000
Silver Lamprey	*Ichthyomyzon unicuspis*	*Ichthyomyzon* spp.	0.972	1.000	0.364	0.140
Silver Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	*Ichthyomyzon* spp.	0.972	1.000	0.364	0.140
Silver Shiner	*Notropis photogenis*	Fathead Minnow	0.035	0.000	0.009	0.000
Spotted Sucker	*Minytrema melanops*	White Sucker	0.139	0.002	0.021	0.000
Unidentified *Ichthyomyzon*	*Ichthyomyzon sp.*	*Ichthyomyzon* spp.	0.972	1.000	0.364	0.140

Table 12. Estimated mortality rates for mussel species of conservation concern based on surrogate species responses from Newton et al. (2017). Surrogate mortality was based on an exposure duration of eight hours.

Species Common Name	Species Scientific Name	Species Used to Estimate Mortality	Mortality Rate from Target gB Concentrations
Fawnsfoot	*Truncilla donaciformis*	Kidneyshell	0.543
Kidneyshell	*Ptychobranchus fasciolaris*	Kidneyshell	0.543
Lilliput	*Toxolasma parvum*	Kidneyshell	0.543
Mapleleaf	*Quadrula quadrula*	Mapleleaf	0.033
Northern Riffleshell	*Epioblasma rangiana*	Kidneyshell	0.543
Rainbow	*Villosa iris*	Rainbow	0.143
Rayed Bean	*Villosa fabalis*	Kidneyshell	0.543
Round Hickorynut	*Obovaria subrotunda*	Round Hickorynut	0.444
Round Pigtoe	*Pleurobema sintoxia*	Round Pigtoe	0.224
Salamander Mussel	*Simpsonaias ambigua*	Kidneyshell	0.543
Snuffbox	*Epioblasma triquetra*	Kidneyshell	0.543
Threehorn Wartyback	*Obliquaria reflexa*	Kidneyshell	0.543
Wavyrayed Lampmussel	*Lampsilis fasciola*	Wavyrayed Lampmussel	0.508

MORTALITY FROM BAYLUSCIDE APPLICATIONS

Overall, the estimated mortality from Bayluscide applications for both fishes and mussels demonstrated a strongly right-skewed probability distribution, with zero Bayluscide-induced mortality as the most likely outcome across the range of species for a single application cycle (see Appendix B). However, higher mortality events, while not the norm, were possible for both fishes and mussels with severity based on whether the median or 95th percentile values were of interest (see Appendix B for species-specific figures and Tables 13 to 16 for median and 95th percentile mortality values, respectively). For non-lamprey fishes, median results did not vary greatly across species. The majority of species were predicted to experience no Bayluscide-induced mortality based on median values and only Blackstripe Topminnow had median mortality of one fish or greater (Table 13). However, the 95th percentile results were more variable and yielded substantially greater mortality values (Table 14) indicating that 5% of the time a substantial number of individuals experienced mortality from a single Bayluscide application cycle. Generally, *Ichthyomyzon* spp. exhibited greatest mortality based on the 95th percentile resulting in > 300 individuals killed for some scenarios (i.e., Silver Lamprey in the Sydenham River as well as Silver Lamprey + unidentified *Ichthyomyzon*, Northern Brook Lamprey + unidentified *Ichthyomyzon*, and unidentified *Ichthyomyzon* in the Thames River). Substantial mortalities were observed for other fish species based on 95th percentiles including 22 individuals (Lake Chubsucker [*Erimyzon sucetta*] in St. Clair River), 19 individuals (Blackstripe Topminnow in Sydenham River), and 3 individuals (Eastern Sand Darter in Detroit River, Sydenham River, and Thames River).

Table 13. Estimated median mortality (number of dead fish) of fish species of conservation concern following simulation modelling of the application of granular Bayluscide at six 500 m² randomly selected Type I and Type II sites within each of the four focal rivers. The range in median mortality reflects the minimum and maximum of the species-specific toxicity scenarios. Outcomes with "-" reflect scenarios where fish species are absent from a focal river.

Species Common Name	Species Scientific Name	Detroit River Median Mortality	St. Clair River Median Mortality	Sydenham River Median Mortality	Thames River Median Mortality
Black Redhorse	*Moxostoma duquesnei*	-	-	-	0–0
Blackstripe Topminnow	*Fundulus notatus*	-	0–0.39	0–1.57	-
Channel Darter	*Percina copelandi*	0–0.07	0–0.08	0–0.08	-
Eastern Sand Darter	*Ammocrypta pellucida*	0–0.9	-	0–0.93	0–0.95
Grass Pickerel	*Esox americanus vermiculatus*	0–0	0 –0	0–0	0–0
Lake Chubsucker	*Erimyzon sucetta*	-	0–0	-	-
Lake Sturgeon	*Acipenser fulvescens*	0–0	0–0	-	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	0–0	-	0–0
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-	1.47–10.51	-	60.64–433.11
Northern Madtom	*Noturus stigmosus*	0–0	0–0	-	0–0
Northern Sunfish	*Lepomis peltastes*	0–0	0–0	0 – 0	0–0
Pugnose Minnow	*Opsopoeodus emiliae*	0–0	0–0	0–0	-
Pugnose Shiner	*Notropis anogenus*	0–0.26	0–0.23	0–0.25	-
River Darter	*Percina shumardi*	-	-	0–0	0 -- 0
River Redhorse	*Moxostoma carinatum*	-	0–0	-	0–0
Silver Chub	*Macrhybopsis storeriana*	-	-	-	0–0
Silver Lamprey	*Ichthyomyzon unicuspis*	0–0	0–0	0–0	0–0
Silver Lamprey + Unidentified *Ichthyomyzon*.	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	0–0	1.48–10.58	0–0	63.61–454.39
Silver Shiner	*Notropis photogenis*	-	-	-	0–0
Spotted Sucker	*Minytrema melanops*	0–0.21	0–0.32	0–0.28	0–0.1
Unidentified *Ichthyomyzon*	*Ichthyomyzon sp.*	-	1.47–10.47	-	61.58–439.86

Table 14. Estimated 95th percentile mortality (number of dead fish) of fish species of conservation concern following simulation modelling of the application of granular Bayluscide at six 500 m² randomly-selected Type I and Type II sites within each of the four focal rivers. The range in mortality reflects the minimum and maximum of the species-specific toxicity scenarios. Outcomes with "-" reflect scenarios where fish species are absent from a focal river.

Species Common Name	Species Scientific Name	Detroit River 95th Percentile Mortality	St. Clair River 95th Percentile Mortality	Sydenham River 95th Percentile Mortality	Thames River 95th Percentile Mortality
Black Redhorse	*Moxostoma duquesnei*	-	-	-	0–0
Blackstripe Topminnow	*Fundulus notatus*	-	0–4.18	0–19.61	-
Channel Darter	*Percina copelandi*	0–0.31	0–0.32	0–0.3	-
Eastern Sand Darter	*Ammocrypta pellucida*	0–3.09	-	0–3.26	0–3.23
Grass Pickerel	*Esox americanus vermiculatus*	0–0.74	0–0.74	0–0.75	0–0.77
Lake Chubsucker	*Erimyzon sucetta*	-	0–21.96	-	-
Lake Sturgeon	*Acipenser fulvescens*	0–0	0–0	-	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-	0–0	-	9.73–69.5
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-	4.77–34.06	-	300.15–2,143.9
Northern Madtom	*Noturus stigmosus*	0–3.11	0–2.87	-	0–3.14
Northern Sunfish	*Lepomis peltastes*	0–0.29	0–0.3	0–0.25	0–0.28
Pugnose Minnow	*Opsopoeodus emiliae*	0–0.8	0–0.83	0–0.82	-
Pugnose Shiner	*Notropis anogenus*	0–2.34	0–2.43	0–2.49	-
River Darter	*Percina shumardi*	-	-	0–0	0–0
River Redhorse	*Moxostoma carinatum*	-		-	0–0
Silver Chub	*Macrhybopsis storeriana*	-	-	-	0–0
Silver Lamprey	*Ichthyomyzon unicuspis*	0.14–1	0–0	0.14–1	0.14–1
Silver Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	13.72–97.97	4.78–34.11	45.13–322.37	291.65–2,083.2
Silver Shiner	*Notropis photogenis*	-	-	-	0–1.18
Spotted Sucker	*Minytrema melanops*	0–0.62	0–0.92	0–0.78	**0–0**.25
Unidentified *Ichthyomyzon*	*Ichthyomyzon sp.*	-	4.78–34.12	-	296.8–2,120.02

The results for mussel species were similar to fishes in that median indicated a lack of Bayluscide-induced mortality for all study species except Kidneyshell (43 individuals in the Sydenham River) (Table 15). The Kidneyshell result illustrated the sensitivity of outputs to the reported statistic where 60th percentiles indicated similar levels of mortality in the Sydenham for Kidneyshell, Northern Riffleshell, and Rayed Bean (*Villosa fabalis*) (also seen in cumulative probability distributions; see Appendix B). The 95th percentile results indicated that some species may experience high mortality 5% of the time including Rayed Bean (1,442 individuals in Sydenham River), Northern Riffleshell (1,304 individuals in Sydenham River), and Kidneyshell (1,131 individuals in Sydenham River) (Table 16). In contrast, some species yielded no mortality based on 95th percentiles for both the Thames and Sydenham rivers including Threehorn Wartyback, Round Hickorynut, and Salamander Mussel (*Simpsonaias ambigua*). Altogether, similar to the fish results, mussel results demonstrated that most Bayluscide application cycles will result in no or relatively low mortality, but substantial mortality (> 500 individuals) to species of conservation concern may occur 5% of the time or less.

Table 15. Estimated median mortality (number of dead mussels) for mussel species of conservation concern following simulation modelling of the application of granular Bayluscide at six 500 m² randomly-selected Type I and Type II sites within the Sydenham and Thames rivers. Outcomes with "-" reflect scenarios where mussel species are absent from a focal river or density values could not be derived.

Species Common Name	Species Scientific Name	Sydenham River Median Mortality	Thames River Median Mortality
Fawnsfoot	*Truncilla donaciformis*	0	0
Kidneyshell	*Ptychobranchus fasciolaris*	43.39	-
Lilliput	*Toxolasma parvum*	-	-
Mapleleaf	*Quadrula quadrula*	0	0.69
Northern Riffleshell	*Epioblasma rangiana*	0	-
Rainbow	*Villosa iris*	0	0
Rayed Bean	*Villosa fabalis*	0	0
Round Hickorynut	*Obovaria subrotunda*	0	-
Round Pigtoe	*Pleurobema sintoxia*	0	0
Salamander Mussel	*Simpsonaias ambigua*	0	-
Snuffbox	*Epioblasma triquetra*	0	-
Threehorn Wartyback	*Obliquaria reflexa*	0	0
Wavyrayed Lampmussel	*Lampsilis fasciola*	0	0

Table 16. Estimated 95th percentile mortality (number of dead mussels) of mussel species of conservation concern following simulation modelling of the application of granular Bayluscide at six 500 m^2 randomly-selected Type I and Type II sites within Sydenham and Thames rivers. Outcomes with "-" reflect scenarios where mussel species are absent from the system or density values could not be derived.

Species Common Name	Species Scientific Name	Sydenham River 95th Percentile Mortality	Thames River 95th Percentile Mortality
Fawnsfoot	*Truncilla donaciformis*	271.5	572.57
Kidneyshell	*Ptychobranchus fasciolaris*	1,131.48	-
Lilliput	*Toxolasma parvum*	-	-
Mapleleaf	*Quadrula quadrula*	62.88	141.57
Northern Riffleshell	*Epioblasma rangiana*	1,304.21	-
Rainbow	*Villosa iris*	71.5	272.46
Rayed Bean	*Villosa fabalis*	1,442.09	955.1
Round Hickorynut	*Obovaria subrotunda*	0	-
Round Pigtoe	*Pleurobema sintoxia*	155.59	190.1
Salamander Mussel	*Simpsonaias ambigua*	0	-
Snuffbox	*Epioblasma triquetra*	740.99	-
Threehorn Wartyback	*Obliquaria reflexa*	0	0
Wavyrayed Lampmussel	*Lampsilis fasciola*	-	601.26

Adjustments to Bayluscide Application Methods

Overall, increasing the number or size of application sites for a single Bayluscide application cycle increased the range of mortality values for a species. Decreasing the number or size of application sites decreased the range of mortality values, provided that non-zero mortality values occurred under baseline conditions, which are provided in Appendix B to illustrate selected trends. In both cases, these relationships appeared non-linear. For most species, increasing the number or size of application sites did not affect the median mortality value (e.g., Lake Chubsucker and Northern Madtom) but when it did (i.e., Northern Brook Lamprey and Silver Lamprey), the distribution remained heavily right skewed and dominated by low mortality values. The most substantial change in median results was observed when increasing the number of application sites for species with high density (e.g., Mapleleaf and Kidneyshell). Although these distributions remained heavily right skewed, the increase in median mortality, specifically when changing the number of application sites, is due to the highly dense, patchy distribution of these species. As the number of application sites increase, the likelihood of encountering a patch of many individuals increases, thereby increasing median mortality. If the density of these species were to decrease, median mortality would likely be unaffected by number of sites, similar to the influence of site area.

Area-per-Individual Calculations

Replacing the empirical density values with API values resulted in notable changes to mortality estimates. Several species experienced increases in median mortality; particularly, Channel Darter (maximum of 18 individuals), Pugnose Shiner *Notropis anogenus* (maximum of 14 individuals), Eastern Sand Darter (maximum of 9 individuals), and Blackstripe Topminnow (maximum of 5 individuals) (Table 17). The 95th percentile results varied across species (Table 18) and were either equal to or greater than the mortality values based on the empirical data (Table 14). The species that were sensitive to Bayluscide applications based on the median results (i.e., Channel Darter, Pugnose Shiner, and Eastern Sand Darter with median results as high as 18.16, 14.42, and 8.74 respectively) yielded some of the greatest mortality values for the 95th percentile results under assumed API densities (95th percentile results as high as 36.33, 28.84, and 26.22 individual fish killed per application cycle, respectively).

Table 17. Estimated median mortality (number of dead fish) of fish species of conservation concern based on simulation modelling of application of granular Bayluscide at six 500 m² randomly-selected Type I and Type II sites within each of the four focal rivers, with fish density estimated using the Area-per-Individual (API) approach. The range in median mortality reflects the minimum and maximum of the species-specific toxicity scenarios. Outcomes with "-" reflect scenarios where mortality estimates could not be derived.

Species Common Name	Species Scientific Name	Median Mortality
Black Redhorse	*Moxostoma duquesnei*	0–0
Blackstripe Topminnow	*Fundulus notatus*	0–4.18
Channel Darter	*Percina copelandi*	0–18.16
Eastern Sand Darter	*Ammocrypta pellucida*	0–8.74
Grass Pickerel	*Esox americanus vermiculatus*	0–0
Lake Chubsucker	*Erimyzon sucetta*	0–0
Lake Sturgeon	*Acipenser fulvescens*	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-
Northern Madtom	*Noturus stigmosus*	0–0
Northern Sunfish	*Lepomis peltastes*	0–0
Pugnose Minnow	*Opsopoeodus emiliae*	0–0
Pugnose Shiner	*Notropis anogenus*	0–14.42
River Darter	*Percina shumardi*	0–0
River Redhorse	*Moxostoma carinatum*	0–0
Silver Chub	*Macrhybopsis storeriana*	0–0
Silver Lamprey	*Ichthyomyzon unicuspis*	-
Silver Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	-
Silver Shiner	*Notropis photogenis*	0–0
Spotted Sucker	*Minytrema melanops*	0–0.64
Unidentified *Ichthyomyzon*	*Ichthyomyzon sp.*	-

Table 18. Estimated 95th percentile mortality of fish species of conservation concern based on simulation modelling of the application of granular Bayluscide at six 500 m^2 randomly-selected Type I and Type II sites within each of the four focal rivers, with fish density estimated using the Area-per-Individual (API) approach. The range in 95th percentile mortality reflects the minimum and maximum of the species-specific toxicity scenarios. Outcomes with "-" reflect scenarios where mortality estimates could not be derived.

Species Common Name	Species Scientific Name	95th Percentile Mortality (Number of Dead Fish)
Black Redhorse	*Moxostoma duquesnei*	0–0
Blackstripe Topminnow	*Fundulus notatus*	0–12.53
Channel Darter	*Percina copelandi*	0–36.33
Eastern Sand Darter	*Ammocrypta pellucida*	0–26.22
Grass Pickerel	*Esox americanus vermiculatus*	0–0.24
Lake Chubsucker	*Erimyzon sucetta*	0–0.97
Lake Sturgeon	*Acipenser fulvescens*	-
Northern Brook Lamprey	*Ichthyomyzon fossor*	-
Northern Brook Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon fossor* and *Ichthyomyzon* sp.	-
Northern Madtom	*Noturus stigmosus*	0–33.8
Northern Sunfish	*Lepomis peltastes*	0–1.58
Pugnose Minnow	*Opsopoeodus emiliae*	0–24.42
Pugnose Shiner	*Notropis anogenus*	0–28.84
River Darter	*Percina shumardi*	0–0
River Redhorse	*Moxostoma carinatum*	0–0
Silver Chub	*Macrhybopsis storeriana*	0–0
Silver Lamprey	*Ichthyomyzon unicuspis*	-
Silver Lamprey + Unidentified *Ichthyomyzon*	*Ichthyomyzon unicuspis* and *Ichthyomyzon* sp.	-
Silver Shiner	*Notropis photogenis*	0–3.92
Spotted Sucker	*Minytrema melanops*	0–1.27
Unidentified *Ichthyomyzon*	*Ichthyomyzon sp.*	-

POPULATION LEVEL EFFECTS OF BAYLUSCIDE

Overall, the population-level effects of Bayluscide applications were greatly affected by the estimated population abundance of each species (calculated based on the amount of habitat assumed to be occupied by the population) as well as Bayluscide application frequency. A non-linear relationship was observed between the proportion of habitat occupied vs. estimated population abundance, where increasing the occupied range from 0.01% to 0.05% had a much greater effect on population abundance than increasing the occupied range from 1% to 5% (e.g., Figure 10a, Northern Madtom). Although the exact values varied across species and focal river, typically there was a relatively lower effect of Bayluscide on population abundance when the occupied range was above 10%. Intuitively, when all other factors were equal, smaller populations experienced greater proportional reductions (and thus higher population-level effects) from Bayluscide applications because the resulting mortality would remove a greater fraction of the total population. The benefit of decreasing the frequency of applications (once every year to once every 10 years) was greater for smaller populations (0.01% of occupied range) compared to larger populations (100% of occupied range). The relationship between the percentage of baseline population abundance remaining after 100 years and application frequency was also non-linear (see Figure 10 for examples). Specific results for Eastern Sand Darter, Northern Madtom, Channel Darter, and *Ichthyomyzon* spp. are provided below.

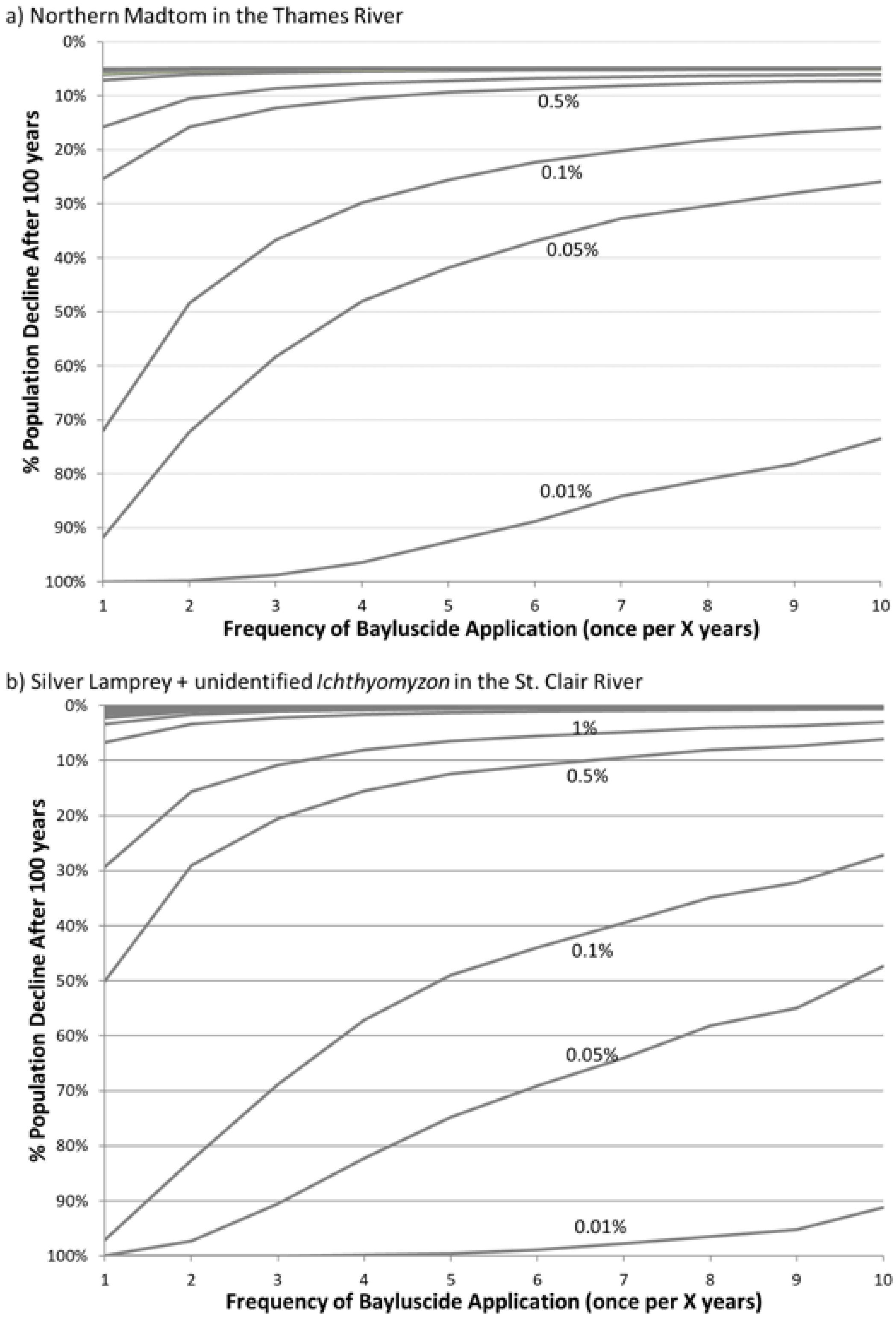

Figure 10. Percentage decline in population abundance of: a) Northern Madtom in the Thames River and b) Silver Lamprey + unidentified Ichthyomyzon *in the St. Clair River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for 100 years. The lines represent uncertainty in results based proportions (0.01% to 100%) of bounded area occupied by the species.*

Eastern Sand Darter

Population-level effects of Eastern Sand Darter varied with assumed species density and proportion of available habitat occupied. When comparing changes in population abundance over time (Figure 11), there was relatively little effect of Bayluscide applications on the Eastern Sand Darter population based on density estimates from Finch et al. (2018). Based on an aggressive Bayluscide application schedule (i.e., a yearly application cycle) and a worst-case scenario of occupied habitat (i.e., 0.01% of bounded range), the population abundance based on density estimates from Finch et al. (2018) declined by 13% after 100 years (Figure 11). Decreasing the frequency of the application cycle had little effect on Eastern Sand Darter abundance under the same density estimate. For example, for a Bayluscide application cycle of three years or greater, there was little change in population abundance. Similar results occurred for annual population growth rates with population growth rates remaining high (0.999) even with an aggressive Bayluscide application schedule (i.e., a yearly application cycle) and a worst-case scenario of occupied habitat (i.e., 0.01% of bounded range) (Figure 12).

When Bayluscide applications were simulated for Eastern Sand Darter based on the Thames density data, population-level effects were greater than those based on density data from Finch et al. (2018) (Figure 11). For this scenario, frequent Bayluscide applications (i.e., a one-year application cycle) had a substantial effect on population abundance, particularly when limited habitat was occupied (e.g., 0.01% of bounded range). This simulation resulted in the population decline of 90% after 100 years. As the frequency of the Bayluscide application cycle decreased, the effect of Bayluscide on the Eastern Sand Darter population was reduced. Although the relationship between frequency of the application cycle and changes in population abundance was similar to the simulation involving data from Finch et al. (2018), an asymptote was not clearly reached based on the 100 year results even when undertaking a 10 year application cycle. Similar results occurred when focusing on annual population growth rates as a measured endpoint where population growth rates declined to 0.977 with a worst case scenario for available habitat and a yearly Bayluscide application schedule (Figure 12).

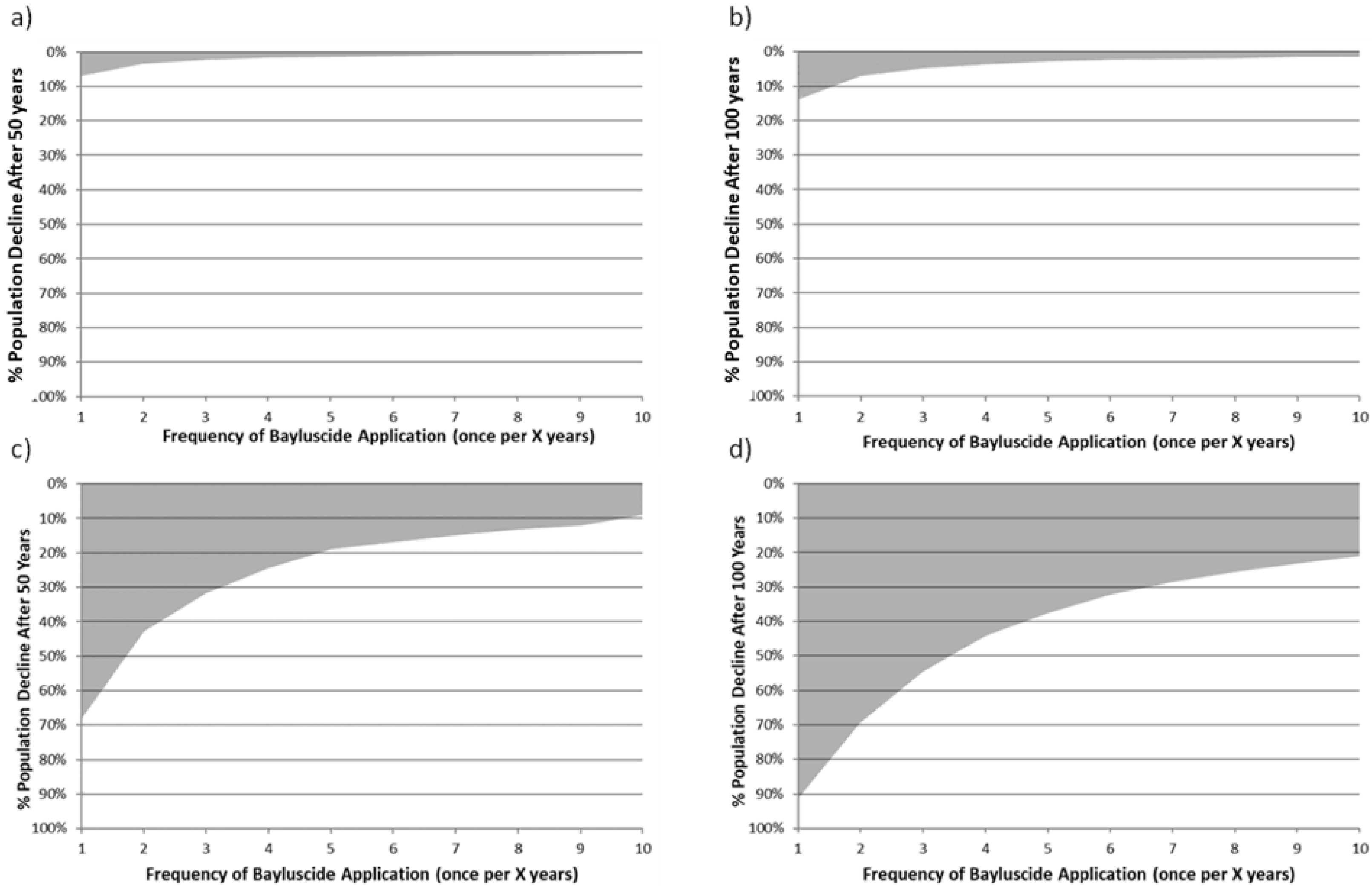

Figure 11. Percentage decline of Eastern Sand Darter population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using Eastern Sand Darter density data from Finch et al. (2018) and for c) 50 years and d) 100 years based on densities from the DFO Biodiversity Science Database. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Eastern Sand Darter.

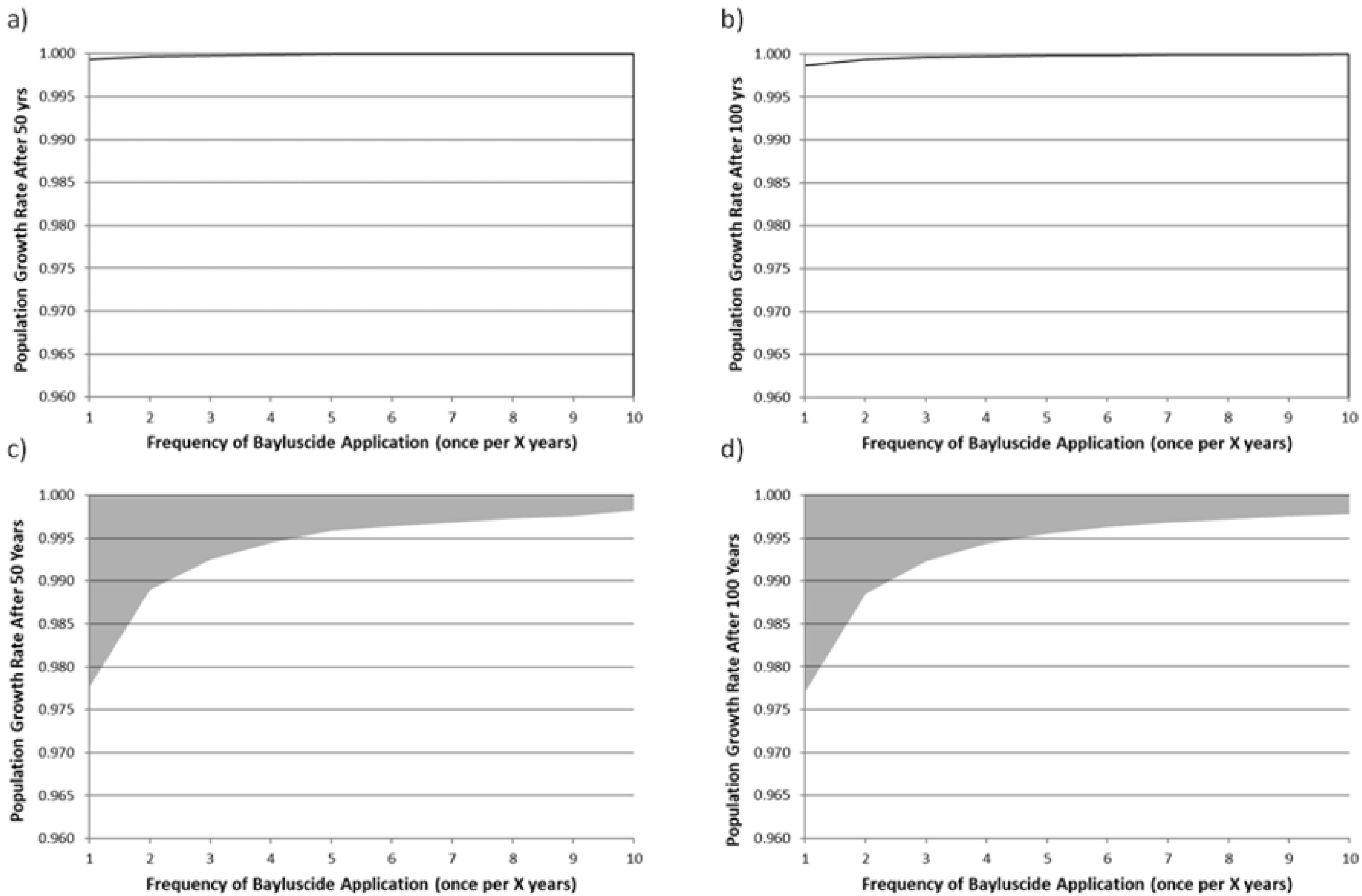

Figure 12. Population growth rates of Eastern Sand Darter in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using data for Eastern Sand Darter in the Thames River from Finch et al. (2018) and for c) 50 years and d) 100 years using density data for Eastern Sand Darter in the Thames River from the DFO Biodiversity Science Database. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Eastern Sand Darter.

Northern Madtom

The effect of Bayluscide applications on Northern Madtom in the Thames River and Detroit River was highly variable, largely based on the amount of critical habitat assumed to be occupied (Figures 13 and 14). For the Thames River, Northern Madtom experienced a near complete population collapse after 100 years when a small amount of habitat was occupied (e.g., 0.01% of identified critical habitat area) and with a Bayluscide application cycle of five years or fewer. When the frequency of Bayluscide applications decreased to once every 10 years, a substantial effect on the Northern Madtom population was still evident after 100 years. Although the effect of applications on population abundance did not reveal a logarithmic trend as with Eastern Sand Darter, a logarithmic trend was observed for annual population growth after 50 and 100 years. Similar results were found for the Detroit River. However, the impact of Bayluscide applications was much greater in the Detroit River than the Thames River. The Detroit River results indicated the potential for a near complete population collapse (> 90% population decline) after 100 years for an application cycle of five years or less. The small population decrease observed when a large amount of habitat was occupied (100% of identified critical habitat area) is an artefact of the Northern Madtom population model. The Northern Madtom population model had a population growth rate that was closer to $\lambda = 0.9999$ than $\lambda = 1.00$ due to parameter rounding issues. This slightly lower population growth rate resulted in small (i.e., < 5%) population declines when little to no mortality from Bayluscide applications occurred.

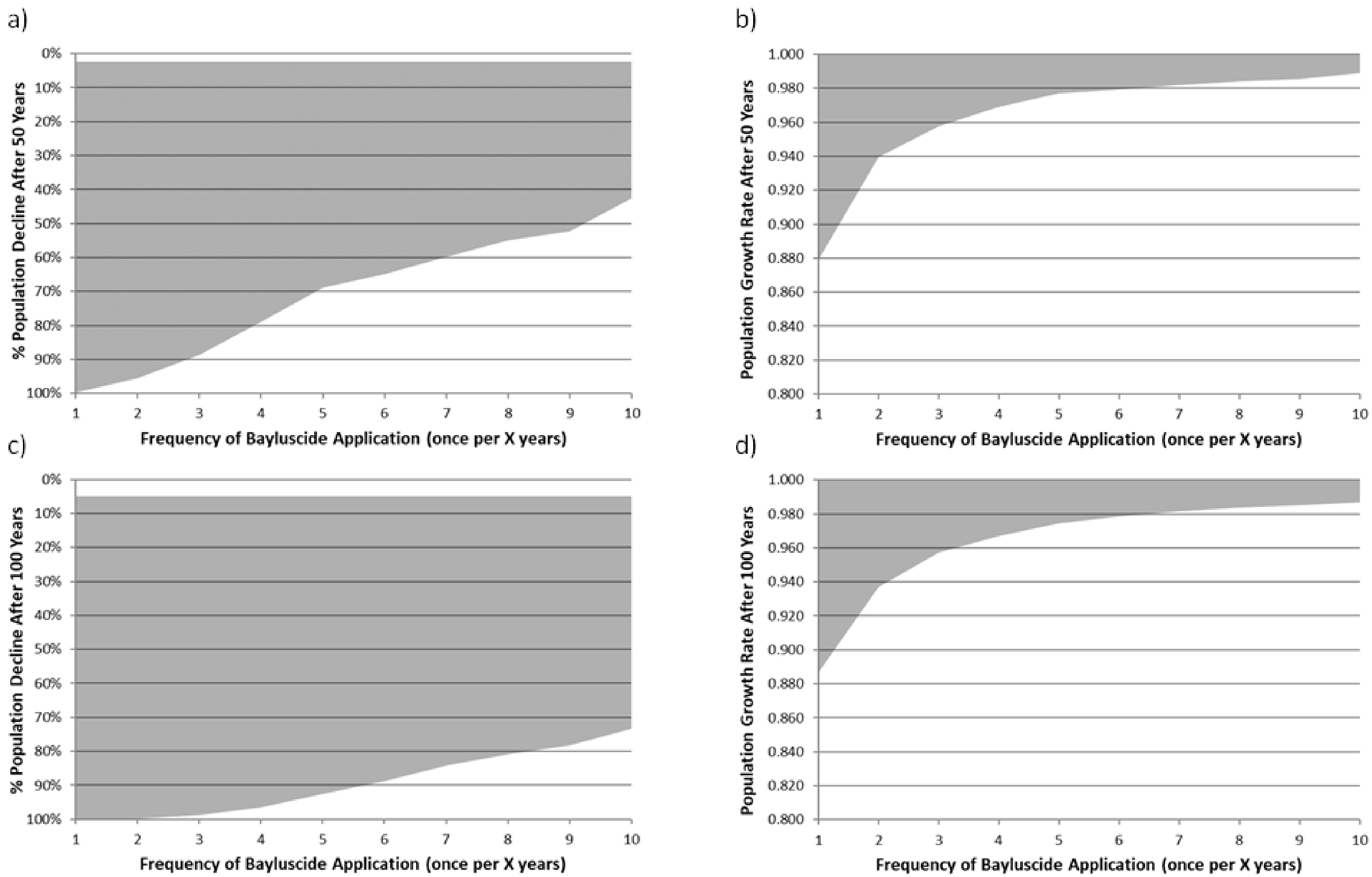

Figure 13. Percentage decline of Northern Madtom population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and c) 100 years using density data for Northern Madtom in the Thames River. Also shown are changes in population growth rates at b) 50 years and d) 100 years for Northern Madtom in the Thames River following applications of granular Bayluscide across multiple yearly cycles. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the identified critical habitat area occupied by Northern Madtom.

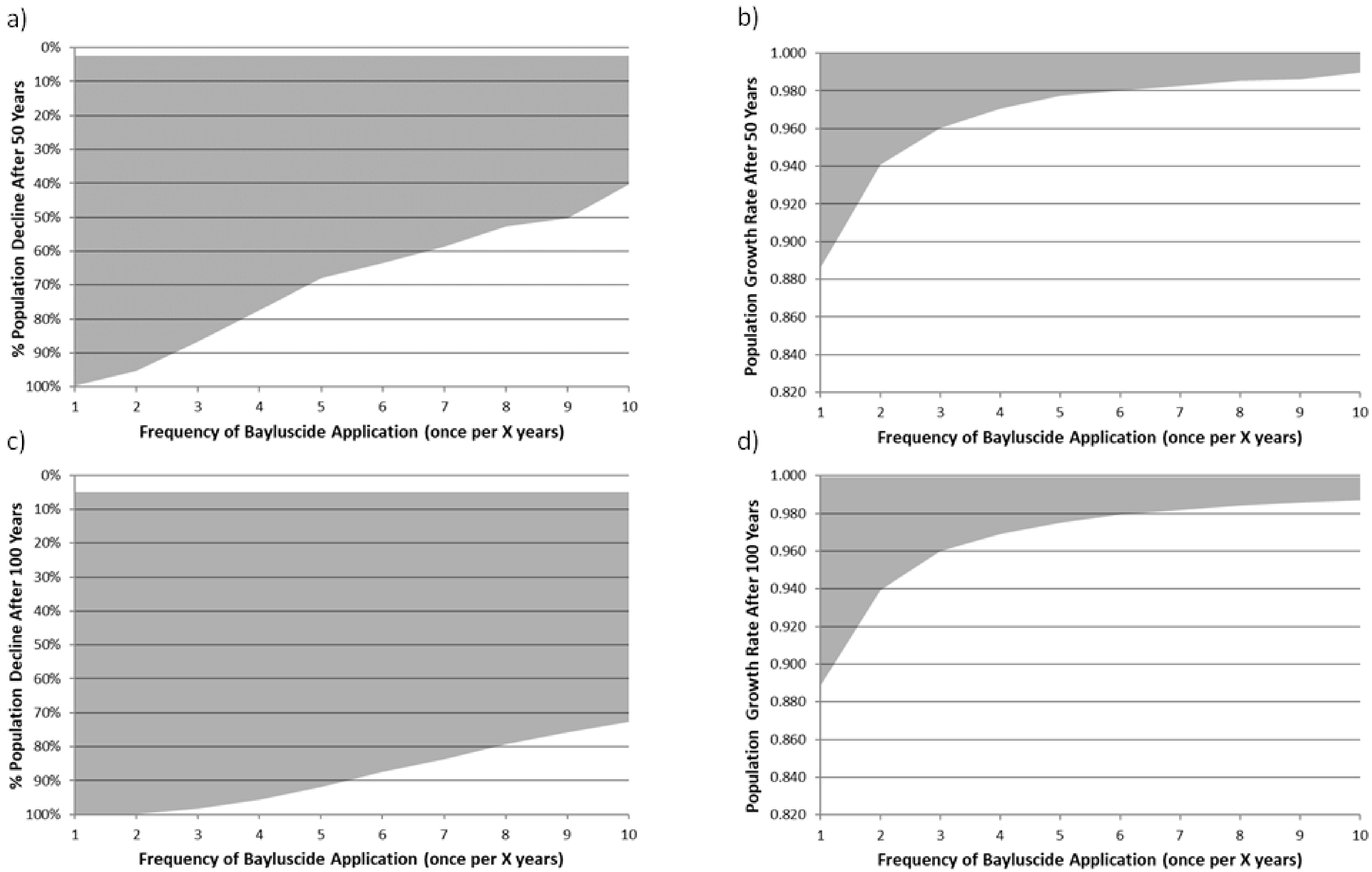

Figure 14. Percentage decline of Northern Madtom population abundance in the Detroit River following simulated granular Bayluscide application cycles of different frequencies (one to ten years) for a) 50 years and c) 100 years using density data for Northern Madtom in the Detroit River. Also shown is the change in population growth rate at b) 50 years and d) 100 years for Northern Madtom in the Detroit River following applications of granular Bayluscide based on the same density data. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the identified critical habitat area occupied by Northern Madtom.

Channel Darter

The effect of Bayluscide applications on Channel Darter in the Detroit River was variable and depended heavily on the amount of occupied habitat within the critical habitat area (Figure 15). Although there was variability in population-level mortality estimates at short application cycles (i.e., once every 1–2 years), the overall effect of Bayluscide applications on Channel Darter was relatively low. As with other species, a logarithmic trend was observed, where decreasing the application frequency beyond once every six years yielded little difference in population abundance or population growth rates.

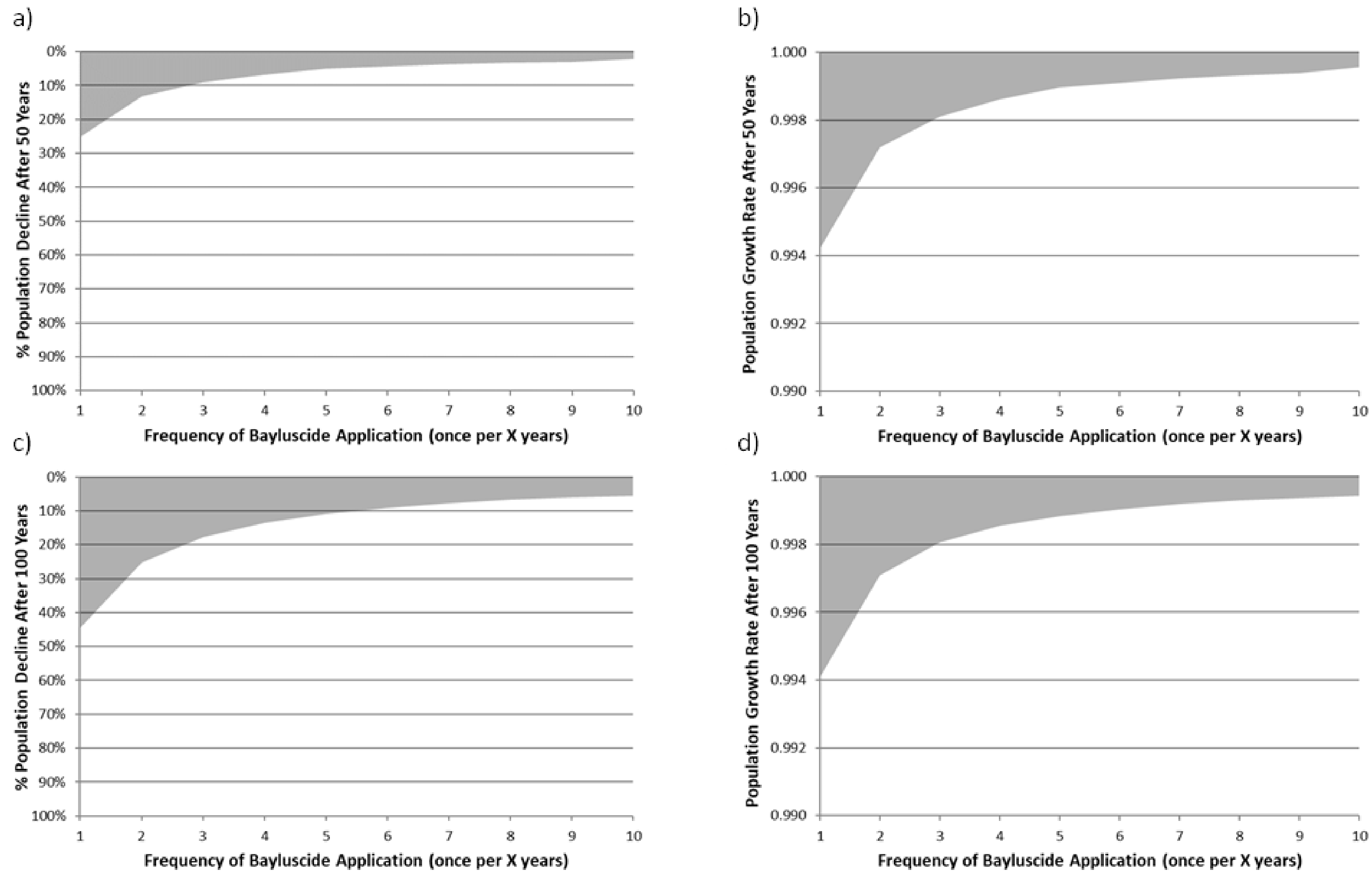

Figure 15. Percentage decline of Channel Darter population abundance in the Detroit River following simulated granular Bayluscide application cycles of different frequencies (one to ten years) for a) 50 years and c) 100 years using density data for Channel Darter in the Detroit River. Also shown is the change in population growth rate at b) 50 years and d) 100 years for Channel Darter in the Detroit River following applications of granular Bayluscide based on the same density data. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the identified critical habitat area occupied by Channel Darter.

Ichthyomyzon spp.

Northern Brook Lamprey combined with unidentified *Ichthyomyzon* experienced a complete or near complete population collapse (i.e., > 90% population declines after 100 years) when limited habitat was occupied (e.g., 0.01% of bounded range) across all Bayluscide application cycles for both the Thames and St. Clair rivers (Figures 16 through 19). When only definitive Northern Brook Lamprey records were used, the effect of Bayluscide applications was substantially reduced and a near complete population collapse (i.e., > 90% population decline after 100 years) occurred only when the Bayluscide application cycle was implemented every year in the Thames River. The same scenario in the St. Clair River yielded a 12% population decline. The large discrepancy between the definitive Northern Brook Lamprey results and the results for Northern Brook Lamprey with unidentified *Ichthyomyzon* is due to the few definitive captures of Northern Brook Lamprey in study rivers. Because unidentified *Ichthyomyzon* were captured substantially more often and in greater densities than definitive captures of Northern Brook Lamprey, incorporating the unidentified collections substantially increased the likelihood of occurrence (Table 6) and density (Table 8) within application sites, resulting in greater mortality (Tables 13 and 14).

Similar results occurred for Silver Lamprey. When combined with unidentified *Ichthyomyzon* occurrences and densities, populations experienced a complete or near complete collapse (i.e., >90% population declines after 100 years) across all application frequencies. Alternatively, population collapse for definitive Silver Lamprey records occurred only when applications took place every year in the Thames River (Figures 20 through 23). Similar to Northern Brook Lamprey, mortality was greatest when limited habitat was available (e.g., 0.01% of bounded range). When the entire habitat polygon was occupied by the species, Bayluscide applications had little effect on Silver Lamprey, regardless of application frequency.

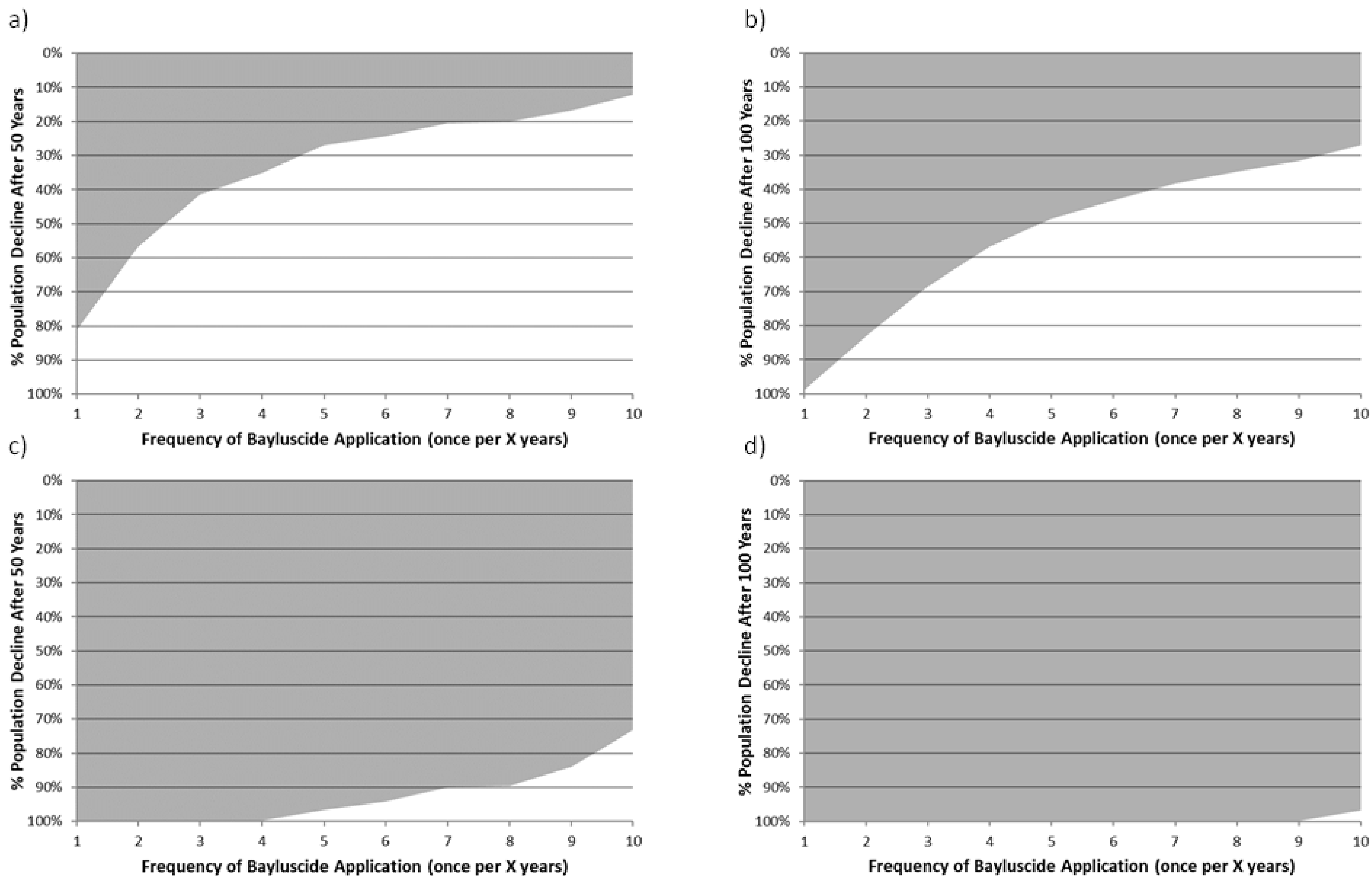

Figure 16. Percentage decline of Northern Brook Lamprey population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Northern Brook Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Northern Brook Lamprey and unidentified Ichthyomyzon*. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Northern Brook Lamprey and unidentified* Ichthyomyzon.

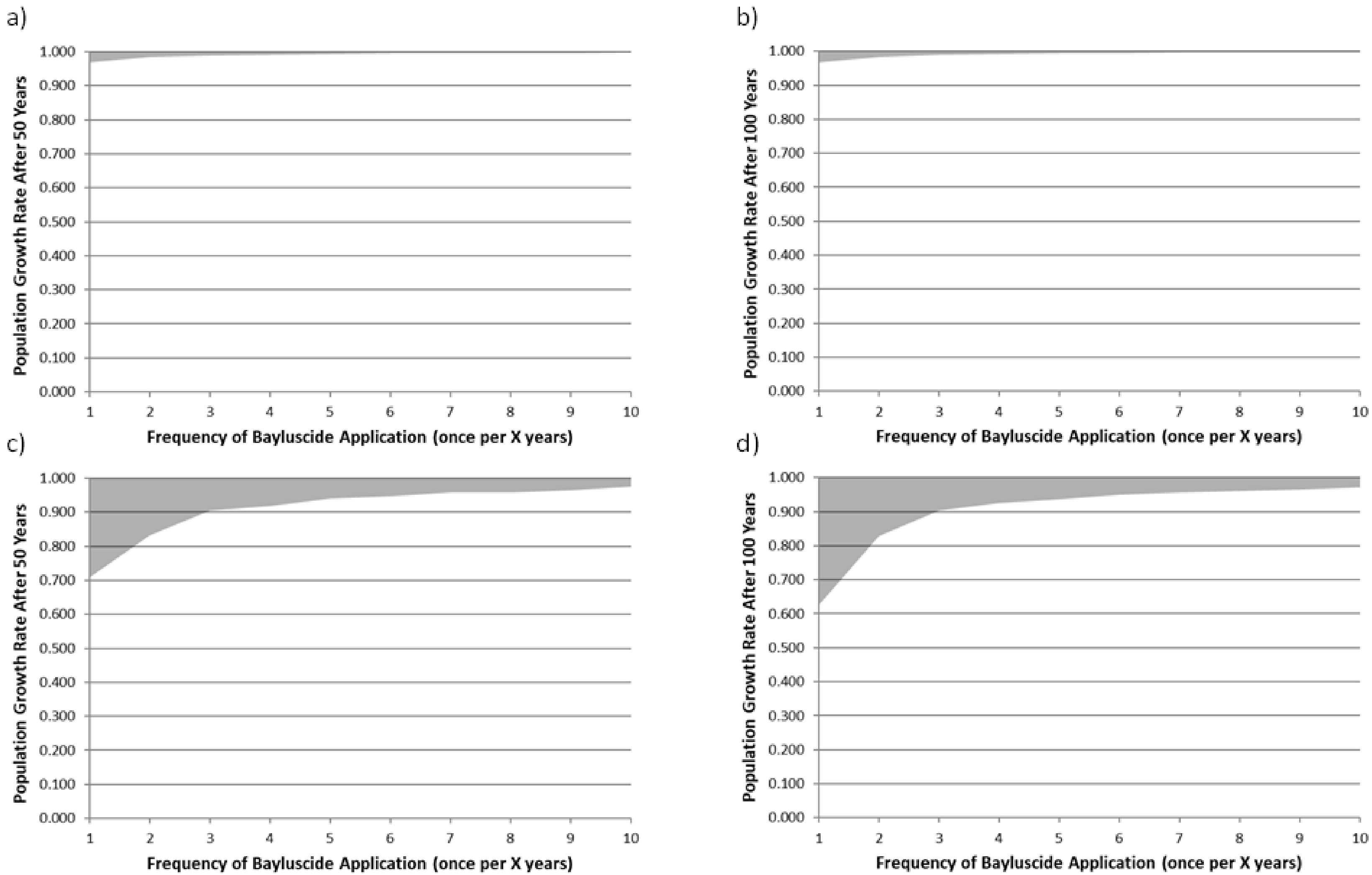

Figure 17. Population growth rates of Northern Brook Lamprey population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Northern Brook Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Northern Brook Lamprey and unidentified Ichthyomyzon*. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Northern Brook Lamprey and unidentified* Ichthyomyzon*.*

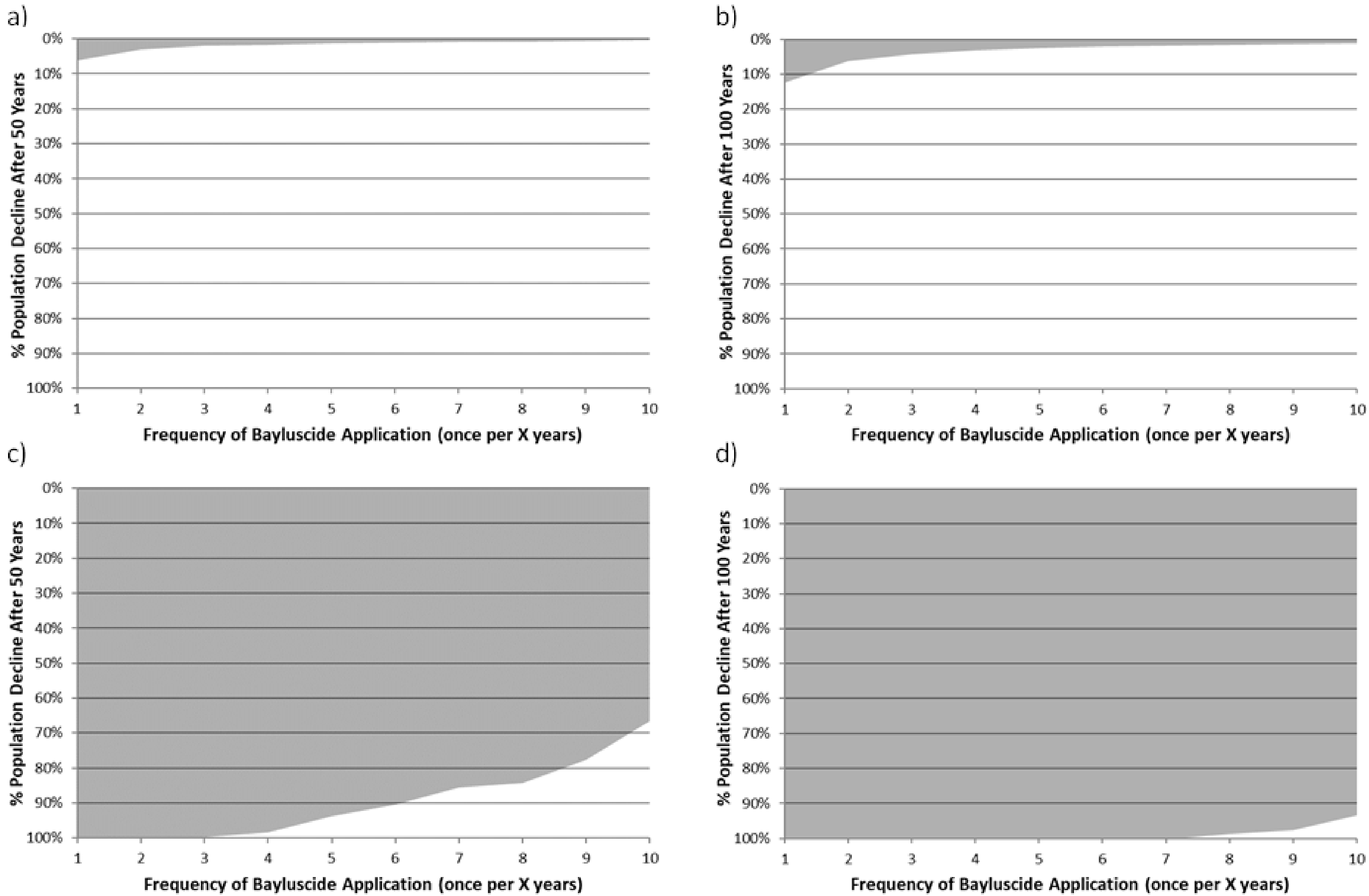

Figure 18. Percentage decline of Northern Brook Lamprey population abundance in the St. Clair River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Northern Brook Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Northern Brook Lamprey and unidentified Ichthyomyzon*. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Northern Brook Lamprey and unidentified* Ichthyomyzon.

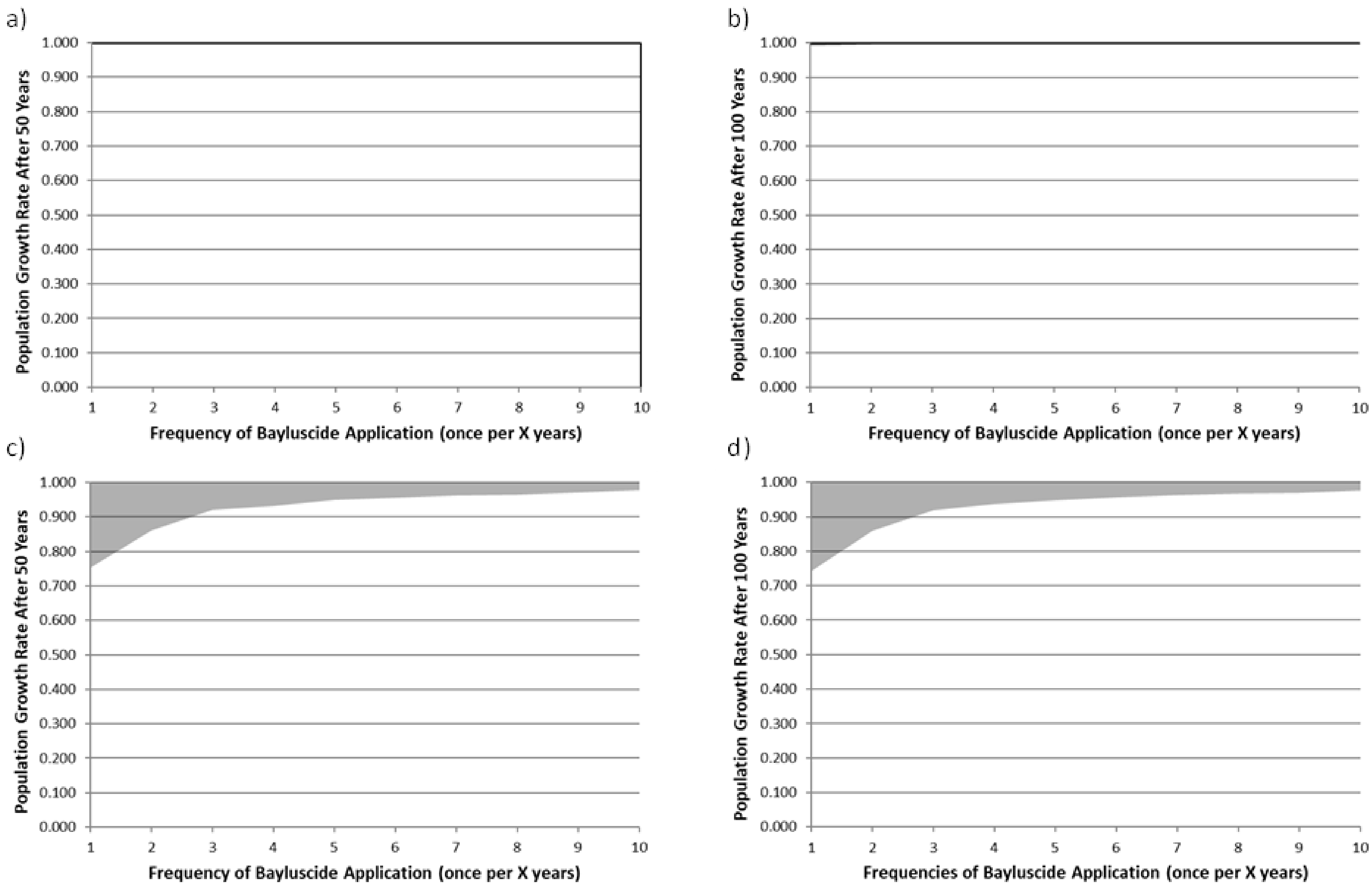

Figure 19. Population growth rates of Northern Brook Lamprey population abundance in the St. Clair River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Northern Brook Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Northern Brook Lamprey and unidentified Ichthyomyzon.*. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Northern Brook Lamprey and unidentified* Ichthyomyzon.

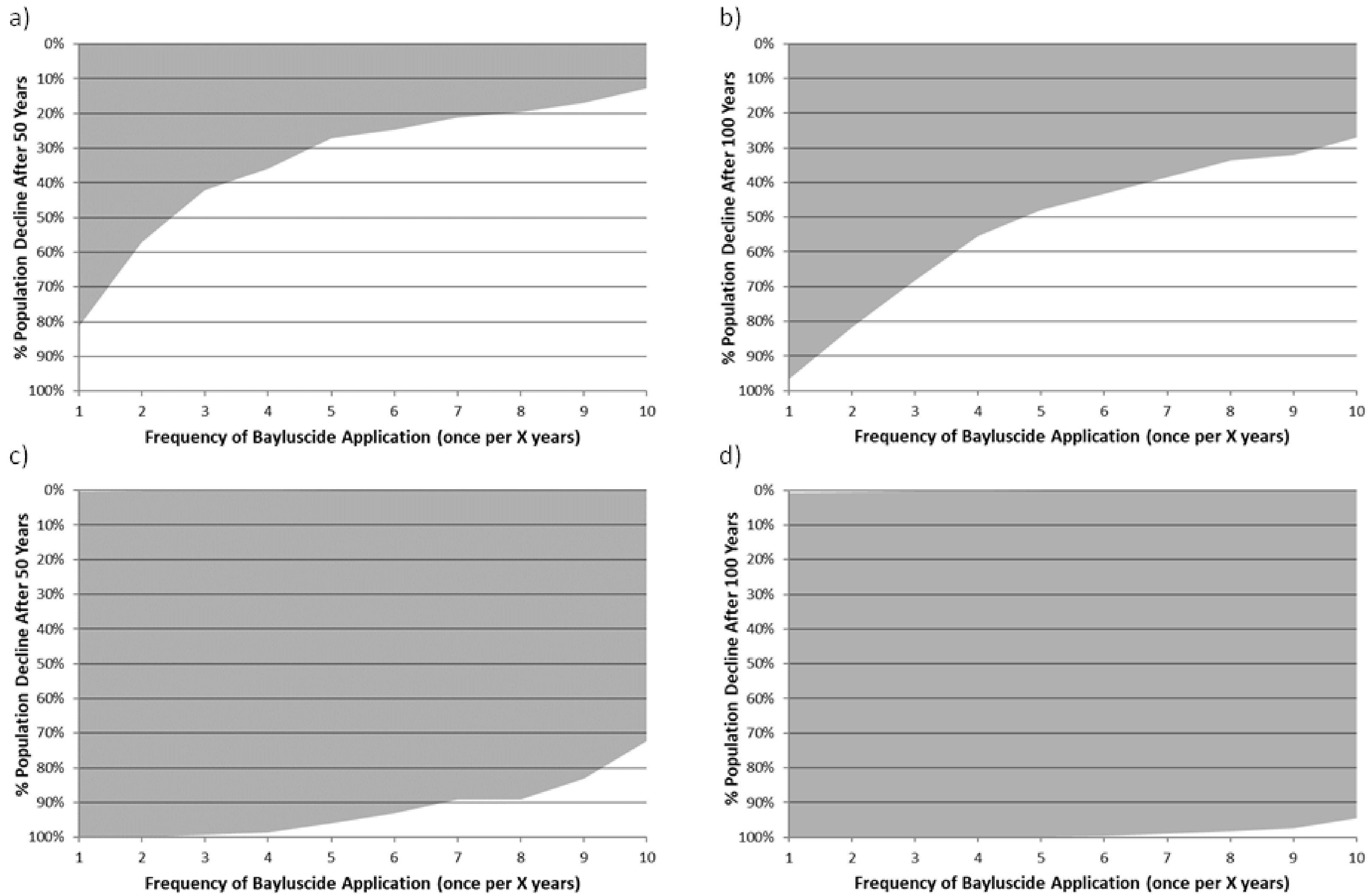

Figure 20. Percentage decline of Silver Lamprey population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Silver Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Silver Lamprey and unidentified Ichthyomyzon. *The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Silver Lamprey and unidentified* Ichthyomyzon.

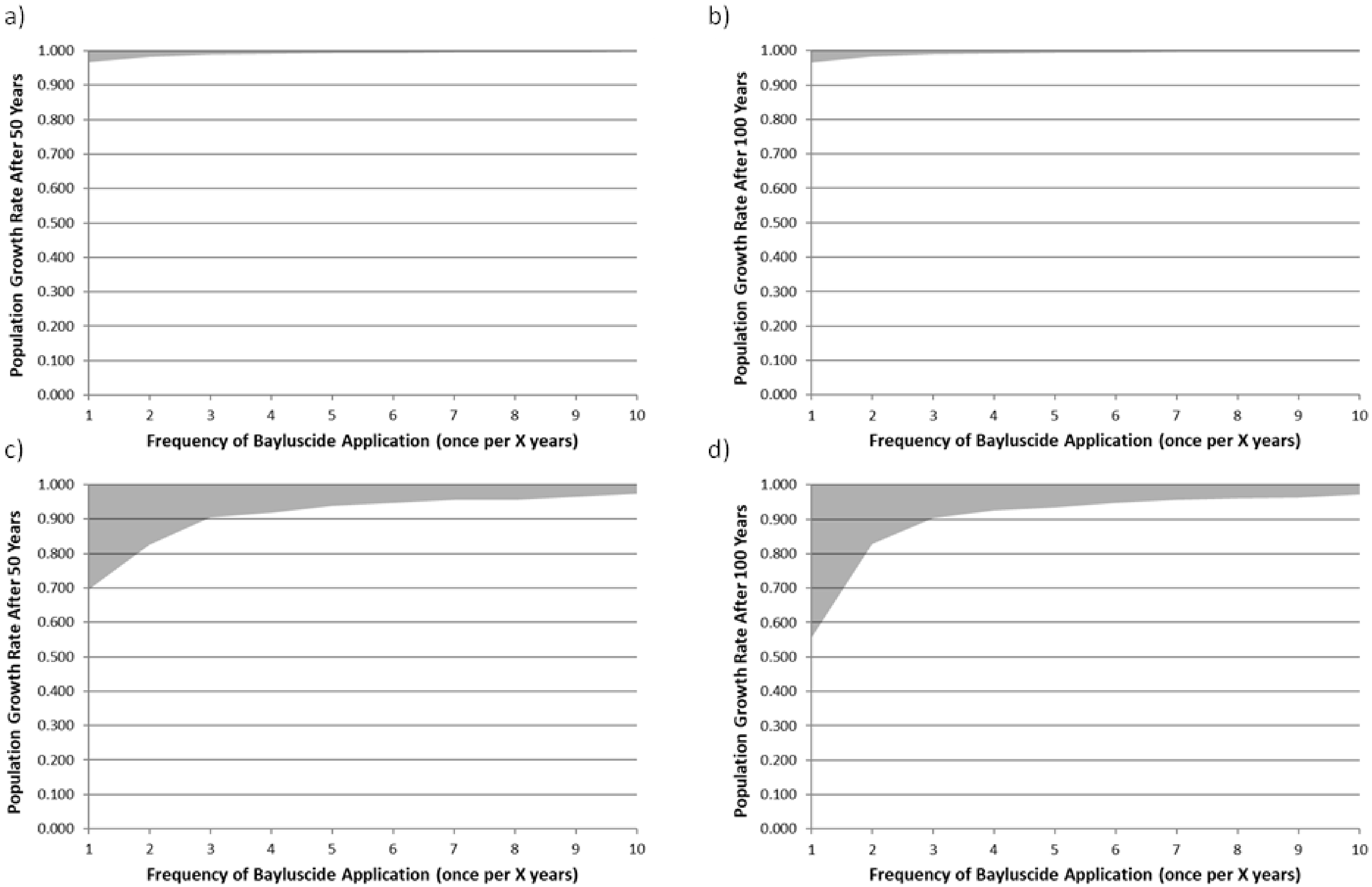

Figure 21. Population growth rates of Silver Lamprey population abundance in the Thames River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Silver Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Silver Lamprey and unidentified Ichthyomyzon. *The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied Silver Lamprey and unidentified* Ichthyomyzon.

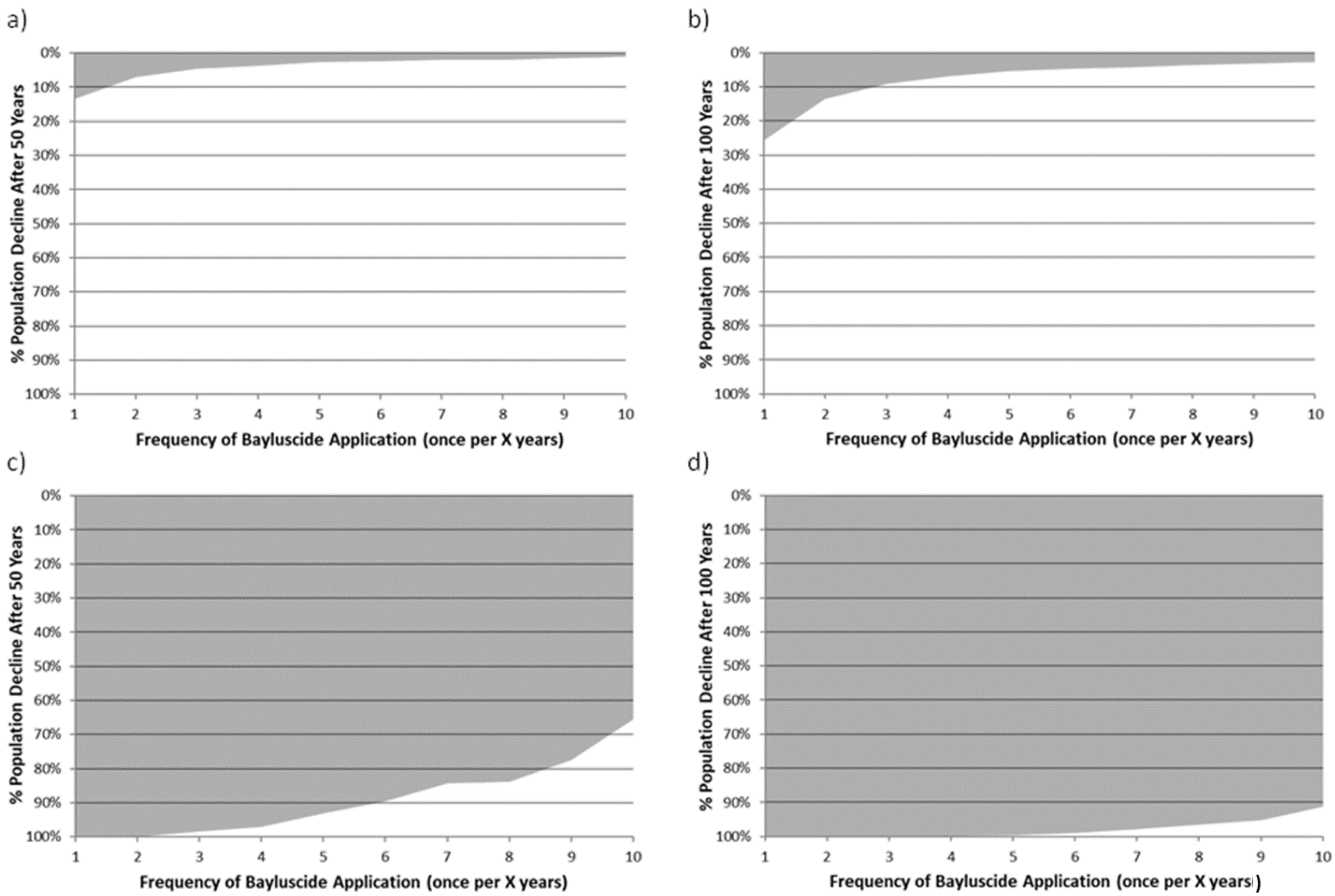

Figure 22. Percentage decline of Silver Lamprey population abundance in the St. Clair River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Silver Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Silver Lamprey and unidentified Ichthyomyzon. *The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied by Silver Lamprey and unidentified* Ichthyomyzon.

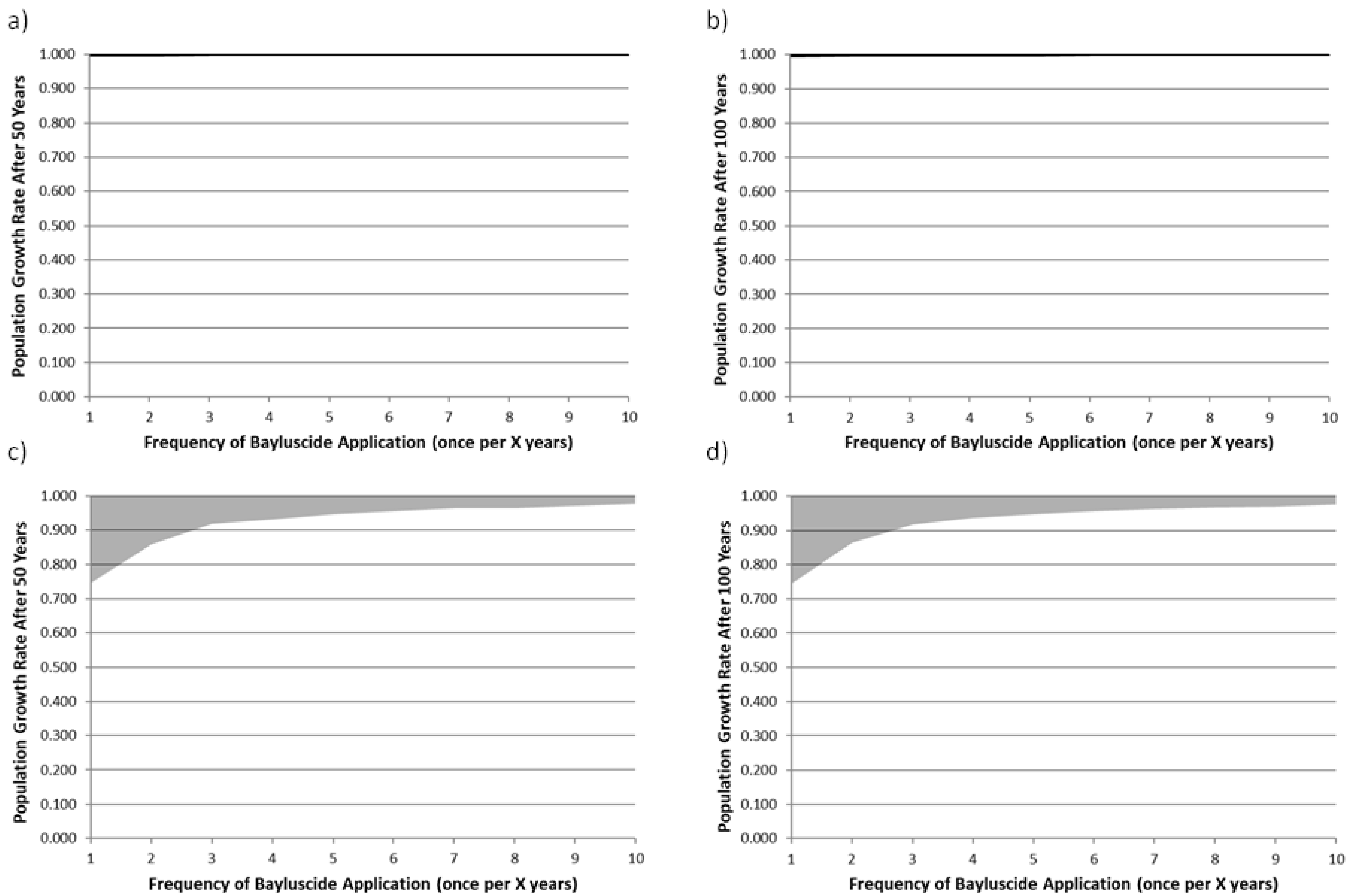

Figure 23. Population growth rates of Silver Lamprey population abundance in the St. Clair River following simulated granular Bayluscide application cycles across different frequencies (one to ten years) for a) 50 years and b) 100 years using definitive Silver Lamprey density and mortality data and for c) 50 years and d) 100 years based on density and mortality data for Silver Lamprey and unidentified Ichthyomyzon*. The grey shaded area represents uncertainty in results based on lower and upper bounds of 0.01% and 100%, respectively, of the bounded area occupied Silver Lamprey and unidentified* Ichthyomyzon*.*

DISCUSSION

The goal of this study was to estimate the likelihood of Bayluscide-induced mortality of fish and mussel species of conservation concern within four rivers of the Huron-Erie corridor, including the potential for population-level effects. This analyses demonstrated that Bayluscide-induced mortality is likely to be low for fishes and mussels of conservation concern in most cases for a single application cycle. These results were driven by low to moderate occurrence and generally low population densities of species in habitats targeted for Bayluscide applications. However, probability distributions of mortality were strongly right-skewed resulting in the possibility of very high mortality (ones to tens of fishes [excluding lampreys] and potentially hundreds of Silver Lamprey, Northern Brook Lamprey, and freshwater mussels) approximately 5% of the time within a single application cycle.

Variation in estimated levels of Bayluscide-induced mortality occurred due to species-specific factors. The likelihood of fishes and mussels of conservation concern occurring within habitats targeted for Bayluscide applications varied strongly (e.g., Spotted Sucker Type I habitat, $p = 0.402$; Threehorn Wartyback Type I habitat, $p = 0.000$). Variation in species occurrence within Type I or Type II habitats reflects the finding that for some species, preferred habitat features may be the target of Bayluscide applications (e.g., Eastern Sand Darter and Spotted Sucker), while in other cases species are susceptible while residing in less preferred habitats. Notably, most fishes and mussels assessed in this study displayed non-zero probabilities of residing within habitats that may be selected for applications and were generally higher for fishes than mussels. The latter result is driven in part by sampling bias in that most mussel sampling occurred in areas dominated by Type III habitat, thereby lowering the opportunity to detect mussel species in habitats selected for Bayluscide application. As the likelihood of occurrence values were not corrected for imperfect detection or other gear biases, it is likely that the true probability of exposure was underestimated. Other factors leading to species-specific variation, such as density estimates and surrogate species, are elaborated on below.

The right skewed distribution of Bayluscide-induced mortality was primarily influenced by the patchy distribution of many fish and mussel species of conservation concern. The likelihood of occurrence values for both fishes and mussels illustrated that there are many instances where species of conservation concern do not occupy an application site as few species had likelihood values > 0.2. The low likelihood of occurrence values demonstrated that the majority of Bayluscide applications may result in no mortality as no non-target individuals would be present at the site. When mortality occurs it is likely occurring at a subset of application sites.

Estimated mortality values must be placed in context with the population abundance of a given species of conservation concern. Potentially large mortality events (e.g., > 100 individuals per application cycle) may yield minor population-level effects if they account for a very small proportion of the population. Likewise, a handful of individuals killed may represent substantial decline if population abundances are very low, as can be the case for imperilled species. Unfortunately, population abundances are unknown for the majority of species assessed in this study, so the interpretation of population-level effects is predicated on assumptions about the proportion of available habitat occupied by a species and its resulting effect on the population size estimate. When a low proportion of the bounded range of a species was assumed (e.g., 0.01% of available habitat occupied), total population sizes were small, and when non-zero occurrence, density, and toxicity occurred, the relative impact of Bayluscide applications was greater as mortality represented a large fraction of total population size. When a relatively high proportion of the bounded range was assumed (e.g., > 50% of available habitat occupied), even large mortality events represented a low proportion of total population size. Unfortunately, habitat occupancy is unknown for most species of conservation concern due to the lack of detailed habitat mapping and occupancy studies. However, it is reasonable to assume that the fraction of the bounded range is well below saturation as species of conservation concern and stream fishes in general often display patchy distributions (Dunham et al. 2002). Density estimates within occupied habitat also influenced population abundances, and for many species, multiple plausible densities were presented. For example, for Eastern Sand Darter, two competing estimates of species density were used to generate population abundances (empirical sampling vs. literature estimates; Finch et al. 2018) and these had strong bearing on population-level effects. Refining the population sizes of the species assessed in this study would help to resolve the population-level consequences associated with Bayluscide applications in the four study rivers.

The mortality of fishes and mussels of conservation concern under different Bayluscide application cycles had varying effects on long-term (i.e., 50 or 100 year) changes in population trajectory and abundance. In some cases, long-term mortality imposed by Bayluscide resulted in ≥ 90% population declines (Northern Madtom, *Ichthyomyzon* spp.) whereas in other cases reductions were smaller (Eastern Sand Darter; 13% population declines). However, as indicated, these results were extremely sensitive to assumptions about the baseline population size of fishes of conservation concern. Moreover, the models assumed worse-case conditions in that population recovery following Bayluscide application was assumed not to occur. Long-term projections of the effect of Bayluscide-induced mortality on freshwater mussels were not incorporated as population models for these species are extremely data-limited. However, the high 95th percentile mortality implies that strong declines of those populations are possible, dependent on assumed population sizes.

Sensitivity analysis indicated that several factors associated with a Bayluscide application cycle had strong influence on the estimated mortality of fishes and mussels. For example, estimated mortality associated with a single application cycle was sensitive to the size and number of treatment sites though non-linear responses were observed. The effect of changes to site size and number was most apparent on the 95th percentile of mortality rather than the median. This indicates that if adjustments to site area or number are pursued as potential mitigation measures, the protective effect would be most apparent for reducing extreme outcomes rather than the average condition. However, species that exhibited little to no mortality under baseline application conditions were unlikely to experience mortality reductions through adjustment to treatment sites. In most cases, the frequency of applications also had bearing on long-term changes in population abundance and trajectory. When the frequency of applications decreased the effect on population abundance also decreased. The relationship between frequency of applications and effect was non-linear, where adjusting the frequency of applications at relatively higher frequencies (i.e., applications every 1–3 years) had a greater effect then adjusting the frequency of applications at lower frequencies. In several cases, including Eastern Sand Darter, an inflection point was reached where decreasing the application frequency had little to no effect on the change in population abundance. This relationship was even further influenced by population abundances. When initial population abundance was small, the magnitude of effect of frequent applications was greater and the application frequency to reach the inflection point was lesser than for large populations. For example, smaller populations (e.g., Northern Madtom) did not display a clear inflection point, indicating that population level effects may still be minimized if Bayluscide applications occurred less frequently than once every ten years. A different trend was observed for larger populations (e.g., Eastern Sand Darter) with an inflection point at or near the three year cycle mark, indicating that population level effects may not be further mitigated by decreasing the frequency of Bayluscide applications beyond once every three years. In the case of the smallest populations, a small mortality event occurring once every ten years may be too substantial for the population to sustain.

The analysis presented in this research document was based on best-available data and knowledge of the Bayluscide application process. However, several assumptions were made during analysis that require elaboration. In general, assumptions and overarching uncertainties relate to: 1) lack of knowledge about environmentally relevant concentrations of Bayluscide across habitat types, including the duration of Bayluscide in the aquatic environment; 2) lack of species-specific Bayluscide toxicity and uncertainty about appropriate surrogates; 3) potential avoidance behavior of non-target species; 4) habitat preferences and species densities that were difficult to estimate through existing field data; 5) uncertainty regarding the underlying composition of *Ichthyomyzon* species in the focal rivers; and, 6) uncertainty in population processes, including unknown population sizes for most species of conservation concern. These issues are elaborated on below.

Because Bayluscide is applied at a fixed density (175 kilograms/hectare), differences in flow conditions, depth, water temperatures, conductivity, and other habitat features (e.g., Type I or Type II habitat) may lead to different realized concentrations of the compound in the aquatic environment per unit space and time, thereby imposing variation in exposure within and among application sites. This analysis assumed that Bayluscide concentrations following an application would be maintained at a constant value within Type I and Type II habitat over the course of an eight hour period. For fishes, two benchmark values were used, which were derived based on the concentrations of Bayluscide that result in Sea Lamprey mortality (50%, 99.9%) following a nine hour exposure (LC50 = 0.035 mg/L; LC99.9 = 0.057 mg/L; Scholefield and Seelye 1992) and estimates from an experimental study for mussels (Newton et al. 2017). Unfortunately, knowledge of the environmental variability of the compound following applications is generally poor (Newton et al. 2017), but if known could significantly refine risk estimates. Variability could result in greater than projected mortality if high concentrations exist due to particular flow conditions or lesser effects if rapid flushing and dilution of the compound occurs.

It was also assumed that environmental concentrations of Bayluscide were contained solely in Type I or Type II habitat. If non-zero Bayluscide concentrations occur in Type III habitat, either because Type III habitat exists in the application sites due to the imprecision of substrate assessment in the field, or due to migration of the compound beyond the application sites, results presented here will underestimate the exposure and potential mortality of species of conservation concern found primarily in coarse substrates (e.g., Black Redhorse, River Redhorse, Rainbow [*Villosa iris*]). It was also assumed that an application cycle led to equal selection of Type I and Type II habitat. If an application cycle is biased towards either habitat type (either due to substrate heterogeneity within the site or intentional weighting of substrate types among sites), mortality will differ from estimates presented here.

Substantial uncertainty surrounds the toxicity of Bayluscide for each focal species owing to a lack of species-specific toxicity trials. Coupled with uncertainty in the environmental concentration of the compound, true exposure and expected mortality is subject to a lack of forecasting precision. Surrogate species were chosen based on hierarchical taxonomic matching to reflect the similarity of physiological responses within genera and families or habitat matching if taxonomic matching was not possible. The suitability of surrogates should be rigorously tested to determine if certain at-risk fishes or mussels are more or less sensitive than proxy species. Given the imperilled status of species resulting from the combined effects of multiple stressors (Richter et al. 1997), it is possible that species-specific responses may be more extreme than those of common proxy species, thereby increasing mortality relative to estimates in this study. In addition, the toxicity of Bayluscide may also be influenced by life stage in that younger individuals exhibiting greater sensitivity than adults as is common for many environmental toxins. The influence of life stage on toxicity was not incorporated as toxicity at the species level could not be completed without the use of surrogates. However, this uncertainty warrants further exploration.

Another critical assumption is that the analysis did not take into account species- or site-specific variation in exposure resulting from behavioural attributes of non-target organisms (e.g., benthic vs. pelagic positioning in the stream; fishes vs. mussels) nor was the incidence or effectiveness of avoidance behaviour accounted for should it occur in the wild. When organisms were present at an application site, they were assumed to be exposed equally to target concentrations (0.035 mg/L and 0.057 mg/L for fishes; 11 mg/L for mussels) for the eight hour period. When granular Bayluscide is applied to the site, granules sink to the bottom and Bayluscide is slowly dissolved with the intention of treating the bottom 5 cm of the water column (Newton et al. 2017). Therefore, burrowing organisms (i.e., mussels and lampreys) will likely experience the highest concentrations of Bayluscide over an extended period. Non-burrowing species are also likely exposed but the rate and consequence of exposure will depend on a suite of factors involving the position of the fish during the trial, the environmental concentration of the compound, and the potential for avoidance behaviours to occur, including the availability of suitable refuge areas. Evidence exists that certain species may detect and actively avoid Bayluscide during laboratory trials (Boogaard et al. 2016) but it is unclear if avoidance responses will occur in the field or the generality of these responses across the suite of non-target species. Large-bodied species may be more likely to vacate an application site due to increased swimming ability, but the relationship between fish size and avoidance of Bayluscide is unknown. Moreover, while avoidance may lessen the importance of the direct physiological pathway in causing Bayluscide-induced mortality (Andrews et al. 2021), avoidance may incur other ecological costs such as increased predation risk or other vital rate shifts resulting from migration to sub-optimal habitat. Other pathways for Bayluscide-induced mortality of fishes and mussels of conservation concern, such as food web effects, are identified in Andrews et al. (2021).

The results of this study were sensitive to assumptions about the density of fishes and mussels in the environment. Although the data used to estimate the density of both fishes and mussels represents the most comprehensive sampling of fish and mussel species of conservation concern in the Canadian waters of the Great Lakes basin, some species were characterized by only a few field collections, indicating that density estimation rests on a limited number of field studies. The approach used to estimate fish density may underestimate true density as field sampling was conducted at a large spatial scale (100's m^2), thereby omitting potentially small but high-density microhabitats. This issue does not apply to mussels where field density estimates were derived at a small scale (1's m^2) and then extrapolated to larger areas. The uncertainty of fish density estimates was assessed by incorporating densities generated by the API approach (Minns 2003). Fish density estimates derived through API relationships generally indicated higher densities than those obtained from field sampling, suggesting that imperfect detection and other gear bias may have led to underestimates of true density based on field data alone. For example, based on API densities, the likelihood of harm was substantially higher for several fish species including Eastern Sand Darter, Blackstripe Topminnow, and Pugnose Shiner where median mortality indicated > 8 fish killed per Bayluscide application cycle. The variation between the API densities and those generated from empirical data demonstrates the need to further evaluate the density of these species in each focal river to better understand the harm imposed by Bayluscide applications.

The limitations of the mussel density data presented an alternative challenge as the mussel densities were extrapolated from small-scale density based field sampling. This extrapolation resulted in scenarios where 100's of mussel mortalities occurred following Bayluscide applications. This extrapolation was necessary to estimate potential mussel densities within

Bayluscide application sites, but may have overestimated the true densities of the species within the entirety of the application site.

Mussel species of conservation concern were considered absent in the St. Clair and Detroit rivers because of evidence that the invasion of dreissenids (Zebra Mussel [*Dreissena polymorpha*] and Quagga Mussel [*D. bugensis*]) has resulted in the functional extirpation of native unionids in the Huron-Erie corridor (Schloesser et al. 2006).The lack of recent detections of native unionids in the St. Clair and Detroit rivers prevented the estimation of Bayluscide-induced mortality in these systems. However, recent sampling in 2019 by Allred et al. (2020) demonstrated that populations of several species-at-risk (Threehorn Wartyback, Round Pigtoe, and Mapleleaf mussels) still occur within the Canadian waters of the Detroit River. The status of these and other species in the St. Clair River remains unknown. Given the highly depressed state of mussel populations that may exist in the St. Clair River and those recently confirmed in the Detroit River, any increases in mortality, even if infrequent, may lead to substantial population level effects.

Uncertainty in population processes, including unknown population growth rates for most assessed species, is expected to influence the consequence of Bayluscide applications at the population level. Given that population growth rates were fixed at $\lambda = 1.0$, fish populations were modelled to not exhibit recovery following mortality from a Bayluscide application. However, if populations of Eastern Sand Darter, Northern Madtom, Channel Darter, or *Ichthyomyzon* spp. experience population growth rates of $\lambda > 1.0$ following application, the long-term declines of these species may be less severe than modelled. Given this situation, populations may potentially reach pre-Bayluscide abundances provided that the net effect of population recovery is greater than the mortality imposed by recurring Bayluscide applications. Conversely, several populations may currently be experiencing declining growth rates ($\lambda < 1.0$), where additional sources of mortality including Bayluscide applications would lead to quicker declines in population abundance and lower population growth rates than those in this analysis. Finally, this analysis did not consider Allee effects, which may lead to the local extirpation of small populations with or without the addition of Bayluscide. In cases where populations were sufficiently small (e.g., usable habitat ~ 0.01% of the bounded range or critical habitat polygon), Allee effects would have resulted in greater potential for population crash.

Finally, there were significant challenges with estimating mortality of Silver Lamprey and Northern Brook Lamprey due to the difficulty of positively identifying these species as larvae. The analytical approach that involved two likelihood of occurrence and density estimates (1: definitive species-level identification; and 2: species-level identification plus the unknown fraction of the genus) likely encompasses the upper and lower bound of expected mortality but by doing so imposed widely different mortality estimates and associated population responses (e.g., Northern Brook Lamprey in the St. Clair River). When the unidentified *Ichthyomyzon* were incorporated, the likelihood of occurrence and density values substantially increased for both species in both the St. Clair and Thames rivers, except for Northern Brook Lamprey density estimates in the St. Clair River, which substantially decreased when unidentified *Ichthyomyzon* were included.

The limitations and assumptions discussed above showcase potential research opportunities to increase the precision of the potential mortality estimates for species of conservation concern following Bayluscide applications. Field experiments to determine the Bayluscide concentration at various locations within and outside the application sites would substantially reduce uncertainty regarding the expected concentration experienced by non-target organisms. Finally, developing Bayluscide dose-response curves for appropriate surrogates and exposure durations would also improve the accuracy of the Bayluscide toxicity estimates. Currently, the only recent work exploring the toxicity of Bayluscide has been completed on mussels (Newton et al. 2017), while the majority of fish toxicity work is greater than 20 years old and lacks LC50 or dose-response data for fishes at exposure durations < 12 hours (Marking and Hogan 1967, Dawson 2003).

Despite these limitations, this analysis provides the first quantitative assessment of the mortality of species of conservation concern resulting from granular Bayluscide applications, including the potential for population-level effects. Several studies have identified species that are sensitive to Bayluscide (Marking and Hogan 1967, Dawson 2003, Schreier et al. 2008, Ali 2012, Newton et al. 2017). However, such studies have rarely considered the suite of factors required to estimate mortality resulting from environmental exposure. This analysis demonstrates that most applications will be characterized by no, or relatively low, mortality of non-target organisms but with potentially much higher mortality (ones to tens of at-risk fishes; potentially hundreds of native lampreys and at-risk freshwater mussels) in certain instances in the St. Clair, Detroit, Thames, and Sydenham rivers. The analysis also demonstrates the potential harm from ongoing Bayluscide applications, particularly for small populations. Overall, the analysis also provides model-based support for the effectiveness of management measures (changes in application frequency, site size or number) that may be implemented to reduce the potential for extreme mortality events; however, the effect of such measures should be rigorously tested, recognizing that key uncertainties identified above may influence perceived effect sizes.

ACKNOWLEDGEMENTS

We acknowledge numerous individuals who helped develop this research document. We thank D. Andrews for generating data from the DFO databases and for providing critical habitat and species distribution information to inform population models. We thank J. Barnucz for guidance about the interpretation of field sampling. We are grateful to M. Steeves, T. Morris, and K. McNichols-O’Rourke for access to, and guidance with, Sea Lamprey and mussel data respectively. Thanks to staff at DFO’s SLCC for fielding numerous questions about granular Bayluscide application procedures and related data. We also thank R. Prosser, P. Gillis, and M. Boogaard for helpful discussion about species-specific Bayluscide sensitivity calculations. N. Wasilik and E. Quider created the maps within this document. We also thank S. Dunn, S. Staton, M. Steeves, B. Morrison, P. Sullivan, and L. Bouvier for helpful comments on the scope and methods contained in this research document. Finally, we thank M. Wilkie and M. Docker for formal reviews of the research document.

REFERENCES CITED

Ali, A.A. 2012. Effects of Bayluscide on the different sizes of *Biomphalaria glbrata*, in the intermediate host *Schistosoma mansoni*. 2nd International Conference on Ecological, Environmental and Biological Sciences: 29–30.

Allred, S.S., Woolnough, D.A., Morris, T.J., and Zanatta, D.T. 2020. Status update for native mussels in the Detroit River. *In* Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3-4, 2019, Burlington, Ontario. Edited by T.J. Morris, K.A. McNichols O'Rourke, and S.M. Reid. Can. Tech. Rep. Fish. Aquat. Sci. 3352: viii + 34 p.

Andrews, D.W., Smyth, E.R.B., Lebrun, D.E., Morris, T.J., McNichols-O'Rourke, K.A. and Drake, D.A.R. 2021. Relative Risk of Granular Bayluscide Applications for Fishes and Mussels of Conservation Concern in the Great Lakes Basin. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/034. viii + 174 p.

Bergstedt, R.A., McDonald, R.B., Mullett, K.M., Wright, G.M., Swink, W.D., and Burnham, K.P. 2003. Mark-recapture population estimates of parasitic Sea Lampreys (*Petromyzon marinus*) in Lake Huron. J. Great Lakes Res. 29 (Suppl. 1): 226–239.

Bills, T.D., and Marking, L.L. 1976. Toxicity of 3-Trifluoromethyl-4-nitrophenol (TFM), 2',5-dichloro-4'-nitrosalicylanilidae (Bayer 73), and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of Coho Salmon. U.S. Fish and Wildlife Service, Investigations in Fish Control 69: 24 p.

Boogaard, M.A., Erickson, R.A., and Hubert, T.D. 2016. Evaluation of avoidance behavior of Tadpole Madtoms (*Noturus gyrinus*) as a surrogate for the endangered Northern Madtom (*Noturus stigmosus*) in response to granular Bayluscide. USGS Report 2016-1130: 11p.

Coker, G.A., Portt, C.B., and Minns, C.K. 2001. Morphological and ecological characteristics of Canadian freshwater fishes. Can. MS Rpt. Fish. Aquat. Sci. 2554: iv + 89 p.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2011. COSEWIC assessment and status report on the Silver Lamprey, Great Lakes – Upper St. Lawrence populations and Saskatchewan – Nelson Rivers populations *Ichthyomyzon unicuspis* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, ON. xiii + 55 p.

Dawson, V.K. 2003. Environmental fate and effects of the lampricide Bayluscide: a Review. J. Great Lakes Res. 29 (Suppl. 1): 475–492

Delignette-Muller, M.L., Dutang, C., Pouillot, R., Denis, J.B., and Siberchicot, A. 2020. Help to Fit of a Parametric Distribution to Non-Censored or Censored Data.

Docker, M.F., Mandrak, N.E., and Heath, D.D. 2012. Contemporary gene flow between "paired" silver (*Ichthyomyzon unicuspis*) and northern brook (*I. fossor*) lampreys: implications for conservation. Conserv. Genet. 13(3): 823–835.

Dunham, J.B., Rieman, B.E., and Peterson, J.T. 2002. Patch-based models to predict species occurrence: lessons from salmonid fishes in streams. *In* Predicting species occurrences: Issues of scale and accuracy. Edited by J. M. Scott, P.J. Heglund, M.L. Morrison, J.B. Haufler, M.G. Raphael, W.A. Wall, and F.B. Samson. Island Press, Washington, D.C. pp. 327–334

Finch, M., Koops, M.A., Doka, S.E., and Power, M. 2018. Population viability and perturbation analyses to support recovery of imperilled Eastern Sand Darter (*Ammocrypta pellucida*). Ecol. Freshw. Fish. 27(1): 378–388.

Fleeger, J.W., Carman, K.R., and Nisbet, R.M. 2003. Indirect effects of contaminants in aquatic ecosystems. Sci. Total. Environ. 317: 207–233.

Hayes, D.B., and Caroffino, D.C. 2012. Michigan's Lake Sturgeon rehabilitation strategy. Michigan Department of Natural Resources, Fisheries Special Report 62: 26 p.

Hutton, M. 2012. Juvenile and young of year Lake Sturgeon in the Detroit River east of fighting island with a focus on macroinvertebrates and substrate. Thesis (Senior Honors) University of New England, Biddeford, N.E. 46 p.

Kessel, S.T., Hondorp, D.W., Holbrook. C.M., Boase, J.C., Chiotti, J.A., Thomas, M.V., Wills, T.C., Roseman, E.F., Drouin. R., and Krueger, C.C. 2018. Divergent migration within Lake Sturgeon (*Acipenser fulvescens*) populations: Multiple distinct patterns exist across an unrestricted migration corridor. J. Anim. Ecol. 87(1): 259–273.

Marking, L.L., and Hogan, J.W. 1967. Toxicity of Bayer 73 to fish. U.S. Fish and Wildlife Service, Investigations in Fish Control 19: 14 p.

Metcalfe-Smith, J.L., Maio, J.D., Staton, S.K., and Mackie, G.L. 2000. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. J. North Am. Benthol. Soc. 19(4): 725–732.

Metcalfe-Smith, J.L., McGoldrick, D.J., Zanatta, D.T., and Grapentine, L.C. 2007. Development of a monitoring program for tracking the recovery of endangered freshwater mussels in the Sydenham River, Ontario. Environment Canada, WSTD Contribution No. 07–510: 63 p.

Minns, C.K. 2003. An Area-Per-individual (API) Model for estimating critical habitat requirements in Aquatic Species-At-Risk. DFO Can. Sci. Advis. Sec. Res. Doc. 2003/074. ii + 21p.

Newton, T.J., Boogaard, M.A., Gray, B.R., Hubert, T.D., and Schloesser, N.A. 2017. Lethal and sub-lethal responses of native freshwater mussels exposed to granular Bayluscide®, a sea lamprey larvicide. J. Great Lakes Res.43(2): 370–378

Peterson, J.L., Jepson, P.C., and Jenkins, J. 2001. Effect of varying pesticide exposure duration and concentration on the toxicity of carbaryl to two field-collected stream invertebrates, *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae). Environ. Toxicol. Chem. 20(10): 2215–2223.

Quinlan, C., and Maaskant, K. 2017. 2017 Upper Thames River Watershed Report Cards. Upper Thames River Conservation Authority, London, ON. vi + 91 p. + appendices.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ren, J., Buchinger, T., Pu, J., Jia, L., and Li, W. 2014. Complete mitochondrial genomes of paired species northern brook lamprey (*Ichthyomyzon fossor*) and silver lamprey (*I. unicuspis*). Mitochondrial DNA 27(3): 1862–1863.

Richter, B.D., Braun, D.P., Mendelson, M.A., and Master, L.L. 1997. Threats to imperiled freshwater fauna. Conserv. Biol. 11(5): 1081–1093.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., and Arnold, K.E. 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology, and evolution of wildlife. Proc. R. Soc. Biol. Sci. Ser. B 285(1885): 20181297.

Schloesser, D.W., Metcalfe-Smith, J.L., Kovalak, W.P., Longton, G.D., and Smithee, R.D. 2006. Extirpation of freshwater mussels (*Bivalvia: Unionidae*) following the invasion of Dressenid mussels in an interconnecting river of the Laurentian Great Lakes. Am. Midl. Nat. 155(2): 307–320.

Scholefield, R.J., and Seelye, J.G. 1992. Toxicity of 2',5-Dichloro-4'-Nitrosalicylanilide (Bayer 73) to Three Genera of Larval Lampreys. GLFC Technical Report 57: pp. 1-6.

Schreier, T.M., Dawson, V.K., and Larson, W. 2008. Effectiveness of piscicides for controlling Round Gobies (*Neogobius melanostomus*). J. Great Lakes Res. 34(2): 253–264.

SCRCA (St. Clair Region Conservation Authority). 2017. Sydenham River Watershed helping species at risk – June 2017. (Accessed October 1 2018)

Smith, B.R., and Tibbles, J.J. 1980. Sea Lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936-78. Can. J. Fish. Aquat. Sci. 37: 1780–1801.

Smyth, E.R.B. 2011. A quantitative evaluation of fish passage options for the dam on the Black Sturgeon River. Thesis (M.Sc.) University of Guelph, Guelph, ON. 117 p.

USFWS (United States Fish and Wildlife Service) and DFO (Fisheries and Oceans Canada). 2016. Procedure for application of Bayluscide 3.2% granular Sea Lamprey larvicide for assessment or control applications. Technical Operating Procedures TOP017.11: 7 p.

Van Ginneken, M., Blust, R., and Bervoets, L. 2017. How lethal concentration changes over time: Toxicity of cadmium, copper, and lead to the freshwater isopod *Asellus aquaticus*. Environ. Toxicol. Chem. 36(10): 2849–2854.

Vélez-Espino, L.A., Randall, R.G., and Koops, M.A. 2009. Quantifying habitat requirements of four freshwater species at risk in Canada: Northern Madtom, Spotted Gar, Lake Chubsucker, and Pugnose Shiner. DFO Can. Sci. Advis. Sec. Res. Doc. 2009/115. iv + 21.

Venturelli, P.A., Vélez-Espino, L.A., and Koops, M.A. 2010. Recovery Potential Modelling of Channel Darter (*Percina copelandi*) in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2010/096. iv + 20 p.

Wilkie, M.P., Hubert, T.D., Boogaard, M.S. and Birceanu, O. 2019. Control of invasive sea lampreys using the piscides TFM and niclosamide: Toxicology, successes & future prospects. Aquat. Toxicol. 211: 235–252.

APPENDIX A: DFO FISH AND MUSSEL FIELD SAMPLING DATA SUMMARIES USED IN BAYLUSCIDE ASSESSMENT

Table A1. Fish sampling in the four focal rivers completed by DFO.

System	Year	Sampling Gear Type	# of Sampling Sites
Detroit River	2004	Boat Electrofishing	9
Detroit River	2007	Boat Electrofishing	3
Detroit River	2009	Trapline	2
Detroit River	2009	Trawl	4
Detroit River	2010	Trawl	7
Detroit River	2011	Boat Electrofishing	3
Detroit River	2011	Trawl	37
Detroit River	2013	Boat Electrofishing	1
Detroit River	2013	Mini Fyke Net	7
Detroit River	2013	Trawl	19
Detroit River	2013	Trammel Net	2
Detroit River	2014	Boat Electrofishing	12
Detroit River	2014	Gill Net	1
Detroit River	2014	Mini Fyke Net	1
Detroit River	2014	Trap Net	4
Detroit River	2014	Trammel Net	2
Detroit River	2015	Boat Electrofishing	18
Detroit River	2015	Mini Fyke Net	6
Detroit River	2015	Trap Net	4
Detroit River	2016	Boat Electrofishing	22
Detroit River	2016	Boat Electrofishing & Trammel	1
Detroit River	2016	Mini Fyke Net	13
Detroit River	2016	Seine	5
Detroit River	2016	Trap Net	4
Detroit River	2016	Trammel Net	2
Detroit River	2017	Boat Electrofishing	19
Detroit River	2017	Mini Fyke Net	2
Detroit River	2017	Trap Net	1
St. Clair River	2003	Boat Electrofishing	1
St. Clair River	2003	Backpack Electrofishing	3
St. Clair River	2003	Seine	9
St. Clair River	2004	Boat Electrofishing	12
St. Clair River	2006	Boat Seine	2
St. Clair River	2007	Boat Electrofishing	16
St. Clair River	2010	Seine	7
St. Clair River	2010	Trawl	10
St. Clair River	2012	Boat Electrofishing	10
St. Clair River	2012	Trawl	34
St. Clair River	2013	Seine	19
St. Clair River	2013	Trawl	28
St. Clair River	2014	Boat Electrofishing	5
St. Clair River	2014	Seine	34
St. Clair River	2014	Trawl	10

System	Year	Sampling Gear Type	# of Sampling Sites
St. Clair River	2015	Trawl	13
St. Clair River	2016	Mini Fyke Net	18
St. Clair River	2016	Seine	59
Sydenham River	2003	Backpack Electrofishing	14
Sydenham River	2003	Seine	15
Sydenham River	2004	Backpack Electrofishing	8
Sydenham River	2004	Seine	1
Sydenham River	2005	Backpack Electrofishing	5
Sydenham River	2007	Seine	3
Sydenham River	2009	Seine	6
Sydenham River	2010	Backpack Electrofishing	8
Sydenham River	2010	Seine	29
Sydenham River	2010	Trawl	4
Sydenham River	2012	Seine	90
Sydenham River	2012	Trawl	14
Sydenham River	2013	Seine	48
Sydenham River	2015	Boat Electrofishing	21
Sydenham River	2015	Mini Fyke Net	3
Sydenham River	2015	Seine	16
Sydenham River	2015	Trap Net	1
Sydenham River	2015	Trammel Net	1
Sydenham River	2016	Boat Electrofishing	20
Sydenham River	2016	Mini Fyke Net	7
Sydenham River	2016	Seine	6
Sydenham River	2017	Boat Electrofishing	23
Sydenham River	2017	Drift Net	6
Sydenham River	2017	Mini Fyke Net	4
Sydenham River	2017	Trap Net	2
Thames River	2003	Boat Electrofishing	3
Thames River	2003	Seine	4
Thames River	2004	Boat Electrofishing	13
Thames River	2004	Backpack Electrofishing	2
Thames River	2004	Seine	93
Thames River	2005	Boat Electrofishing	15
Thames River	2005	Backpack Electrofishing	1
Thames River	2005	Seine	231
Thames River	2006	Seine	21
Thames River	2007	Seine	4
Thames River	2008	Seine	1
Thames River	2009	Seine	3
Thames River	2009	Trawl	37
Thames River	2010	Trawl	6
Thames River	2011	Seine	124
Thames River	2012	Trawl	42
Thames River	2013	Boat Electrofishing	3
Thames River	2013	Hoop Net	4
Thames River	2013	Mini Fyke Net	1
Thames River	2013	Trawl	42

System	Year	Sampling Gear Type	# of Sampling Sites
Thames River	2013	Trammel Net	2
Thames River	2014	Boat Electrofishing	9
Thames River	2014	Trap Net	1
Thames River	2014	Trawl	32
Thames River	2014	Trammel Net	3
Thames River	2015	Boat Electrofishing	16
Thames River	2015	Mini Fyke Net	4
Thames River	2015	Seine	1
Thames River	2015	Trap Net	2
Thames River	2015	Trawl	91
Thames River	2015	Trammel Net	10
Thames River	2016	Boat Electrofishing	17
Thames River	2016	Mini Fyke Net	12
Thames River	2016	Seine	7
Thames River	2016	Trap Net	1
Thames River	2016	Trawl	18
Thames River	2017	Boat Electrofishing	15
Thames River	2017	Mini Fyke Net	2
Thames River	2017	Seine	12

Table A2. Timed search mussel sampling conducted in Sydenham River.

Year	Sampling Period (d-m-y)	Number of Sites
1997	18-21-Aug-97	7
1997	25-Sep-97	2
1998	23-Jun-98	1
1998	24-28-Aug-98	8
1999	18-May-99	1
1999	28-29-Jul-99	2
1999	5-6-Oct-99	3
2000	6-Jul-00	1
2001	-[1]	14
2001	21-Jun-01	1
2002	28-31-May-02	4
2002	6-Jun-02	1
2002	10-Jun-02	1
2002	18-19-Jun-02	2
2002	4-5-Jul-02	3
2002	9-11-Jul-02	5
2002	22-24-Jul-02	3
2002	30-31-Jul-02	4
2002	6-7-Aug-02	3
2002	13-Aug-02	1
2002	20-Aug-02	1
2002	29-30-Aug-02	5
2003	11-Jun-03	2
2003	16-17-Jul-03	2
2003	23-24-Jul-03	2
2003	6-Aug-03	1
2003	19-Aug-03	2
2004	28-Jul-04	1
2004	9-Aug-04	1
2005	1-Jan-05	6
2005	7-Jul-05	1
2005	26-Jul-05	1
2005	11-Aug-05	1
2006	1-Jan-06	4
2006	24-May-06	1
2006	17-Aug-06	1
2007	7-Jun-07	1
2007	1-Aug-07	1
2008	1-Jan-08	6
2008	17-Jun-08	1
2008	29-Jul-08	1
2008	13-Aug-08	1
2008	25-26-Aug-08	2
2008	4-Sep-08	3
2008	11-Sep-08	1
2009	12-Jun-09	1
2009	25-26-Jun-09	2
2009	20-22-Jul-09	3
2009	24-Jul-09	1
2009	28-Jul-09	1

Year	Sampling Period (d-m-y)	Number of Sites
2009	18-Aug-09	2
2009	15-Sep-09	4
2010	29-Jul-10	1
2010	12-Aug-10	1
2010	18-Aug-10	1
2011	15-Jun-11	1
2011	2-Aug-11	2
2011	17-Aug-11	2
2011	24-Aug-11	1
2011	12-Sep-11	1
2012	8-Jun-12	1
2012	20-Jun-12	1
2012	3-Aug-12	1
2012	13-Aug-12	1
2012	30-Aug-12	1
2013	8-May-13	1
2013	14-15-May-13	2
2013	19-Jun-13	1
2013	19-Jul-13	1
2013	27-Jul-13	1
2013	30-Jul-13	1
2013	29-30-Aug-13	2
2014	28-May-14	1
2014	13-Jun-14	1
2014	18-Jun-14	1
2014	29-Jul-14	1
2015	7-Jul-15	1
2015	15-Jul-15	1
2015	12-Aug-15	1
2015	28-Aug-15	1
2015	3-Sep-15	1
2016	5-May-16	1
2016	25-May-16	1
2016	14-Jun-16	2
2016	15-Jun-16	1
2016	9-Aug-16	2
2016	29-Aug-16	1
2016	9-Sep-16	1
2016	14-Sep-16	1
2016	6-Oct-16	1
2017	6-9-Jun-17	8
2017	12-Jun-17	3
2017	14-Jun-17	3
2017	21-Jun-17	1
2017	18-20-Jul-17	5
2017	24-26-Jul-17	9
2017	31-Jul-17	1
2017	2-Aug-17	1
2017	8-11-Aug-17	5
2017	22-24-Aug-17	5

[1] Exact date not provided for these sampling events

Table A3. Timed search mussel sampling conducted in Thames River.

Year	Sampling Period (d-m-y)	Number of Sites
1997	11-15-Aug-97	9
1997	24-Sep-97	1
1997	26-Sep-97	1
1998	11-Apr-98	1
1998	17-May-98	1
1998	31-May-98	1
1998	22-Jun-98	2
1998	12-14-Aug-98	5
2003	30-Oct-03	1
2004	5-9-Jul-04	9
2004	12-16-Jul-04	12
2004	19-20-Jul-04	4
2004	14-Sep-04	1
2004	25-Oct-04	1
2005	16-17-Aug-05	2
2005	23-Aug-05	2
2005	24-Aug-05	1
2005	12-Sep-05	1
2005	14-15-Sep-05	4
2005	7-Oct-05	2
2006	1-Jan-06	1
2006	12-Apr-06	2
2006	26-Sep-06	1
2007	7-Jul-07	1
2007	-[1]	1
2008	16-May-08	1
2008	1-Jul-08	5
2008	3-4-Jul-08	2
2008	7-11-Jul-08	5
2008	25-28-Sep-08	7
2009	26-May-09	1
2009	20-Jul-09	1
2009	-[1]	1
2010	8-Jun-10	1
2010	16-Jun-10	1
2010	18-Jun-10	1
2010	10-Jul-10	1
2010	14-Jul-10	1
2010	17-Jul-10	1
2010	19-Aug-10	1
2010	26-Aug-10	1
2010	27-Oct-10	1
2011	17-Jun-11	2
2011	29-Jun-11	1
2011	7-Jul-11	1
2011	14-Jul-11	2
2011	22-Jul-11	1
2011	16-Aug-11	3

Year	Sampling Period (d-m-y)	Number of Sites
2011	25-Aug-11	2
2011	1-Sep-11	1
2012	3-Jan-12	1
2012	23-24-Jul-12	4
2012	25-Aug-12	2
2012	31-Aug-12	2
2012	23-Sep-12	3
2013	25-Jun-13	1
2013	16-18-Jul-13	3
2013	22-Jul-13	1
2013	30-Jul-13	2
2013	6-Aug-13	6
2013	9-Aug-13	1
2013	27-Aug-13	2
2013	6-Sep-13	1
2013	19-Sep-13	1
2014	4-Jun-14	1
2014	17-Jun-14	1
2014	22-Jul-14	1
2014	24-Jul-14	1
2014	8-Aug-14	2
2015	27-Apr-15	1
2015	26-May-15	1
2015	4-Jun-15	1
2015	18-Jun-15	1
2015	12-Aug-15	2
2016	17-Jun-16	1
2016	22-Jun-16	1
2016	24-Jun-16	1
2016	23-Aug-16	1
2016	7-Oct-16	1
2016	21-Oct-16	1
2017	30-Nov-16	1
2017	10-Sep-17	1
2017	14-Sep-17	2

[1] No exact date provided for these sampling events

Table A4. Quadrat mussel sampling conducted in Sydenham River.

Year	Sampling Period (d-m-y)	Sampled Sites	Sampled Blocks	Sampled Quadrats
1999	27-Jul-99	1	26	78
1999	9-Aug-99	1	23	69
2001	30-Jul-01	1	27	80
2001	7-Aug-01	1	25	75
2001	12-Sep-01	1	20	80
2002	22-Jul-02	1	26	78
2002	12-Aug-02	1	24	72
2002	26-Aug-02	1	24	72
2002	4-Sep-02	1	27	81
2003	12-Aug-03	1	25	75
2003	13-Aug-03	1	23	69
2003	26-Aug-03	1	27	81
2003	2-Sep-03	3	77	231
2012	25-26-Jun-12	1	24	72
2012	4-Jul-12	1	25	75
2012	9-10-Jul-12	1	25	75
2012	25-26-Jul-12	1	25	75
2012	7-14-Aug-12	1	25	73
2013	17-18-Jul-13	1	25	75
2013	22-23-Jul-13	1	25	75
2013	29-30-Jul-13	1	25	75
2013	14-15-Aug-13	1	25	75
2013	19-20-Aug-13	1	25	75
2015	27-29-Jul-15	1	25	75
2015	10-13-Aug-15	1	25	73

Table A5. Quadrat mussel sampling conducted in Thames River.

Year	Sampling Period (d-m-y)	Sampled Sites	Sampled Blocks	Sampled Quadrats
2004	3-5-Aug-04	2	39	135
2004	9-13-Aug-04	3	67	201
2005	13-Sep-05	1	23	69
2015	22-23-Jun-15	1	25	75
2015	6-8-Jul-15	1	25	75
2015	13-15-Jul-15	1	25	75
2015	17-Aug-15	1	25	75
2016	7-9-Jun-16	1	25	75
2016	27-29-Jun-16	1	25	75
2016	11-13-Jul-16	1	25	75
2016	25-27-Jul-16	1	25	75
2016	22-24-Aug-16	1	25	75
2017	26-28-Jun-17	1	25	75

APPENDIX B: CUMULATIVE MORTALITY OUTPUTS FOR SPECIES OF CONSERVATION CONCERN FOR EACH FOCAL RIVER. SENSITIVITY RESULTS ARE PRESENTED FOR SPECIES OF CONSERVATION CONCERN WITH SUBSTANTIAL MORTALITY ESTIMATES

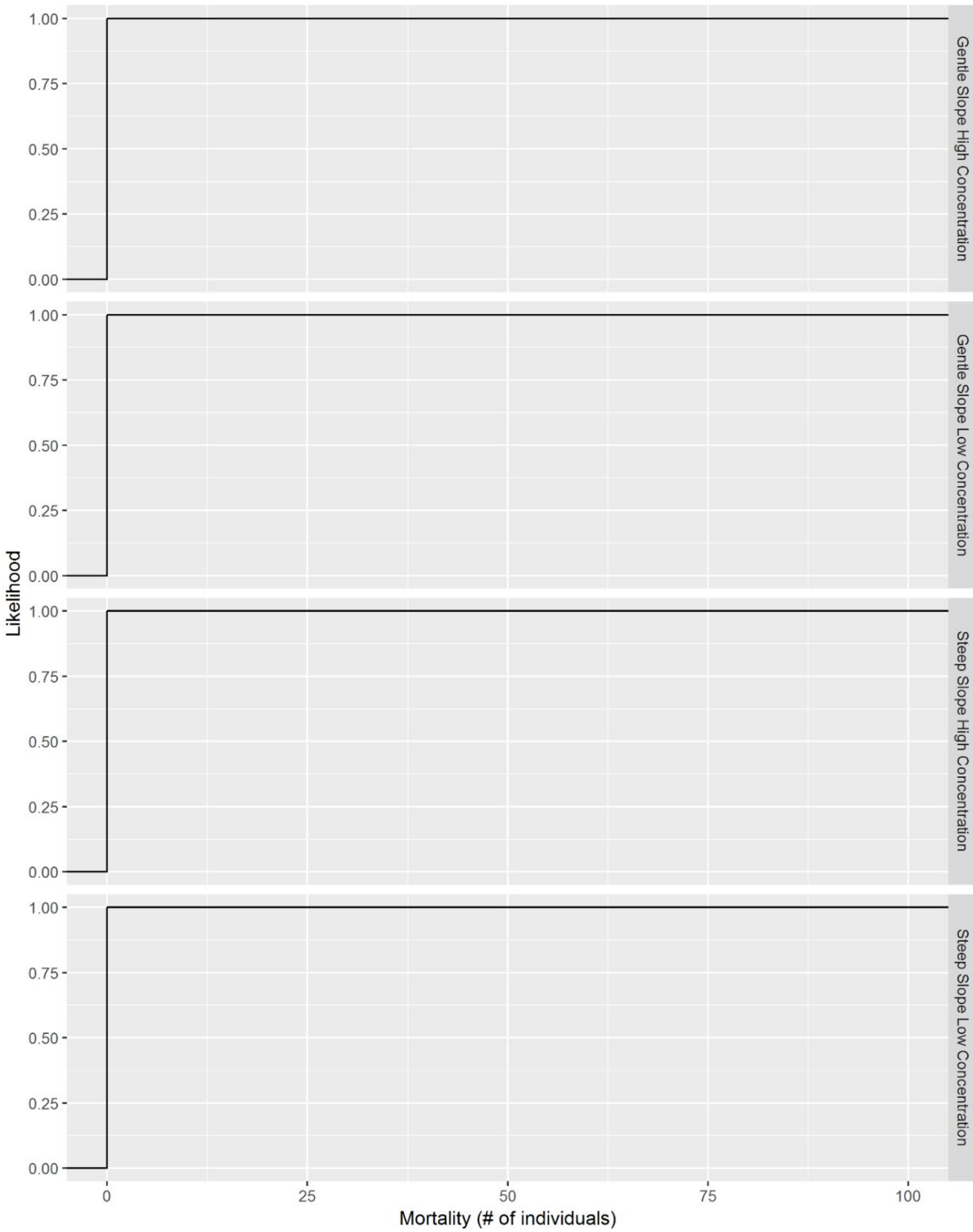

Figure B1. Cumulative likelihood results for Black Redhorse mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

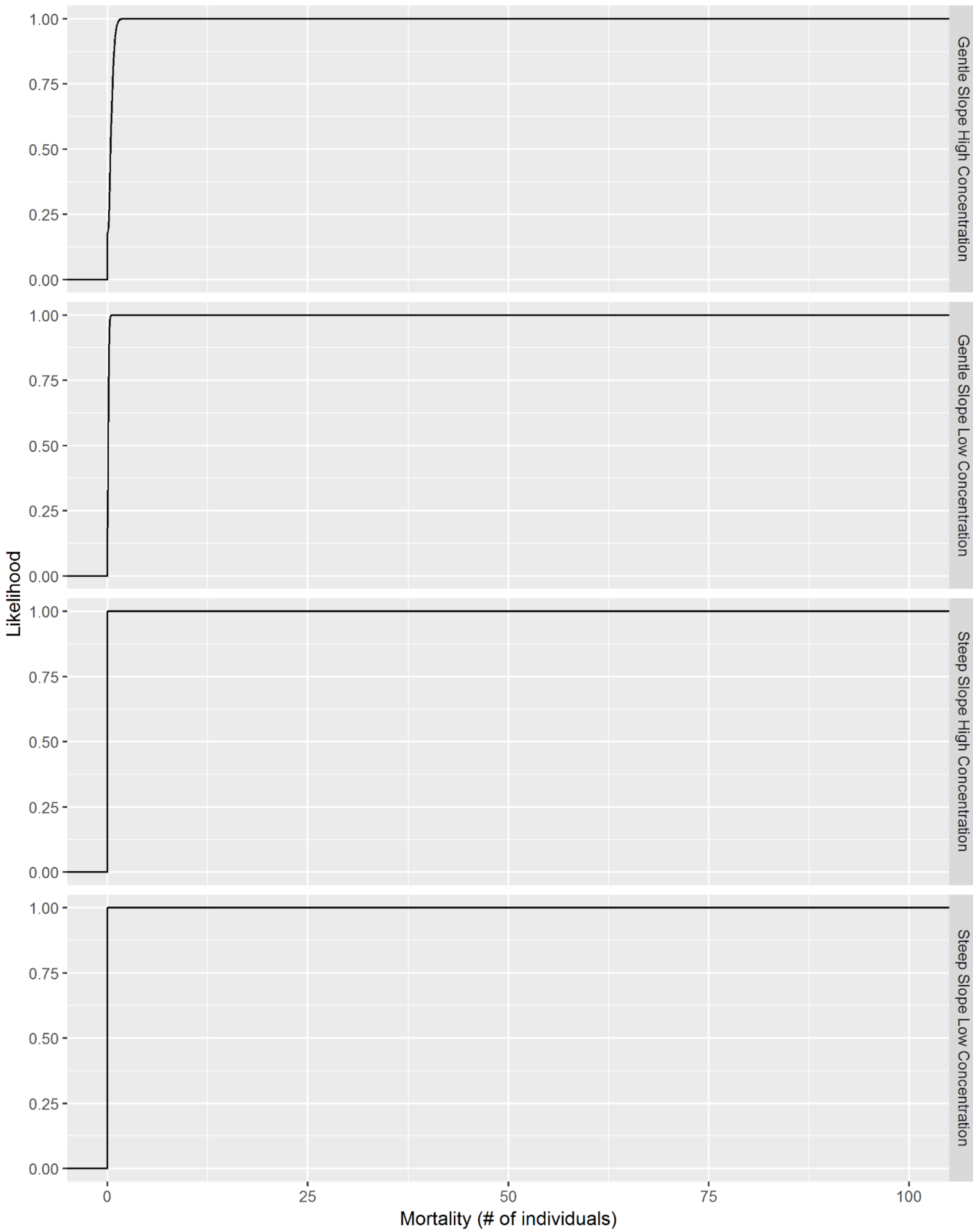

Figure B2. Cumulative likelihood results for Blackstripe Topminnow mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

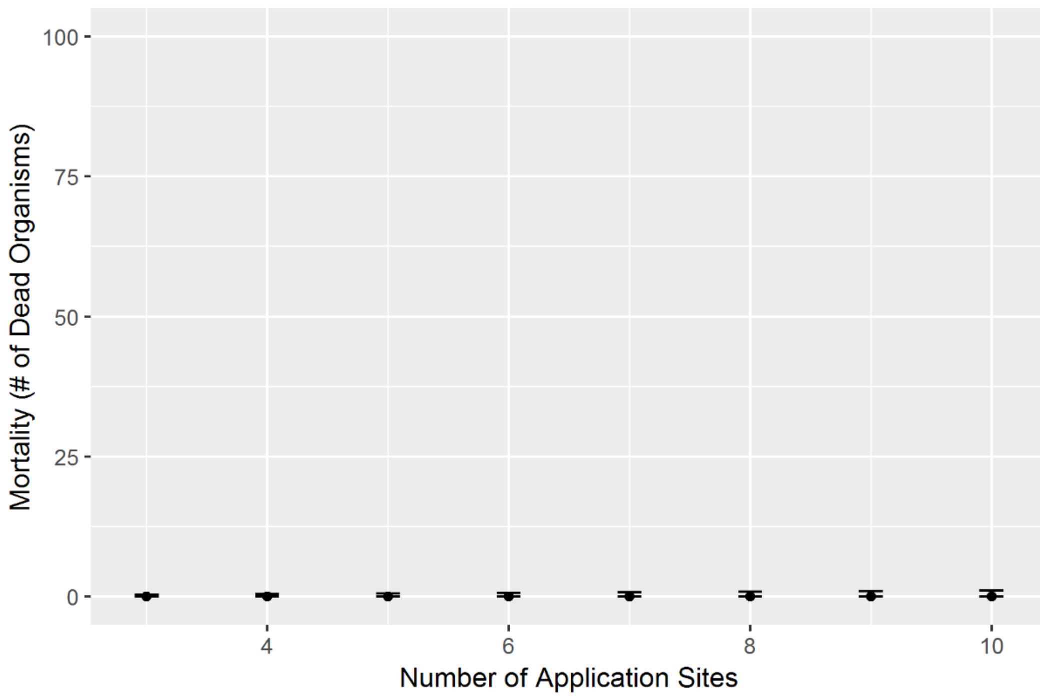

Figure B3. The influence of the number of 500 m^2 application sites on Blackstripe Topminnow mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

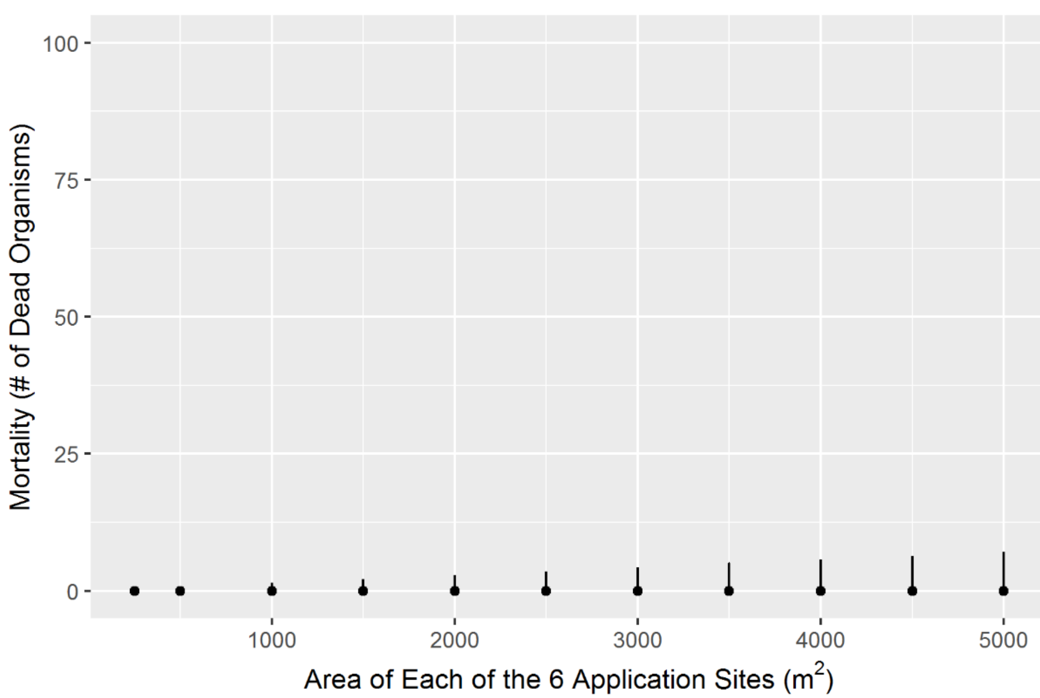

Figure B4. The influence of the size of six application sites on Blackstripe Topminnow mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

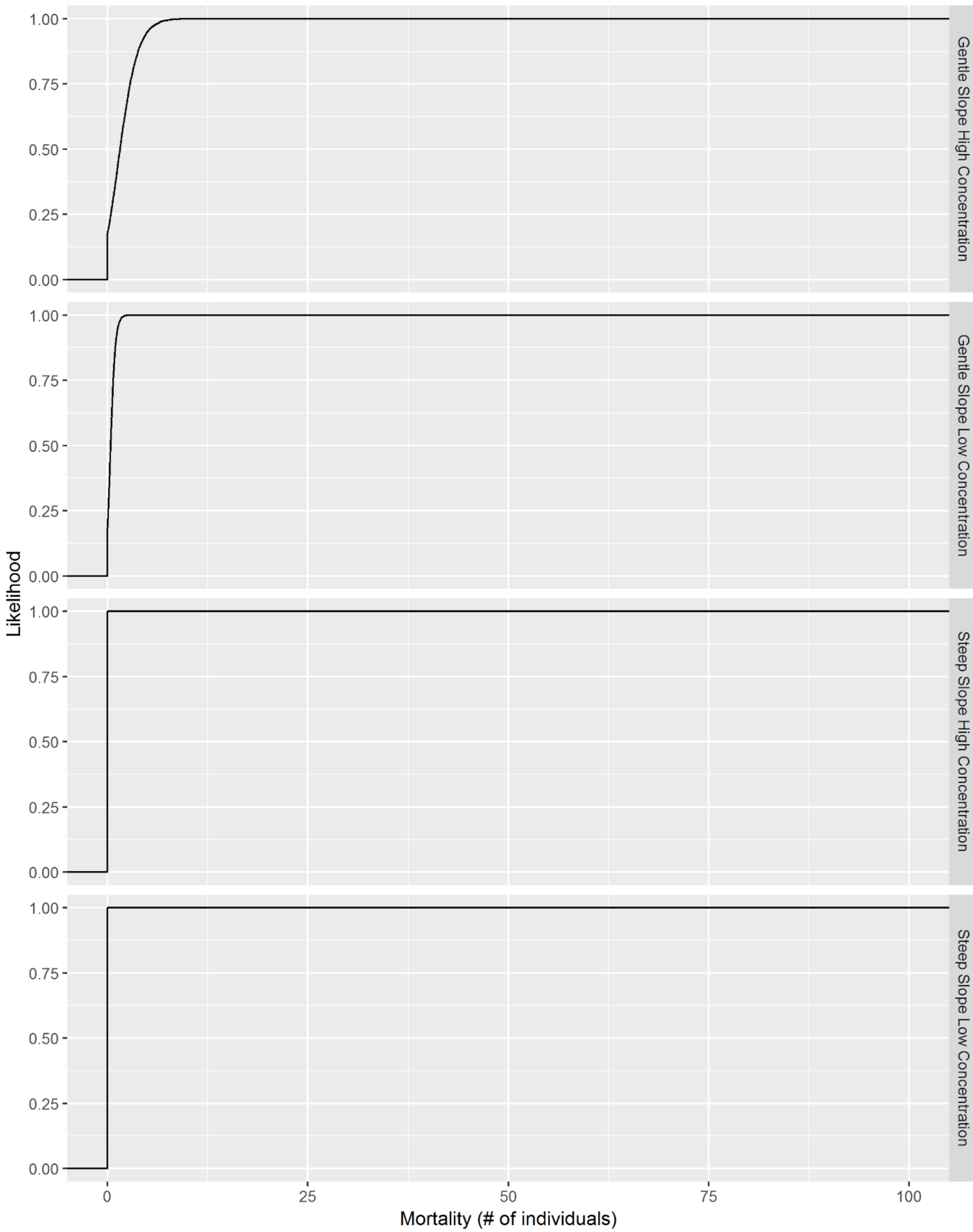

Figure B5. Cumulative likelihood results for Blackstripe Topminnow mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

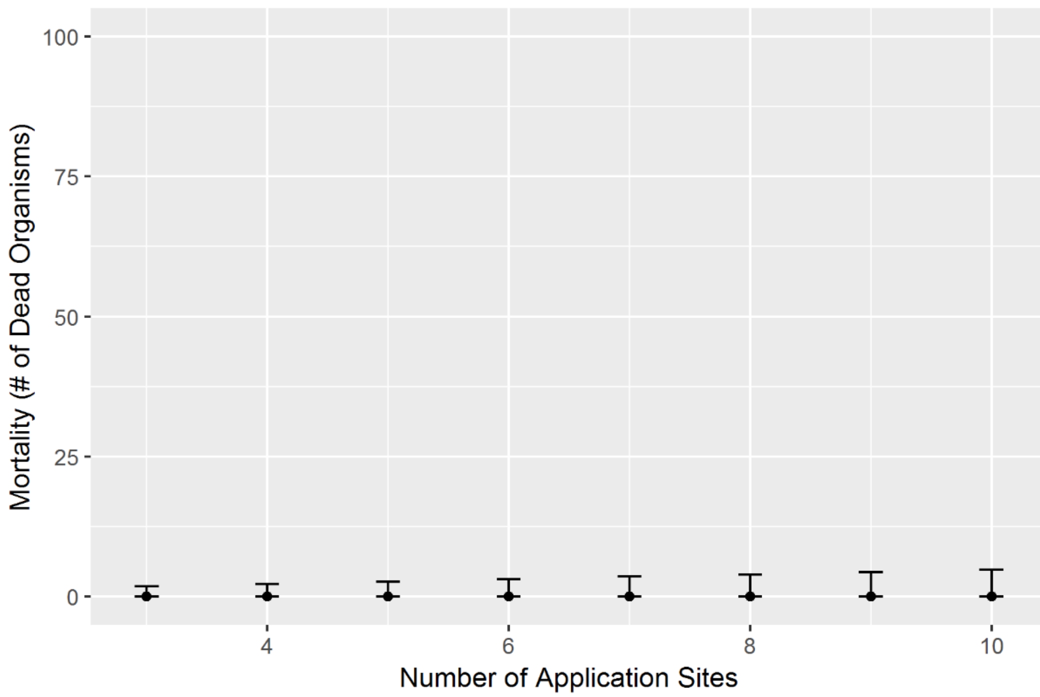

Figure B6. The influence of the number of 500 m² application sites on Blackstripe Topminnow mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

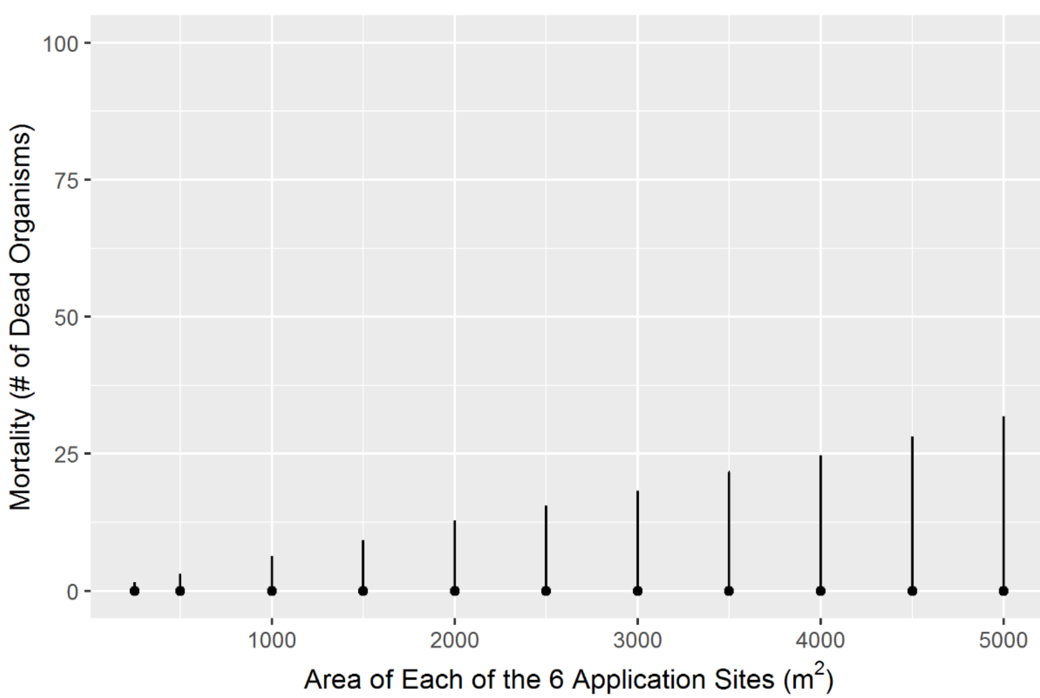

Figure B7. The influence of the size of six application sites on Blackstripe Topminnow mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

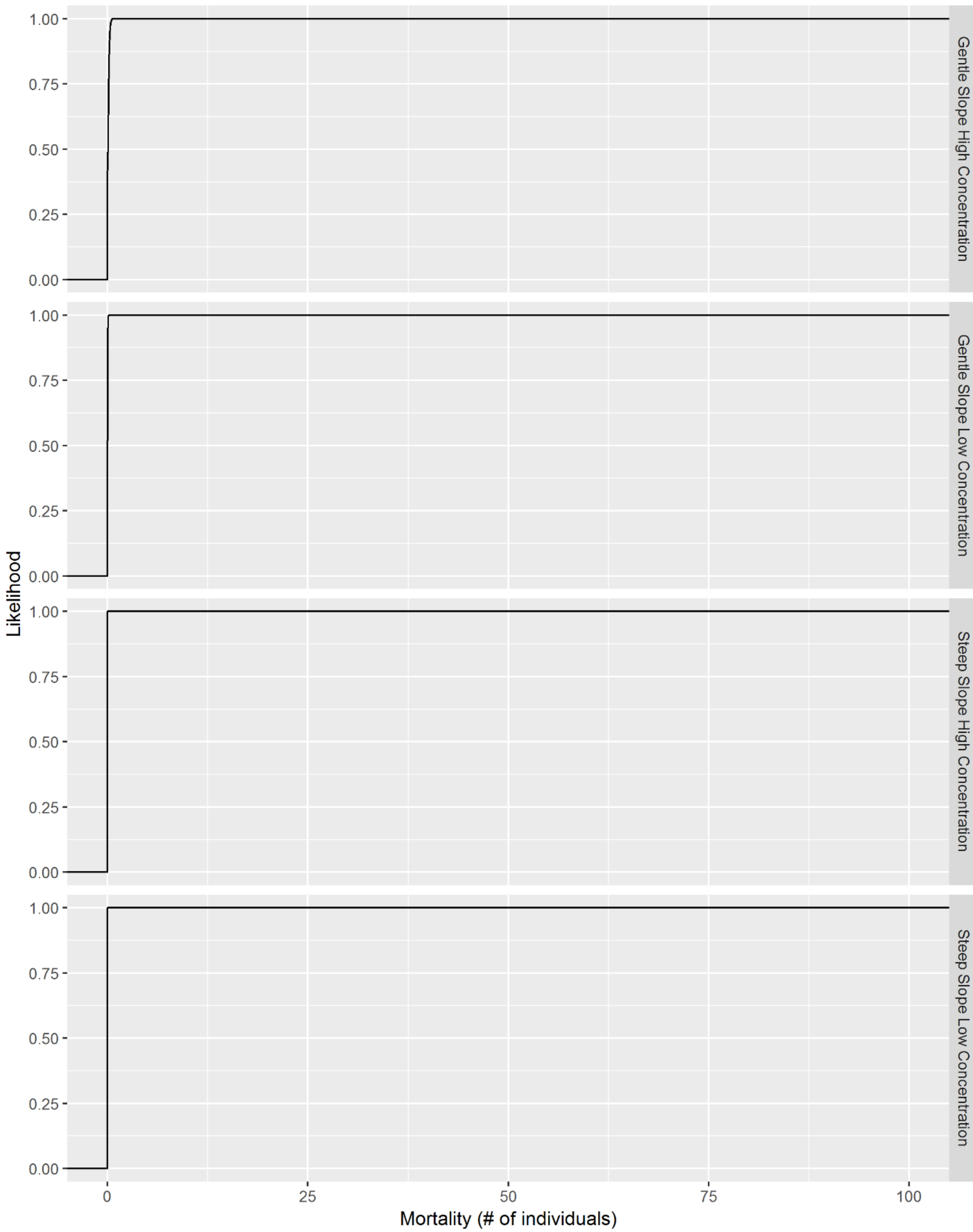

Figure B8. Cumulative likelihood results for Channel Darter mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

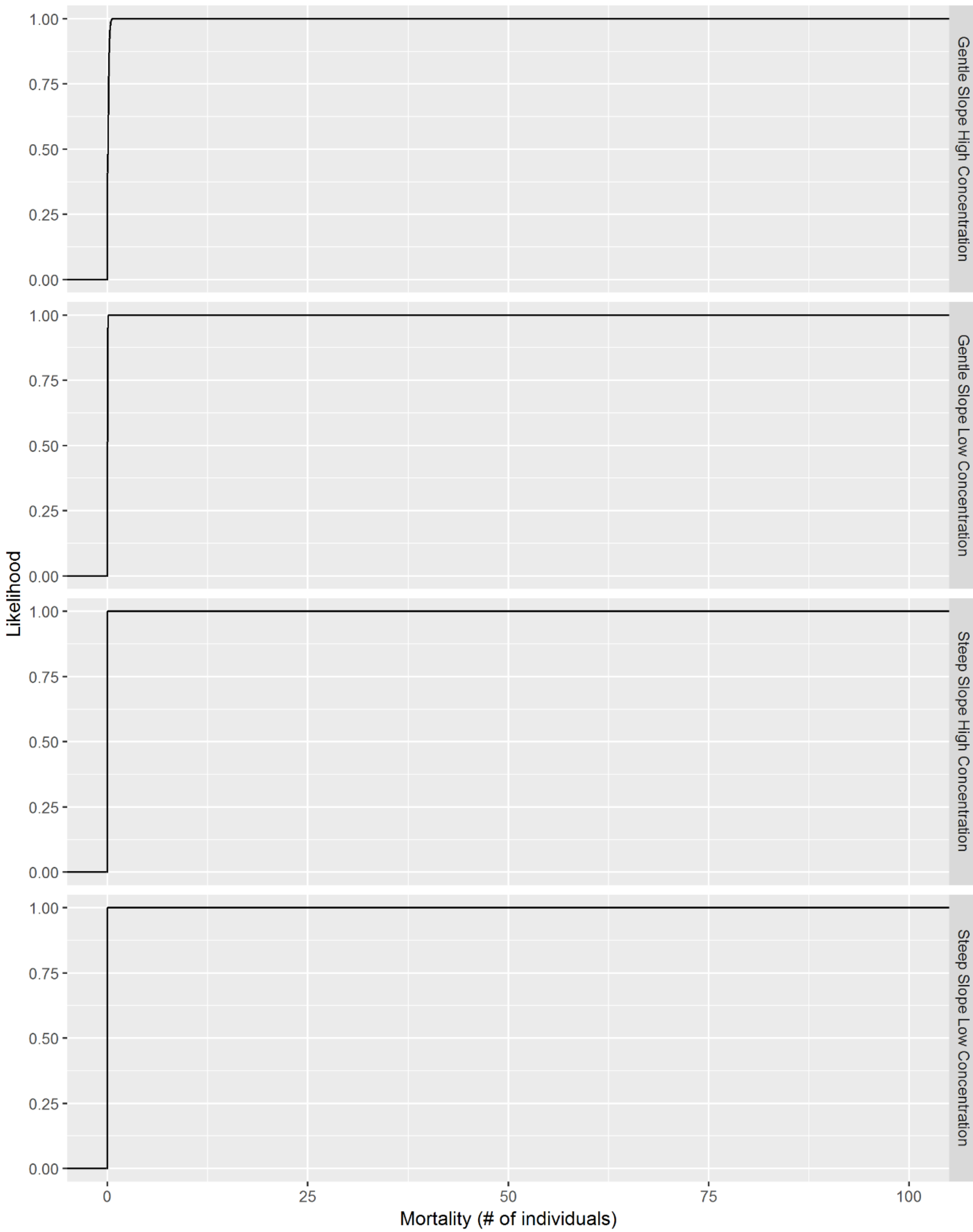

Figure B9. Cumulative likelihood results for Channel Darter mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

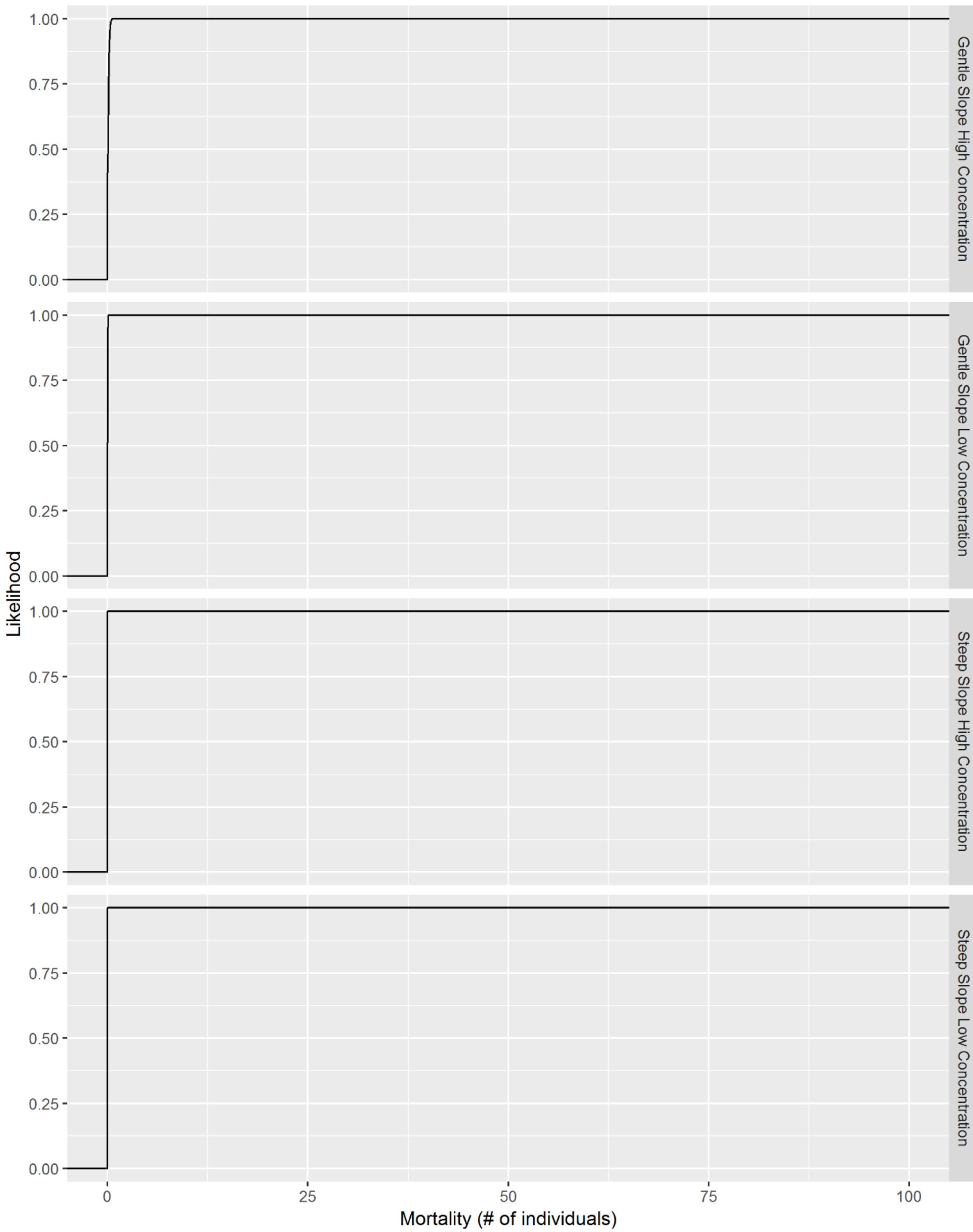

Figure B10. Cumulative likelihood results for Channel Darter mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

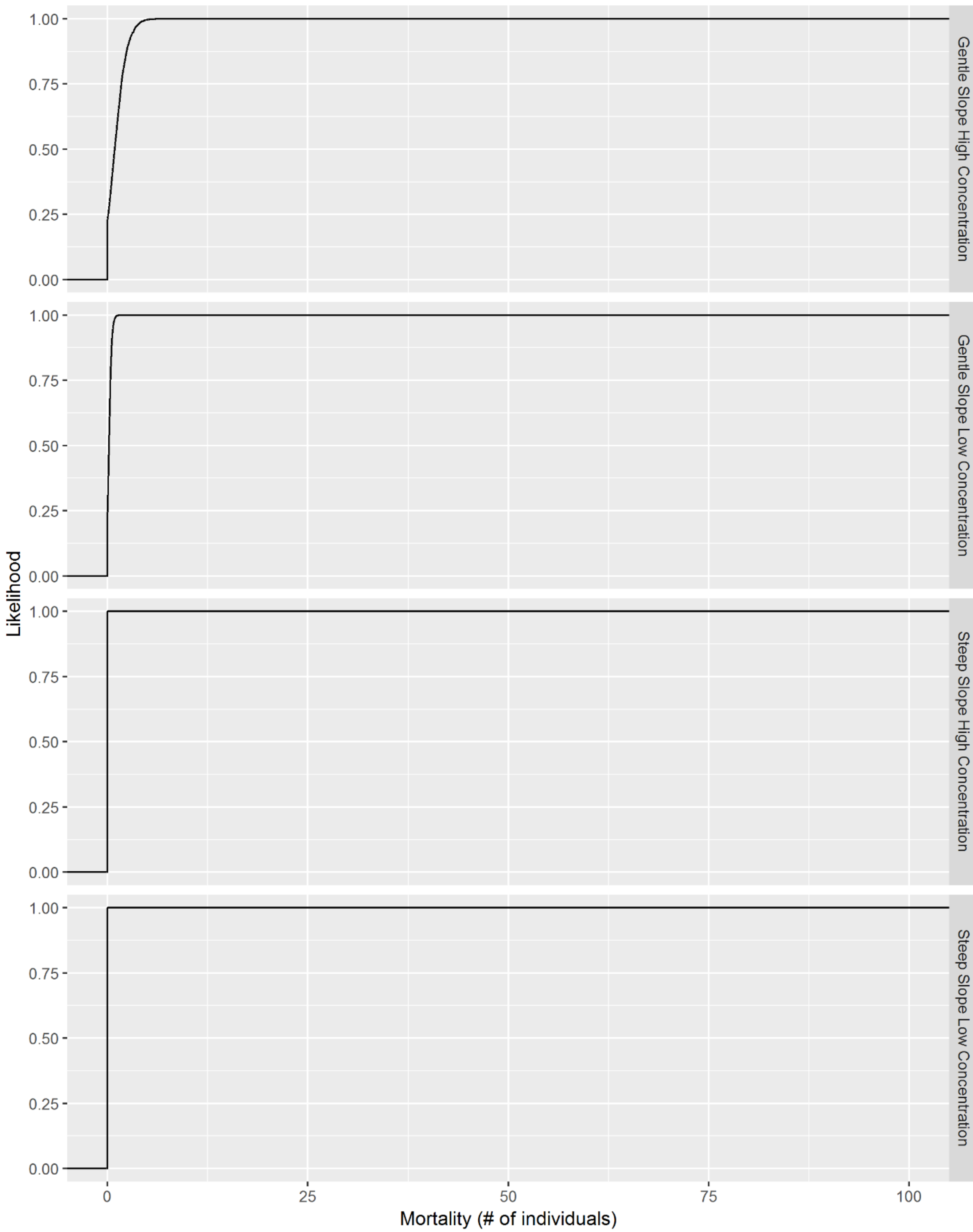

Figure B11. Cumulative likelihood results for Eastern Sand Darter mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

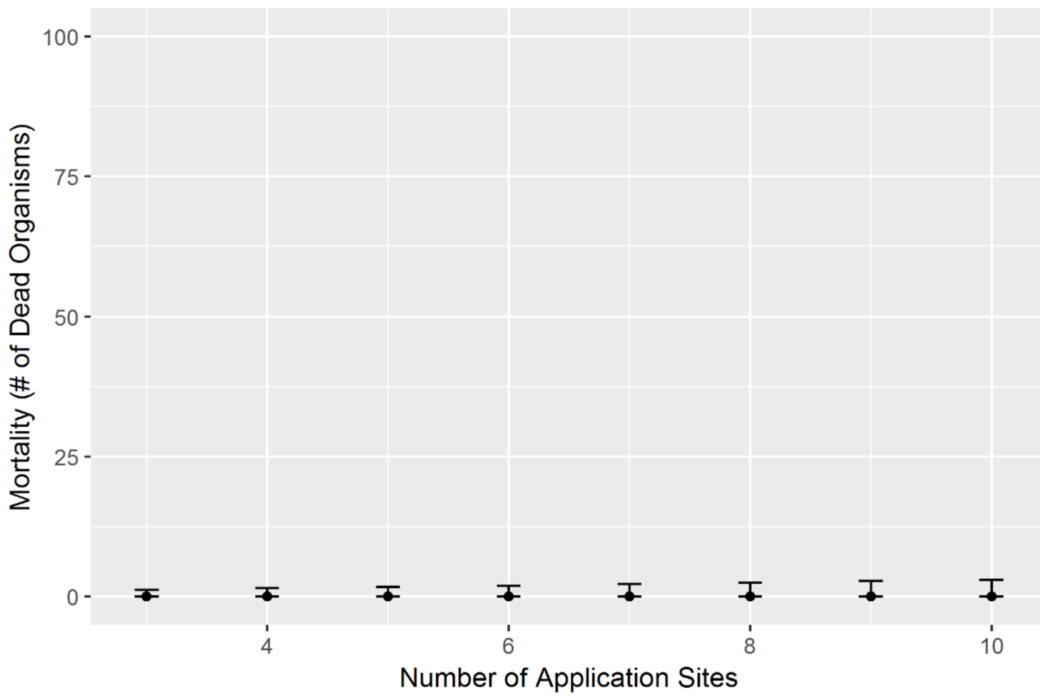

Figure B12. The influence of the number of 500 m^2 application sites on Eastern Sand Darter mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

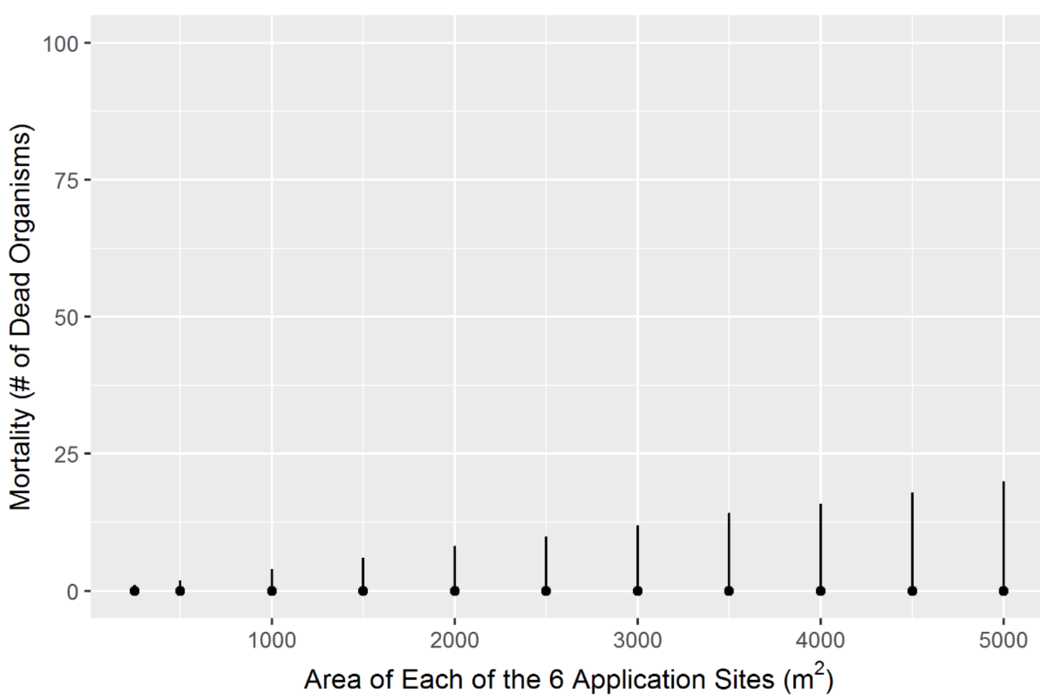

Figure B13. The influence of the size of six application sites on Eastern Sand Darter mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

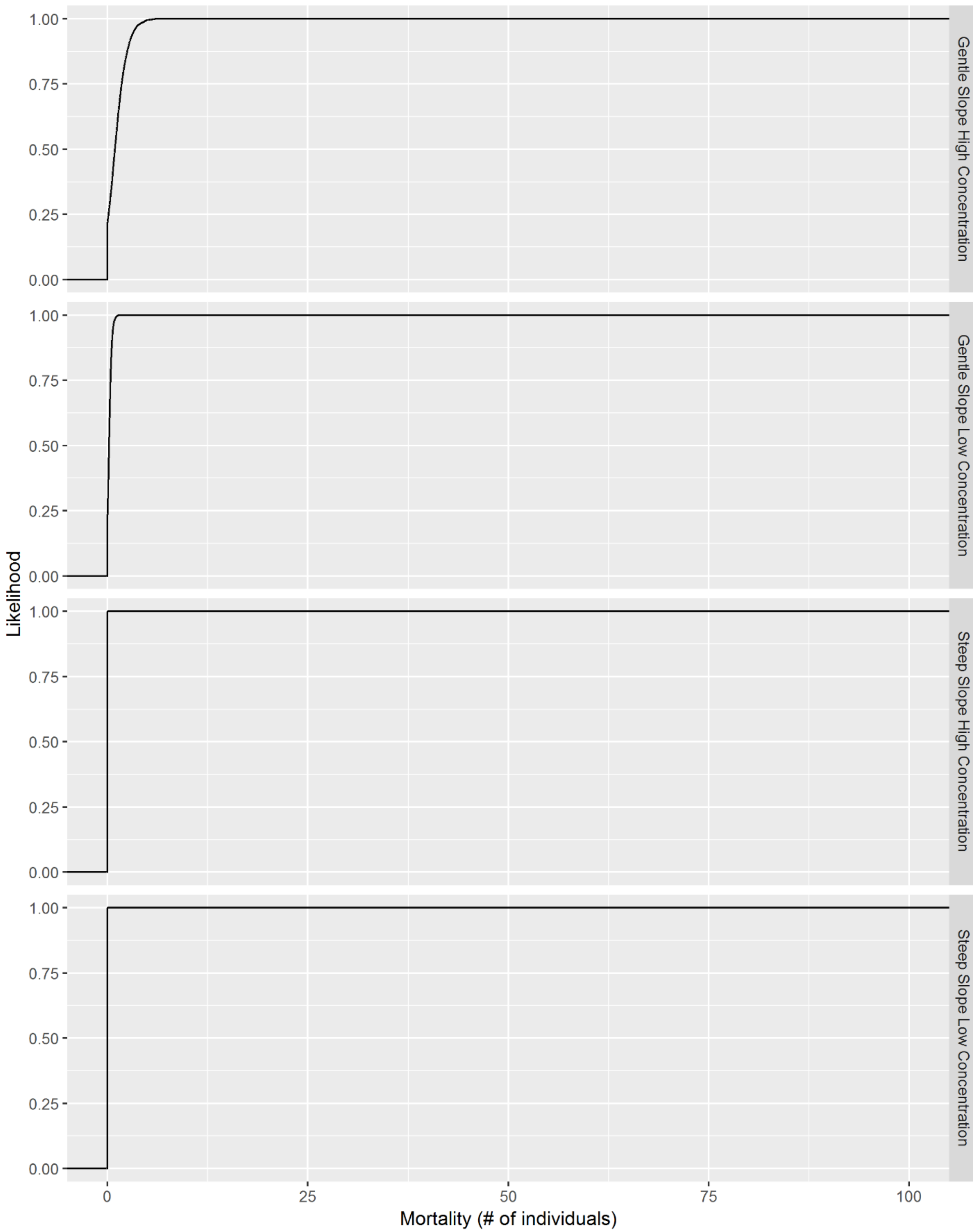

Figure B14. Cumulative likelihood results for Eastern Sand Darter mortality in the Sydenham River following Bayluscide application at six 500 m^2 sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

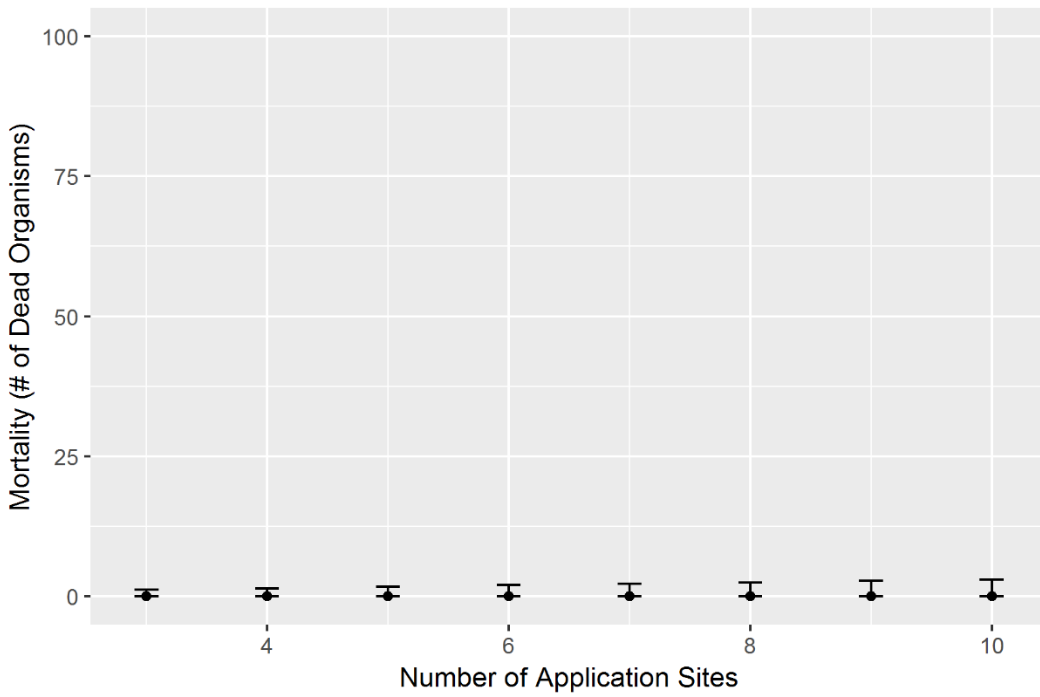

Figure B15. The influence of the number of 500 m² application sites on Eastern Sand Darter mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

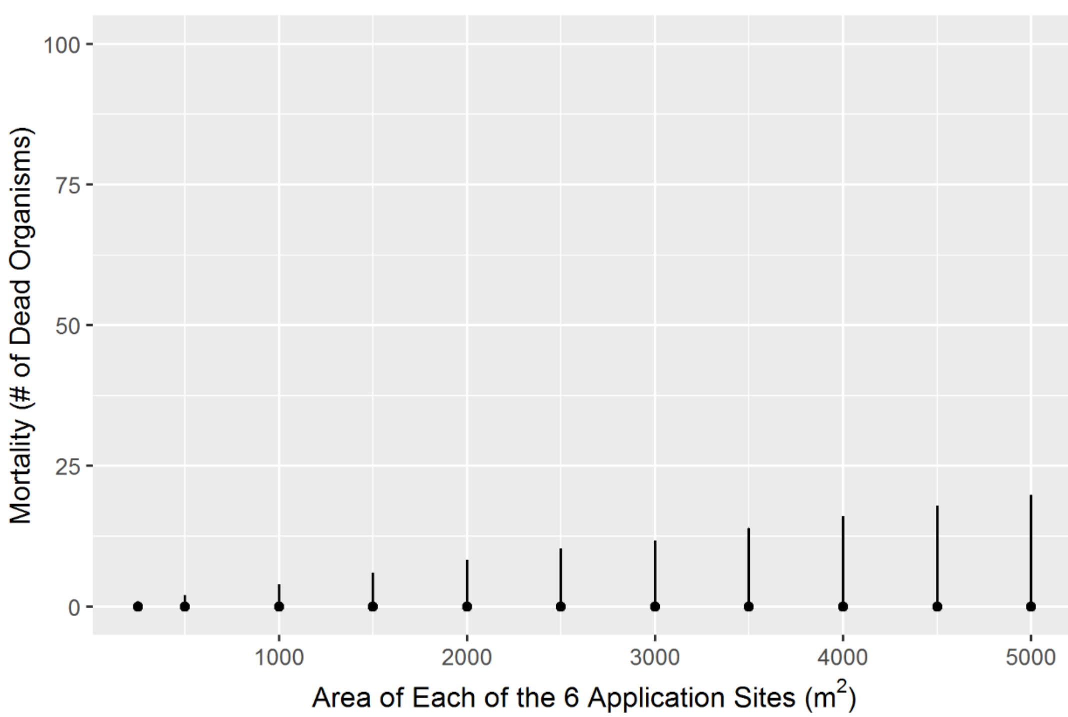

Figure B16. The influence of the size of six application sites on Eastern Sand Darter mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

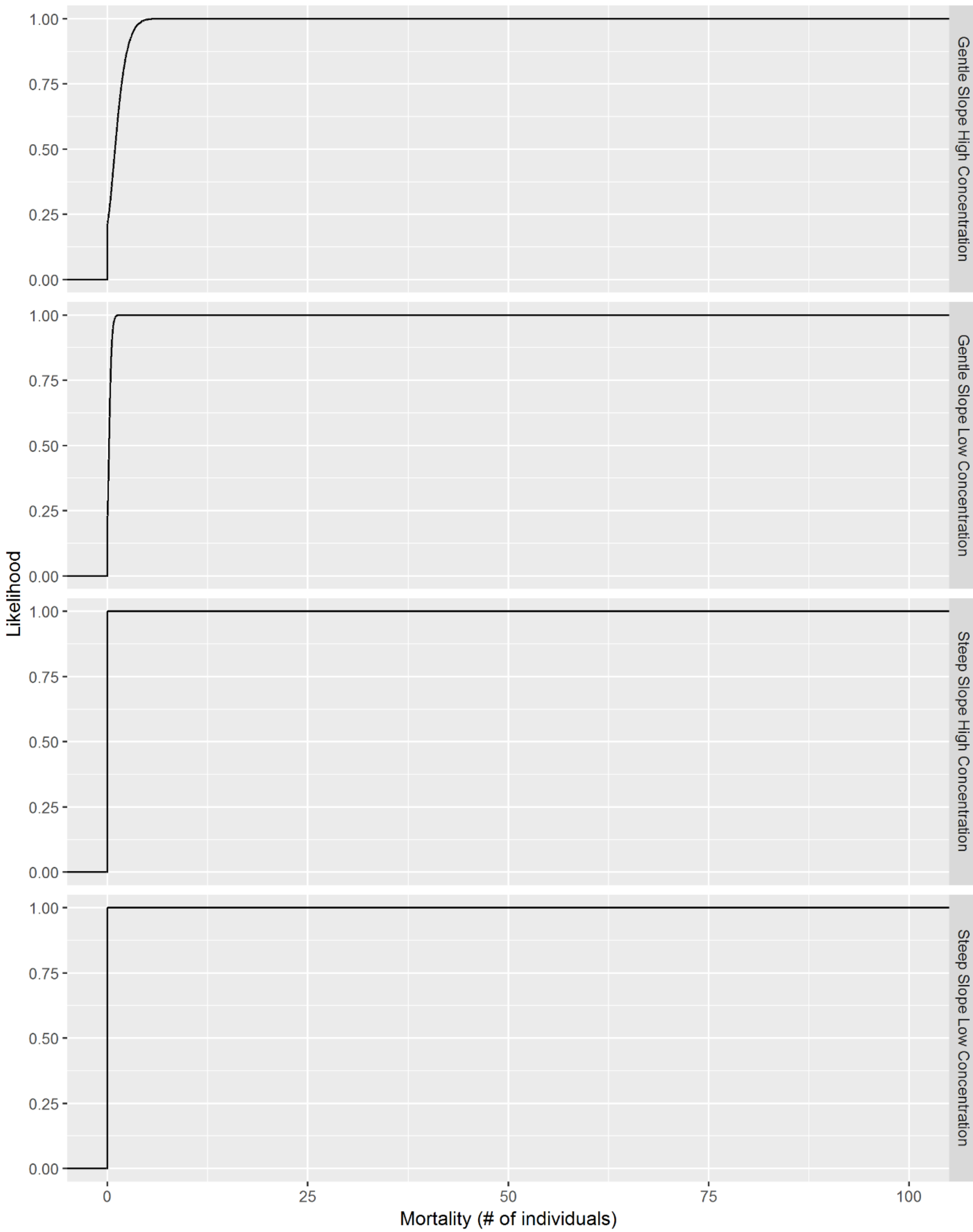

Figure B17. Cumulative likelihood results for Eastern Sand Darter mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

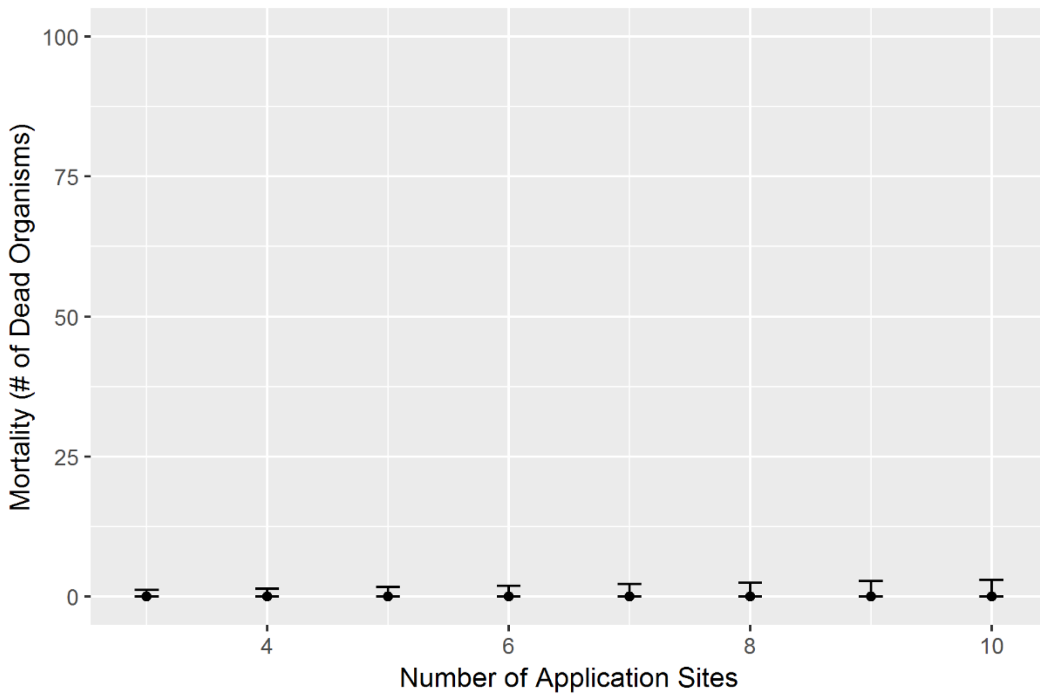

Figure B18. The influence of the number of 500 m^2 application sites on Eastern Sand Darter mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

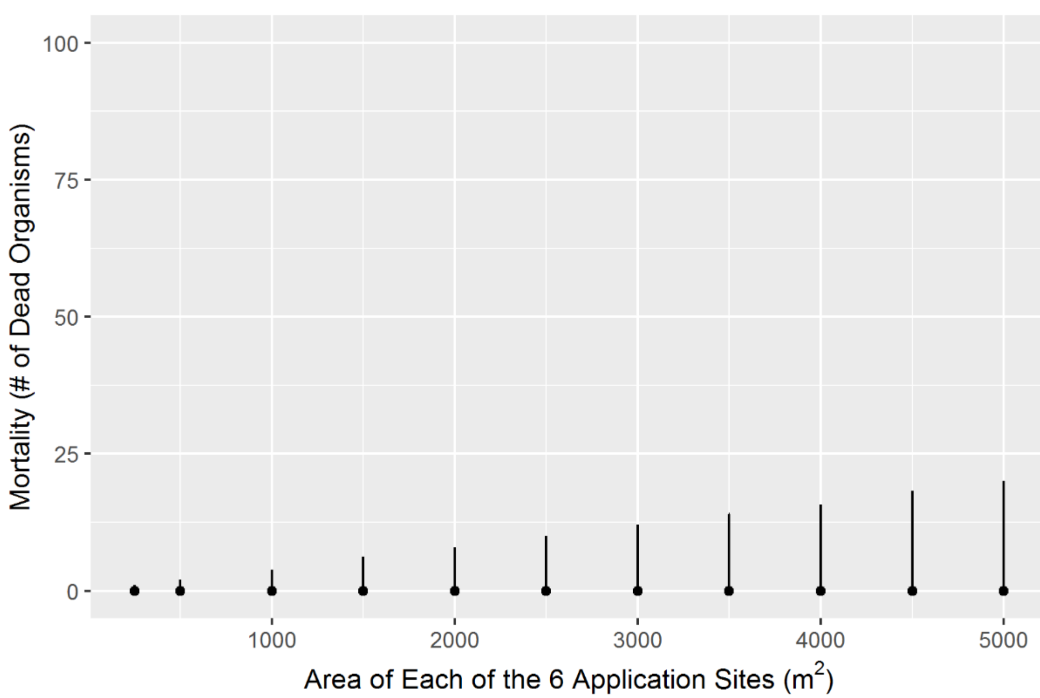

Figure B19. The influence of the size of six application sites on Eastern Sand Darter mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

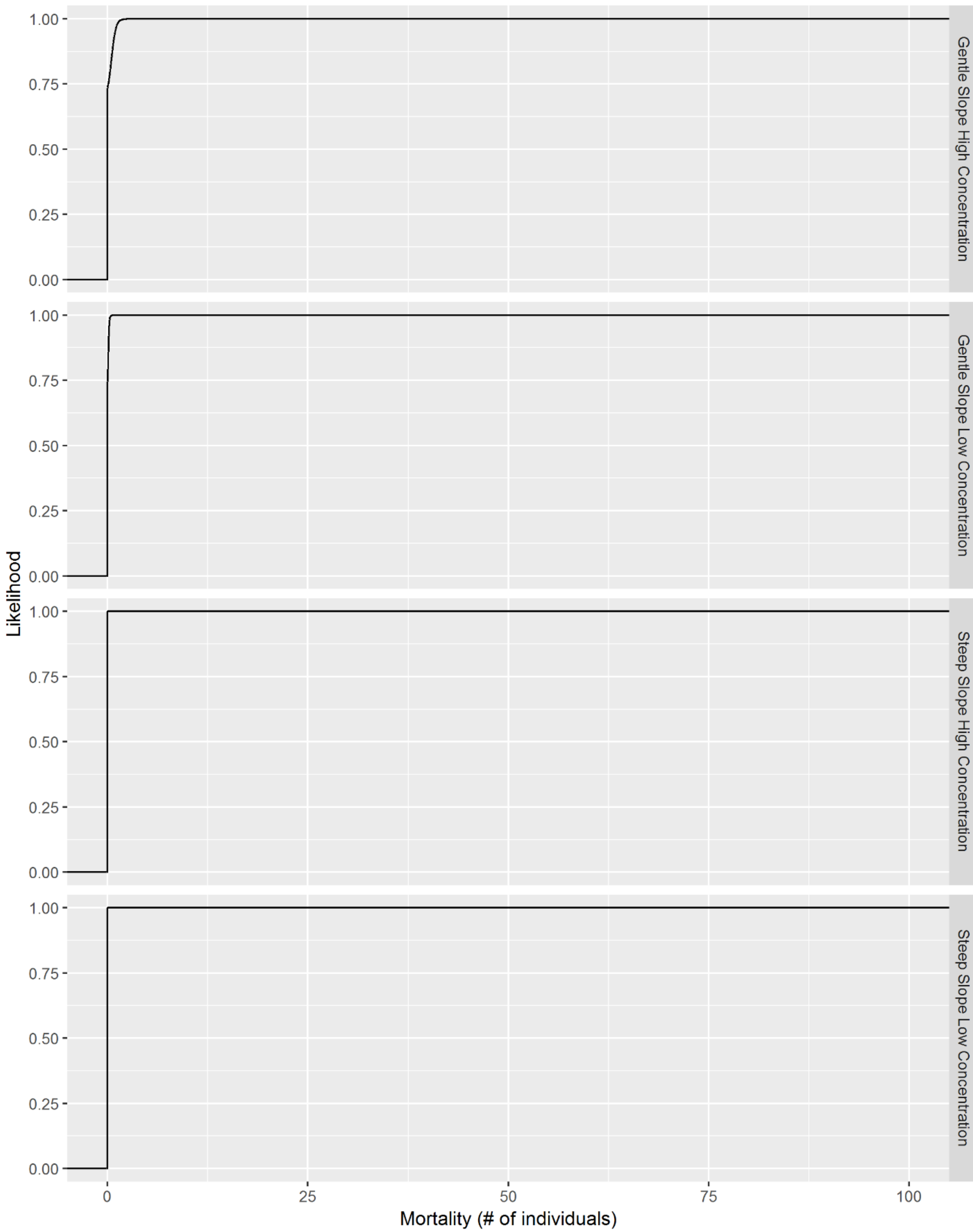

Figure B20. Cumulative likelihood results for Grass Pickerel mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

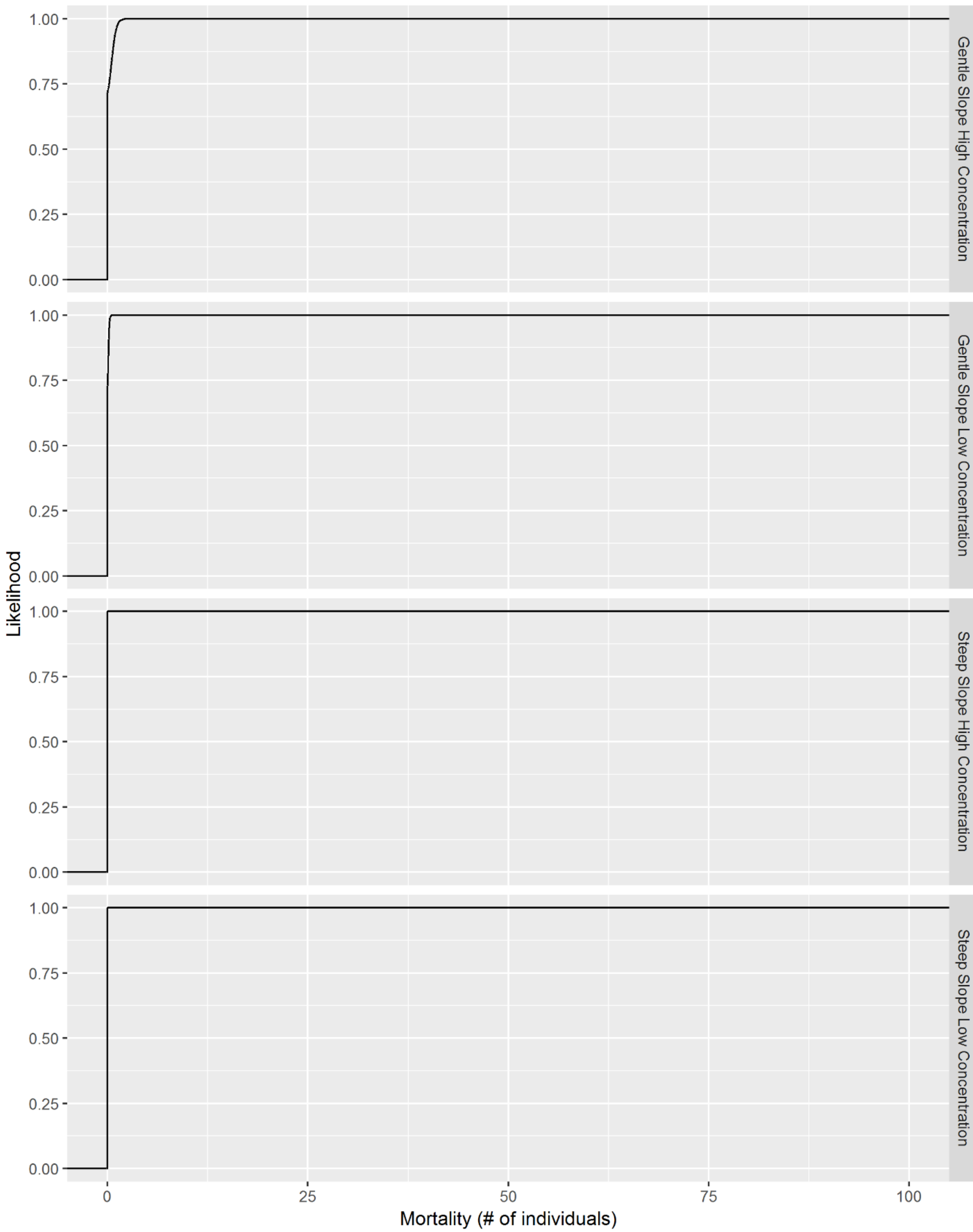

Figure B21. Cumulative likelihood results for Grass Pickerel mortality in the St. Clair River following Bayluscide application at six 500 m² sites across the different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

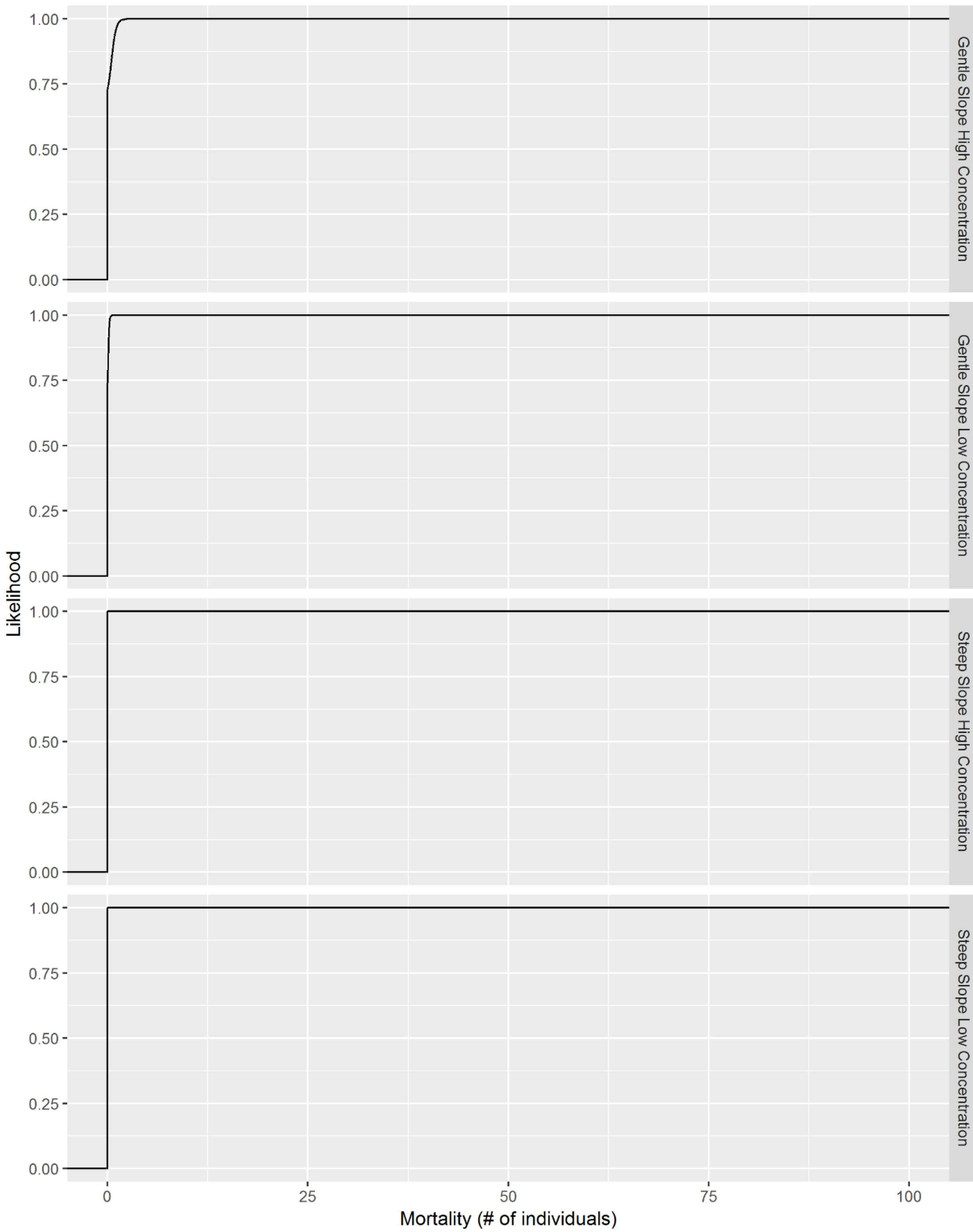

Figure B22. Cumulative likelihood results for Grass Pickerel mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

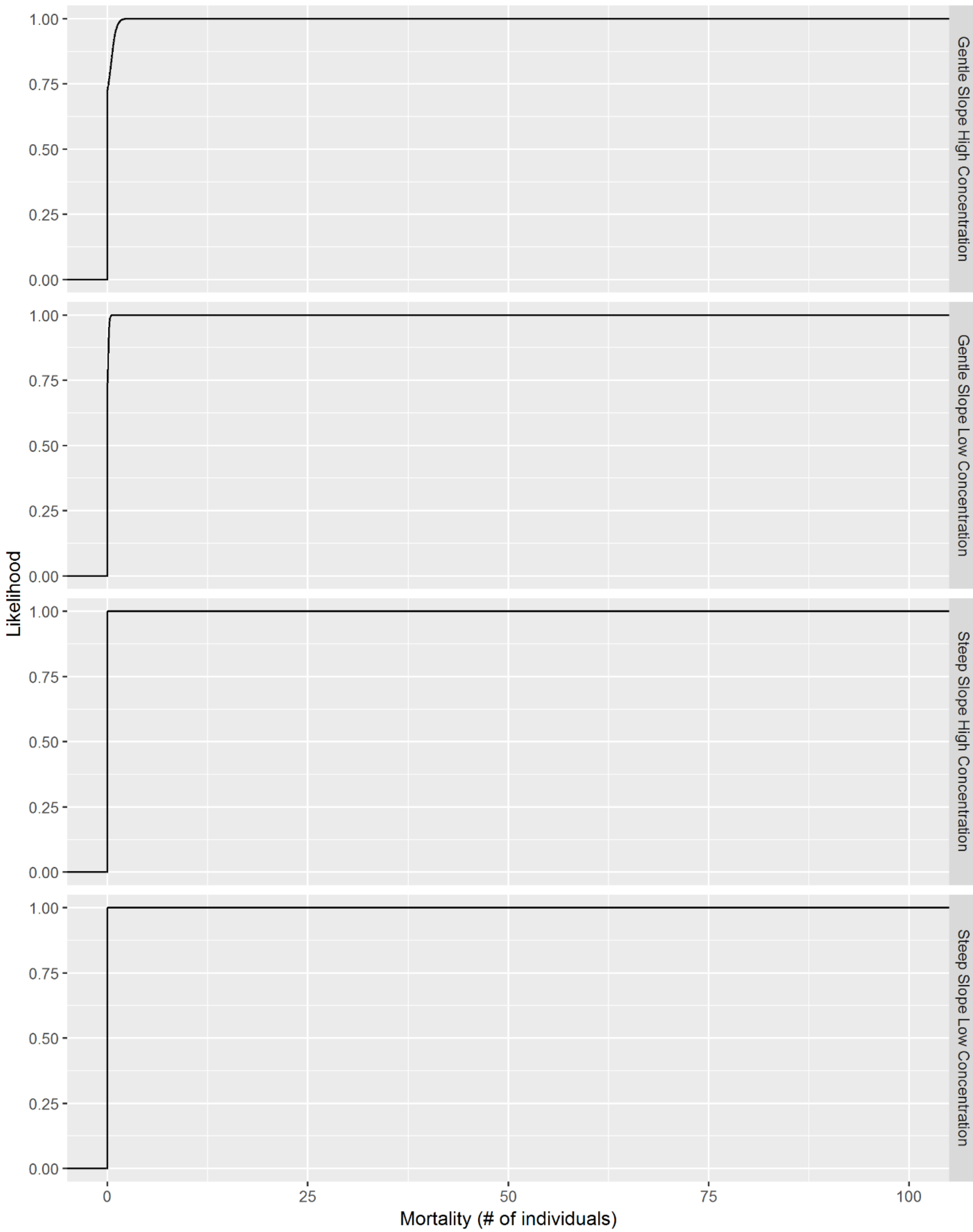

Figure B23. Cumulative likelihood results for Grass Pickerel mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

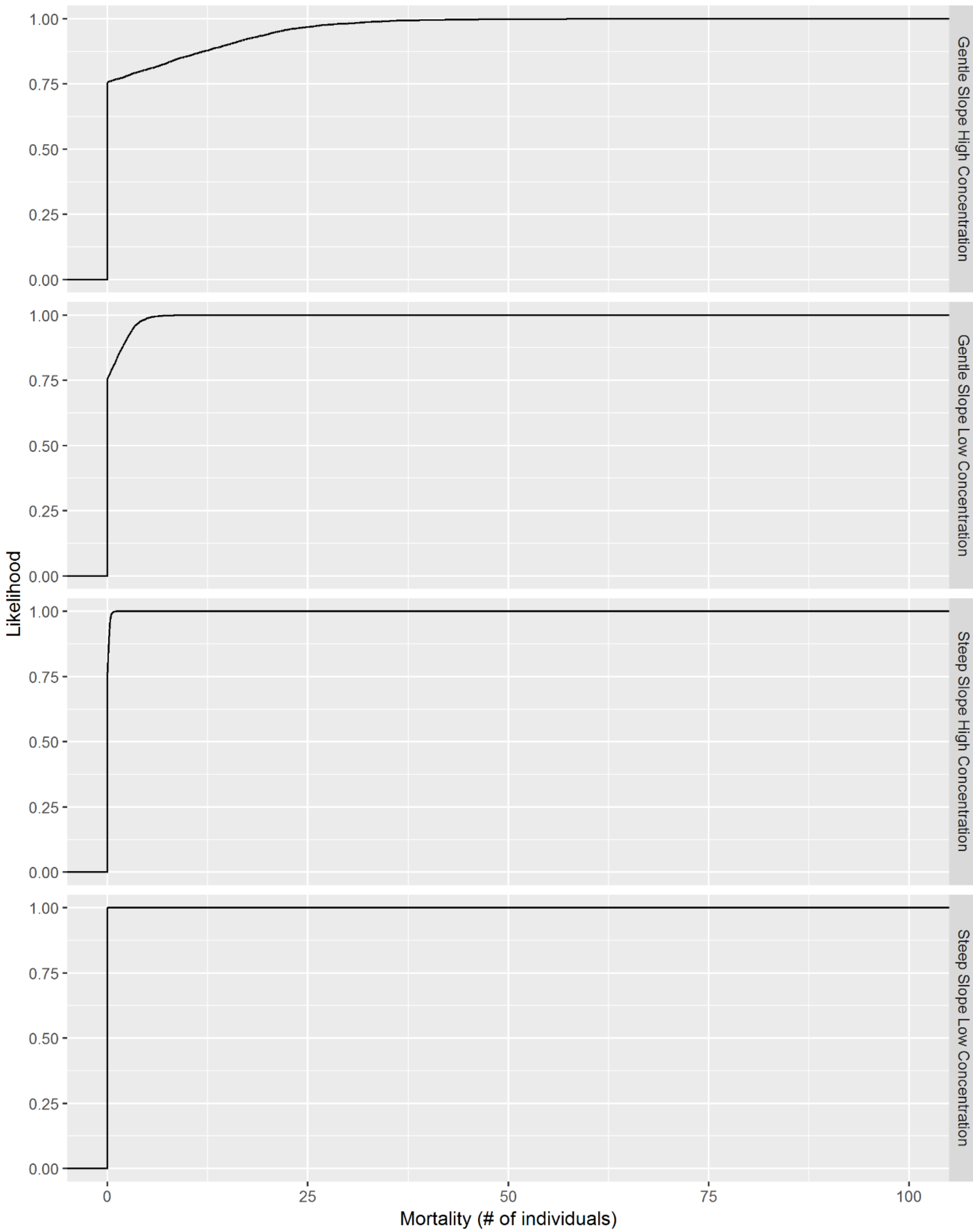

Figure B24. Cumulative likelihood results for Lake Chubsucker mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

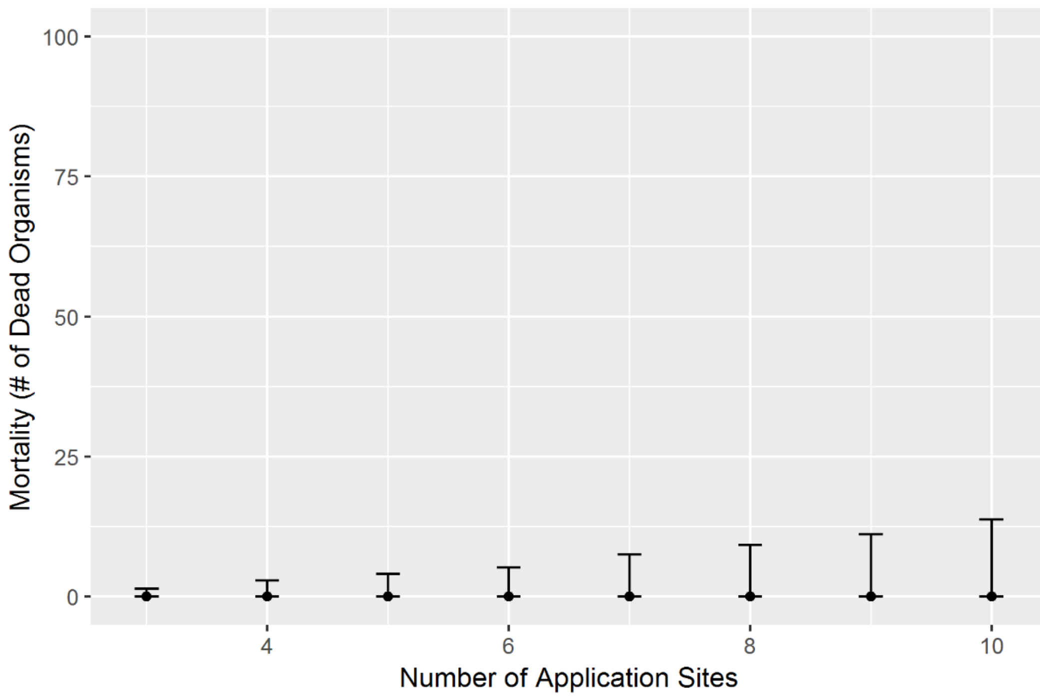

Figure B25. The influence of the number of 500 m^2 application sites on Lake Chubsucker mortality in the St. Clair River. Error bars represents the upper and lower 95^{th} percentile.

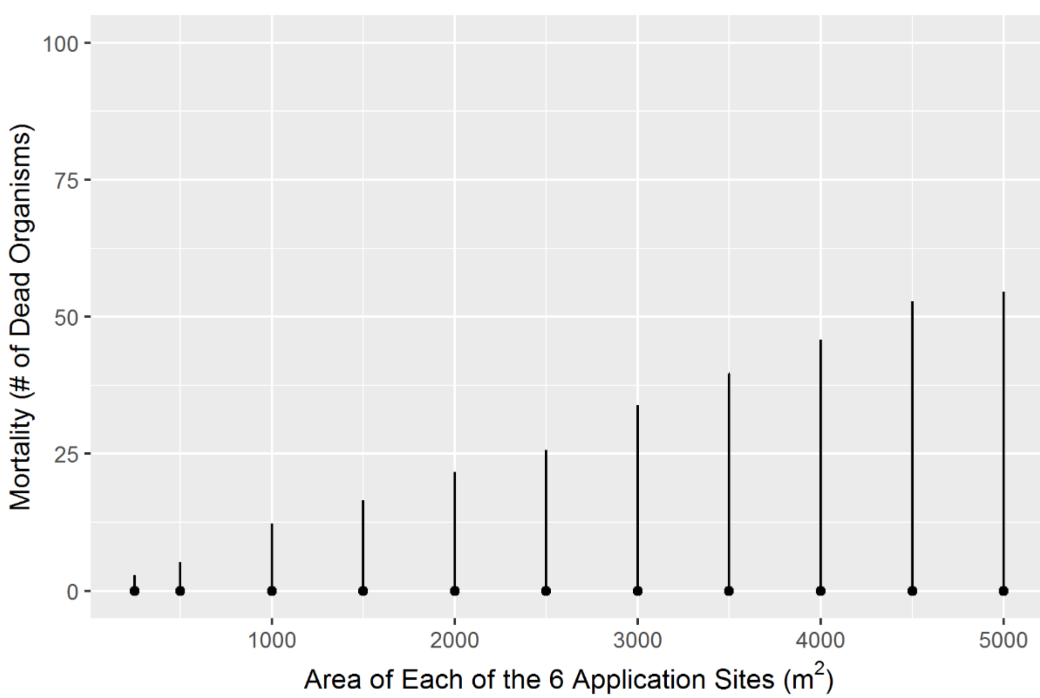

Figure B26. The influence of the size of six application sites on Lake Chubsucker mortality in the St. Clair River. Error bars represents the upper and lower 95^{th} percentile.

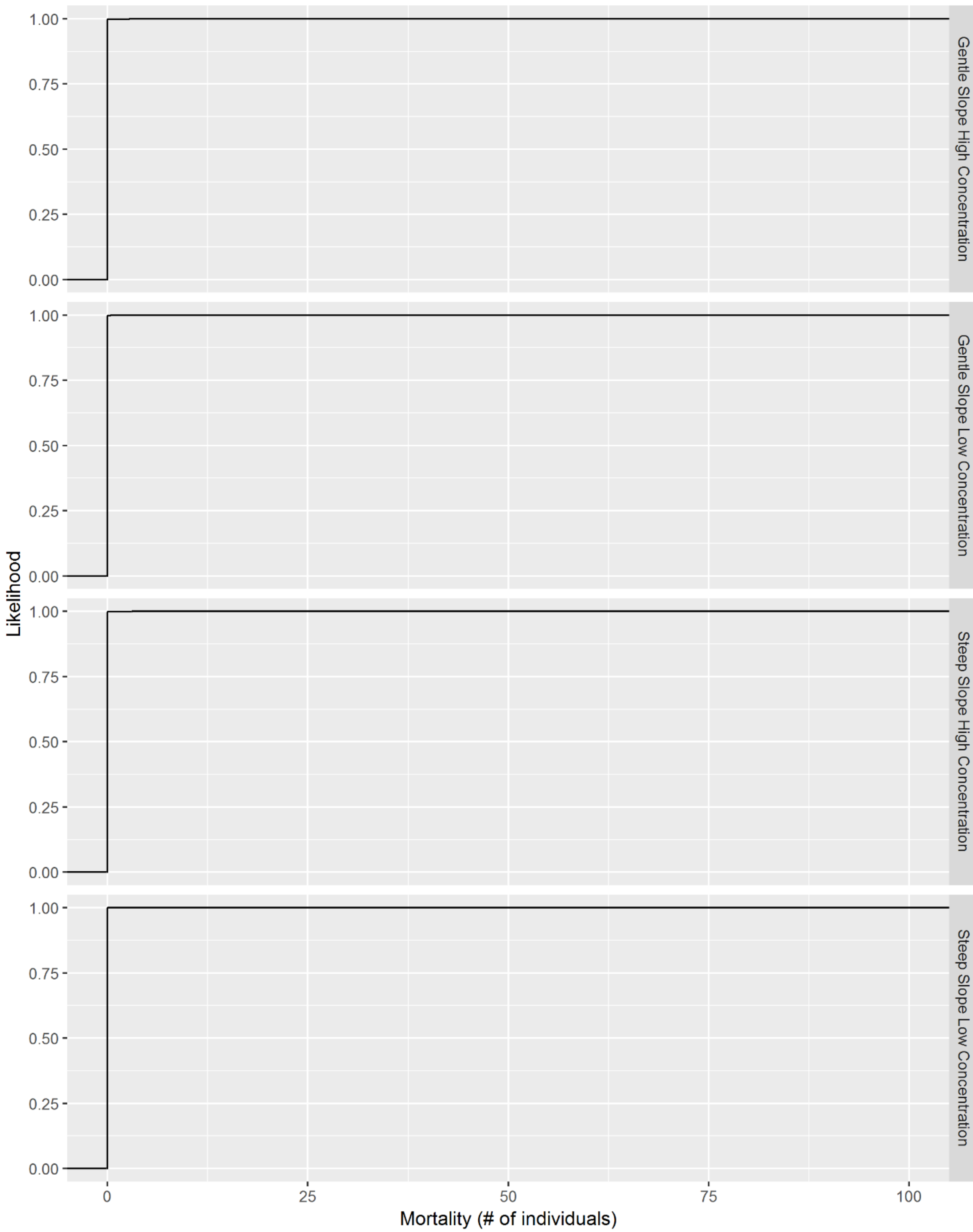

Figure B27. Cumulative likelihood results for Lake Sturgeon mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

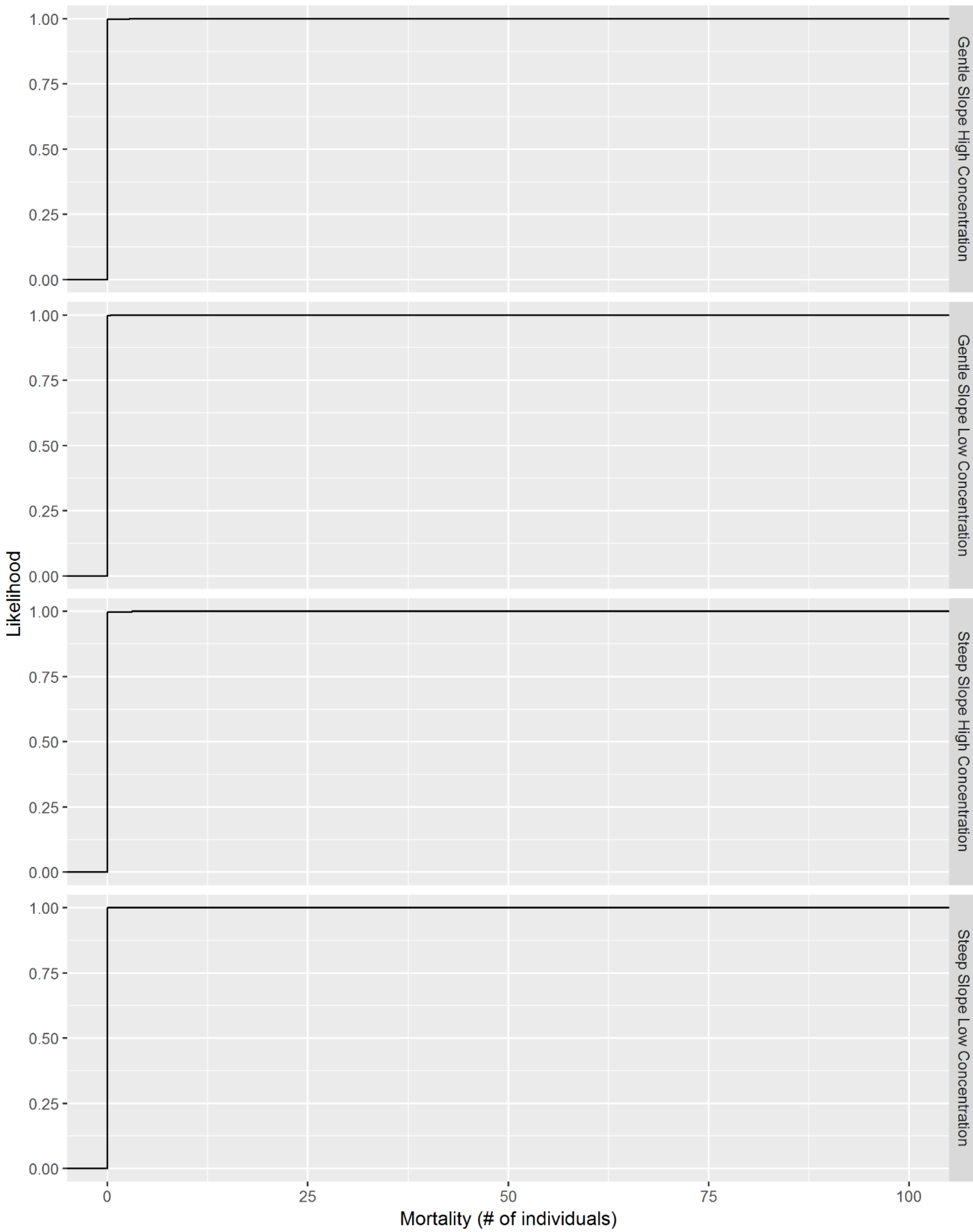

Figure B28. Cumulative likelihood results for Lake Sturgeon mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

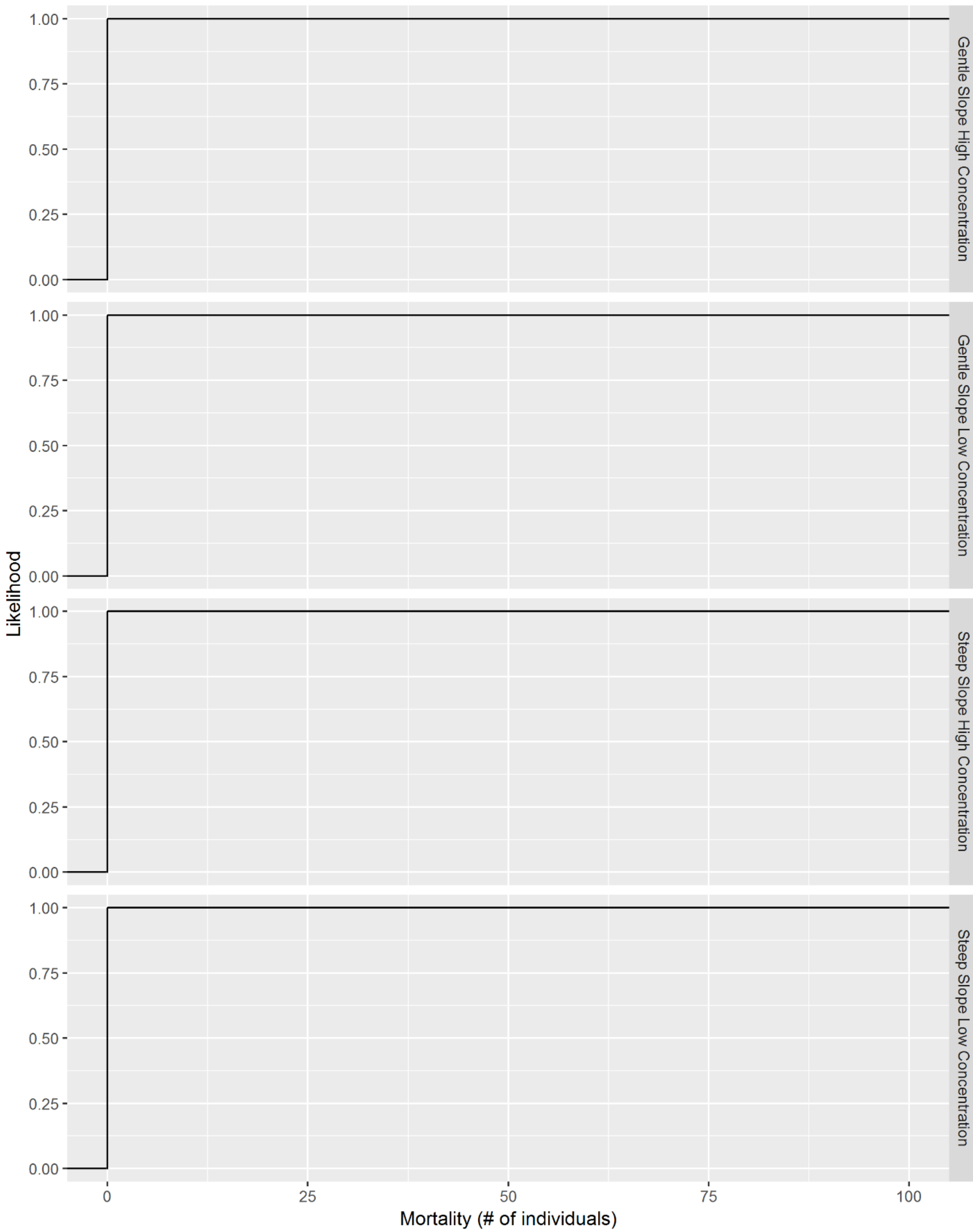

Figure B29. Cumulative likelihood results for Northern Brook Lamprey mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

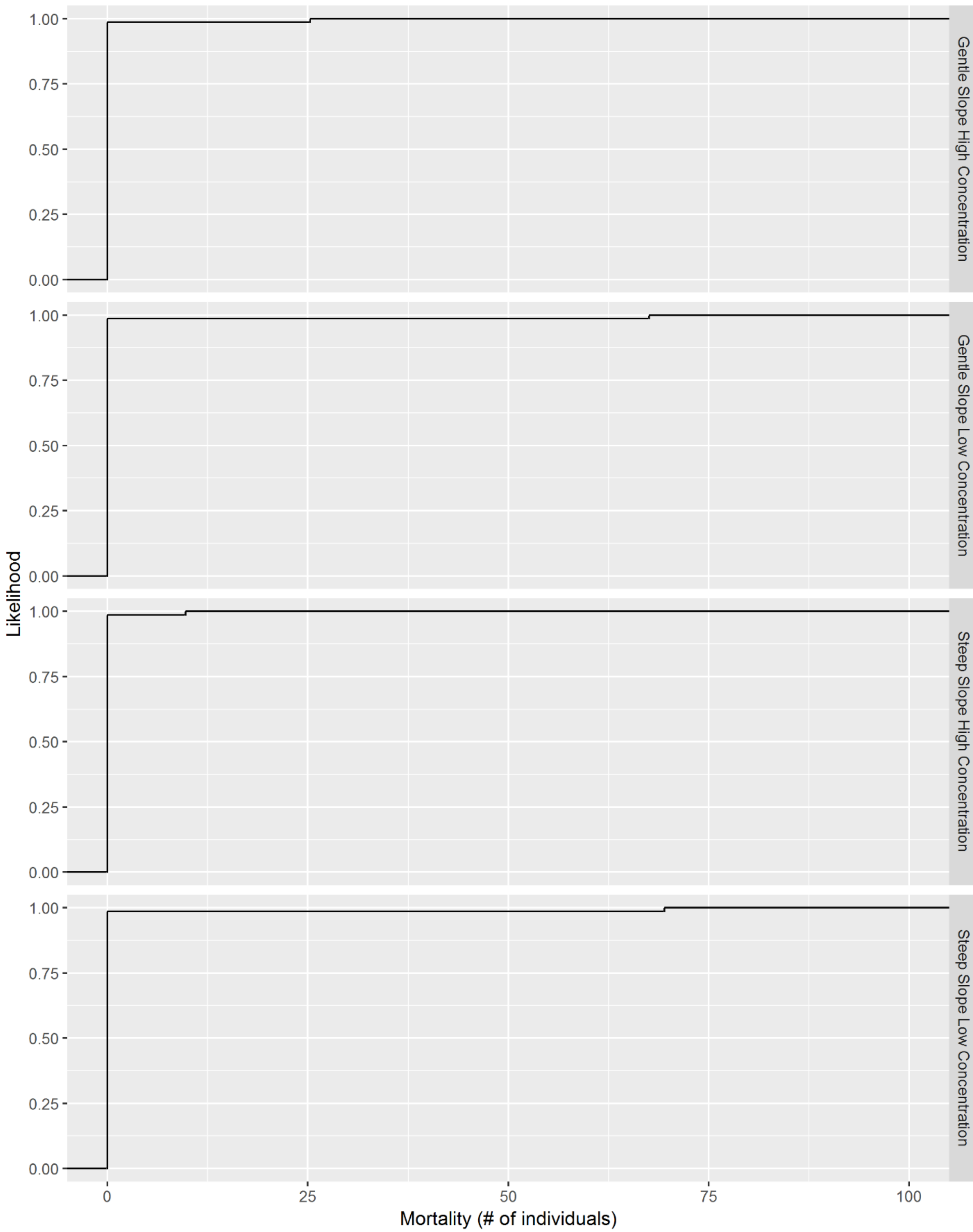

Figure B30. Cumulative likelihood results for Northern Brook Lamprey mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

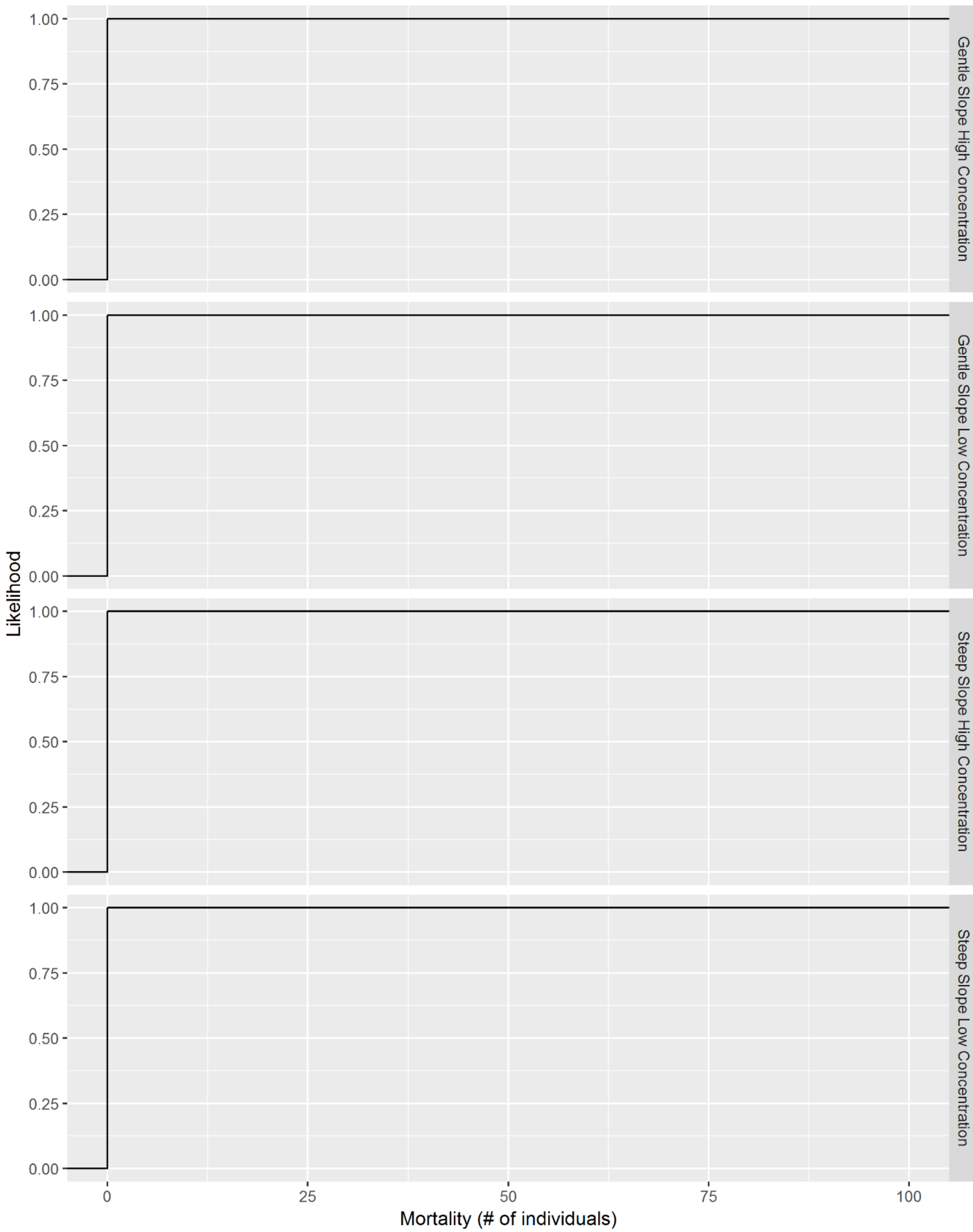

Figure B31. Cumulative likelihood results for Northern Brook Lamprey mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

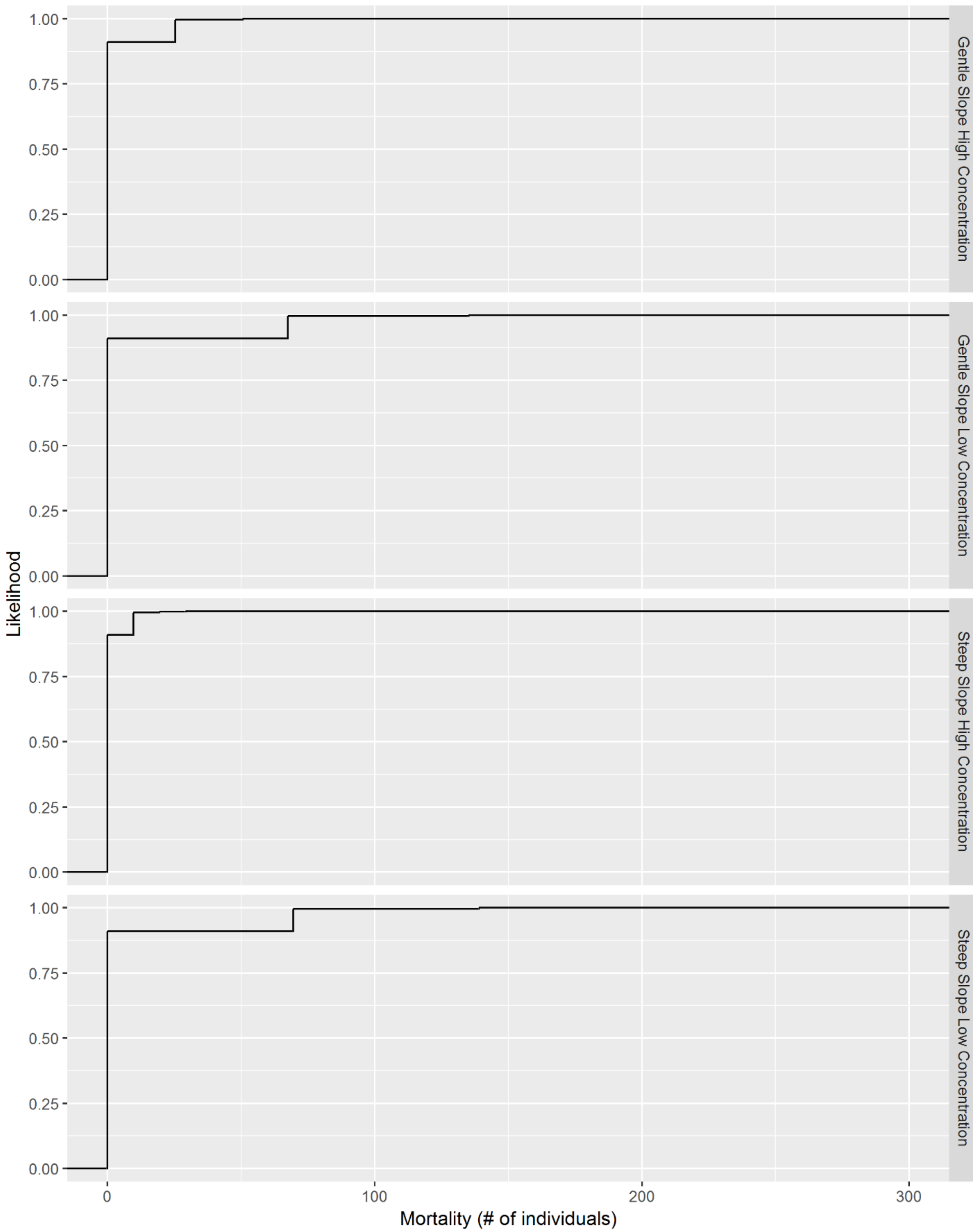

Figure B32. Cumulative likelihood results for Northern Brook Lamprey mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

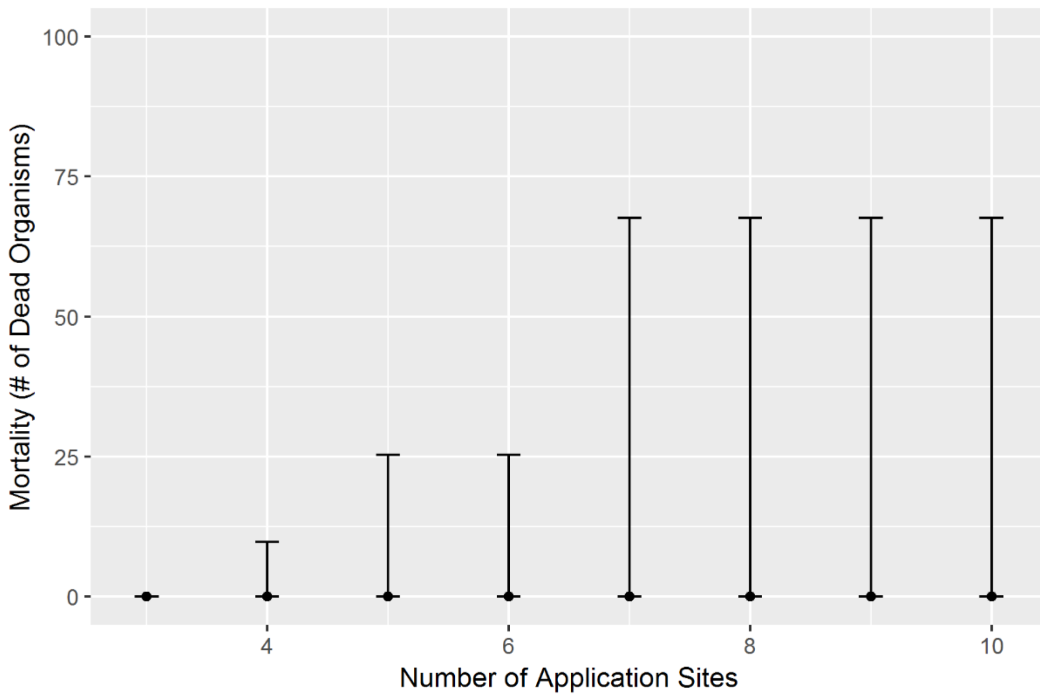

Figure B33. The influence of the number of 500 m^2 application sites on Northern Brook Lamprey mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

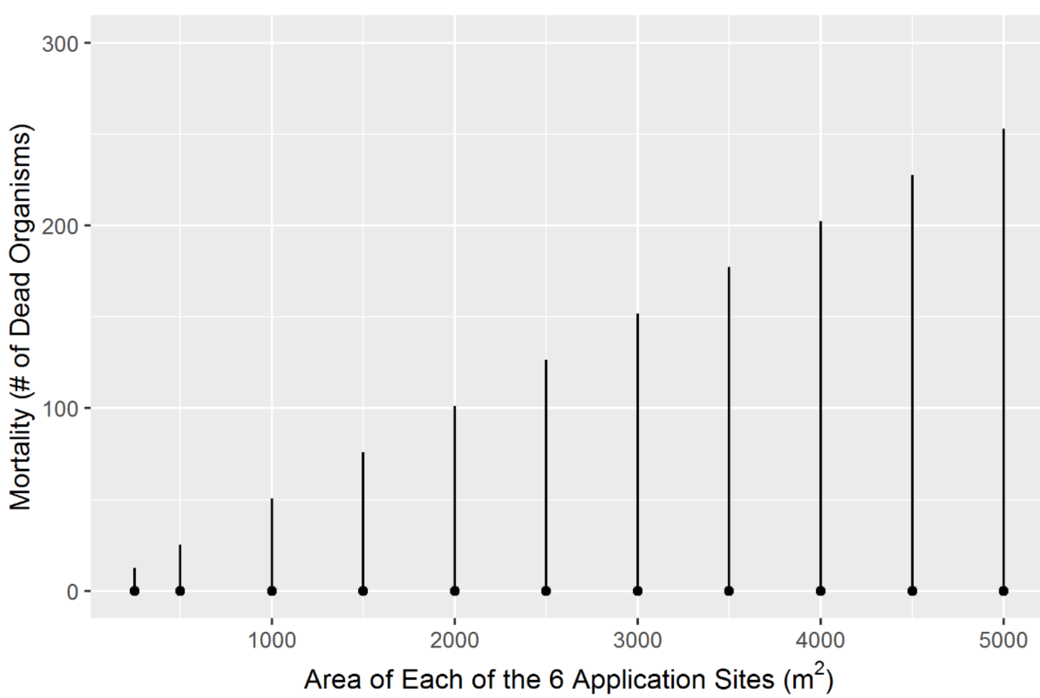

Figure B34. The influence of the size of six application sites on Northern Brook Lamprey mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

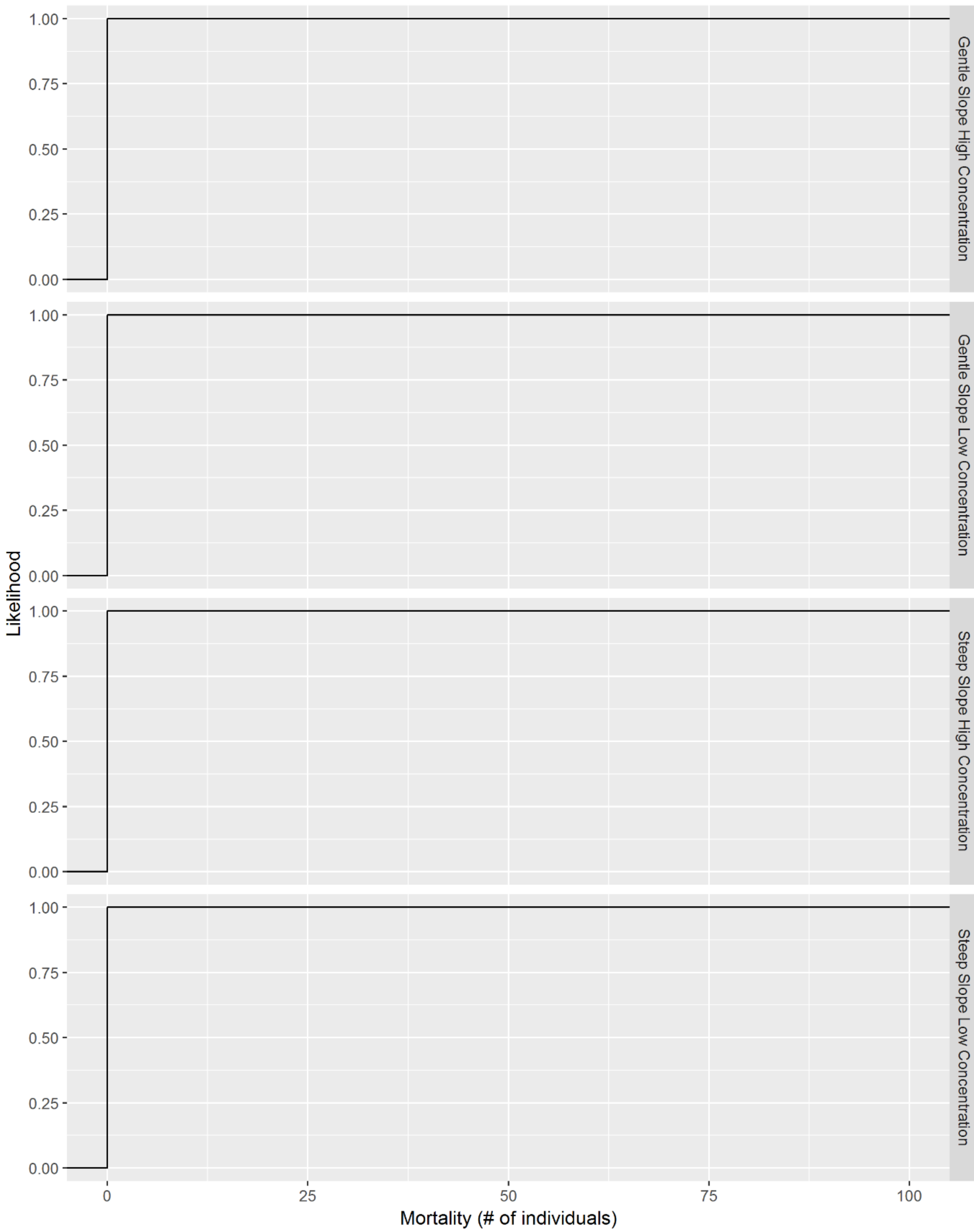

Figure B35. Cumulative likelihood results for Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the Detroit River following Bayluscide application at six 500 m^2 sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

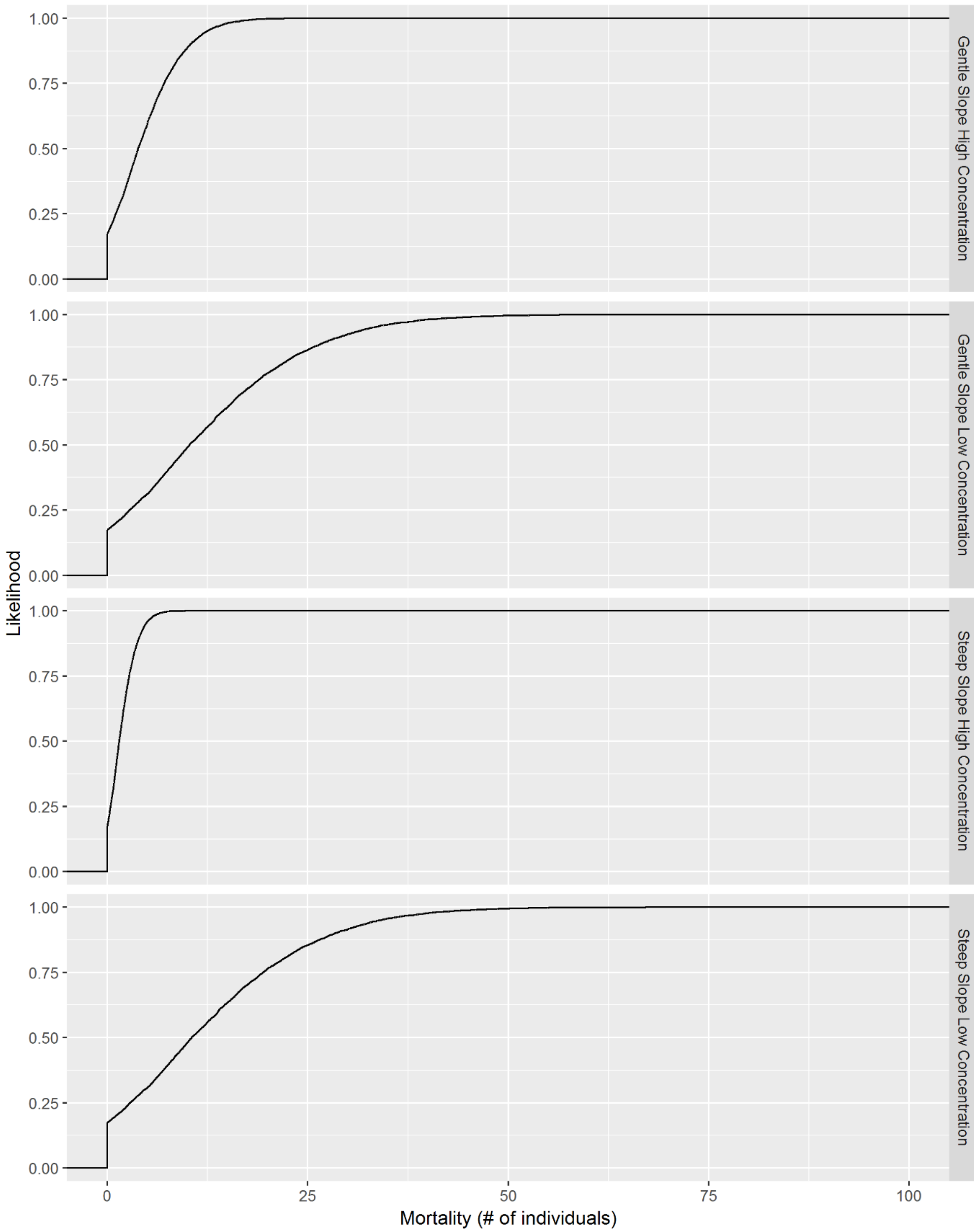

Figure B36. Cumulative likelihood results for Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

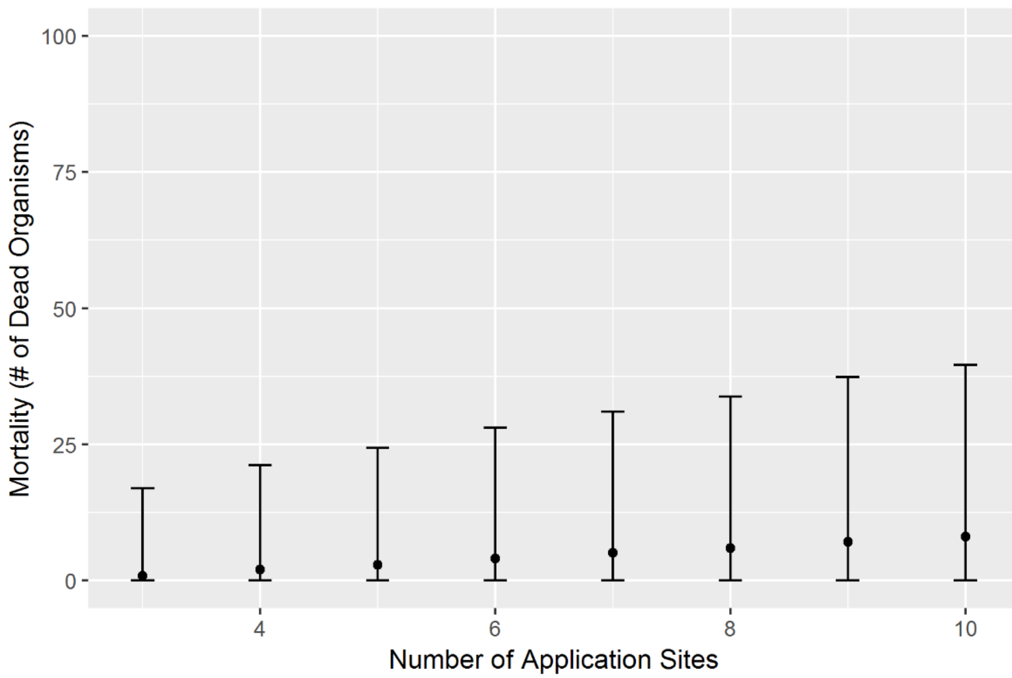

Figure B37. The influence of the number of 500 m² application sites on Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.*

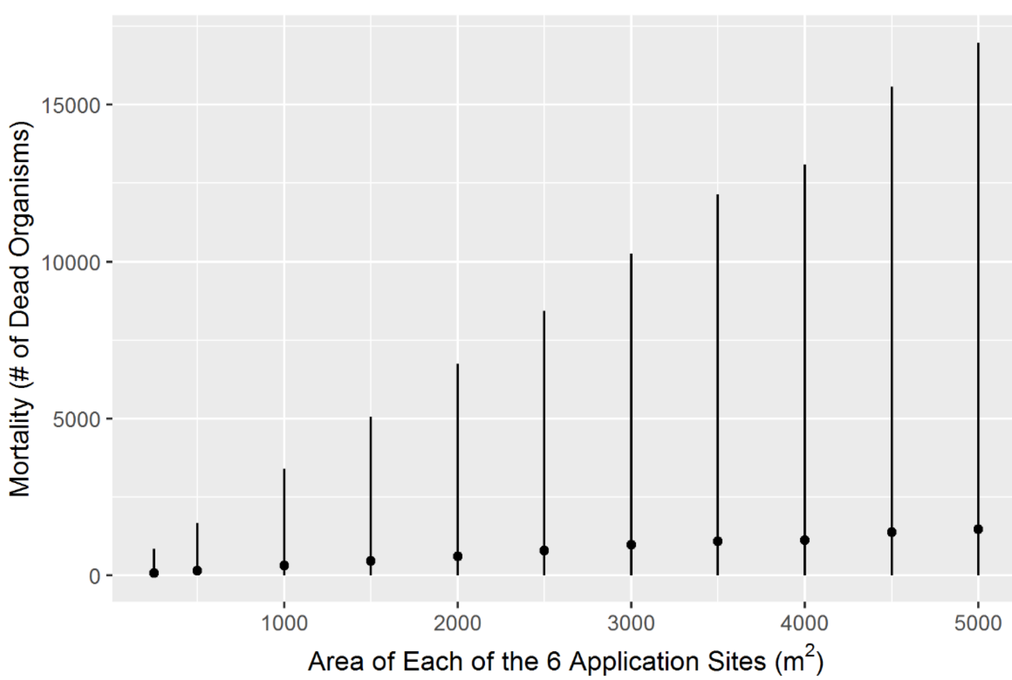

Figure B38. The influence of the size of six application sites on Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.*

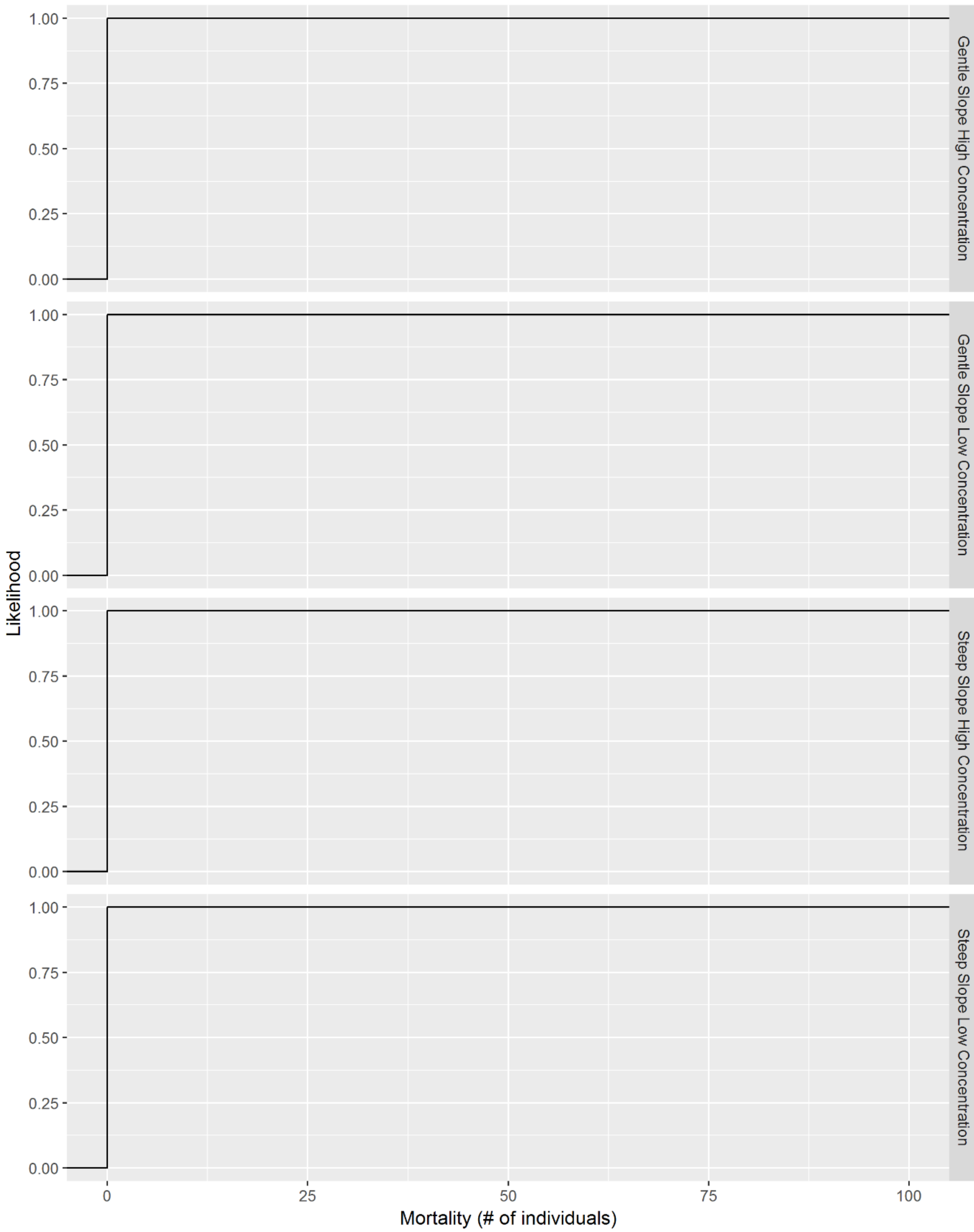

Figure B39. Cumulative likelihood results for Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

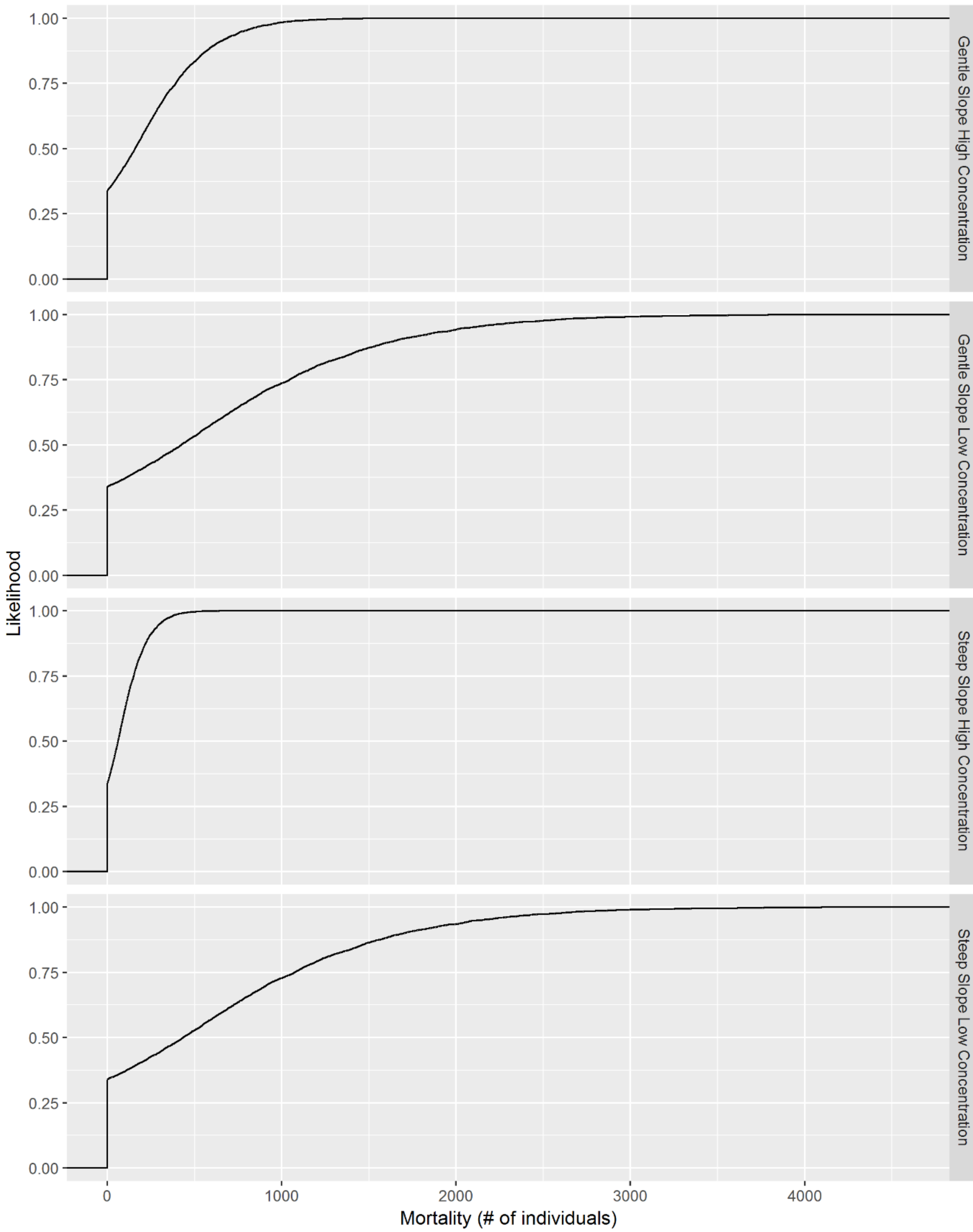

Figure B40. Cumulative likelihood results for Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

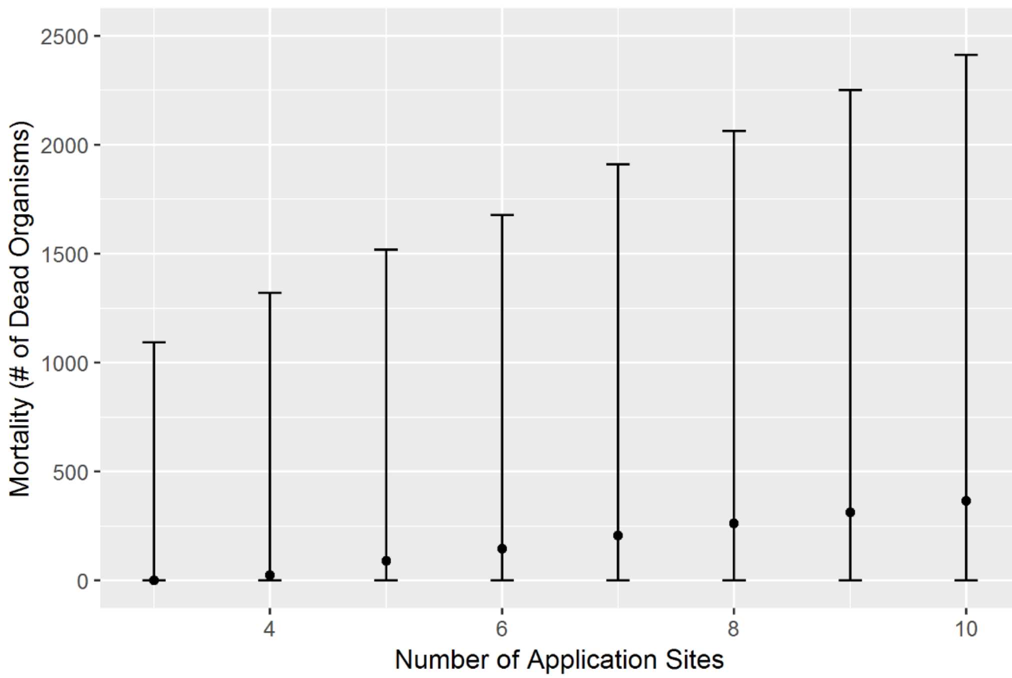

Figure B41. The influence of the number of 500 m² application sites on Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the Thames River. Error bars represents the upper and lower 95th percentile.*

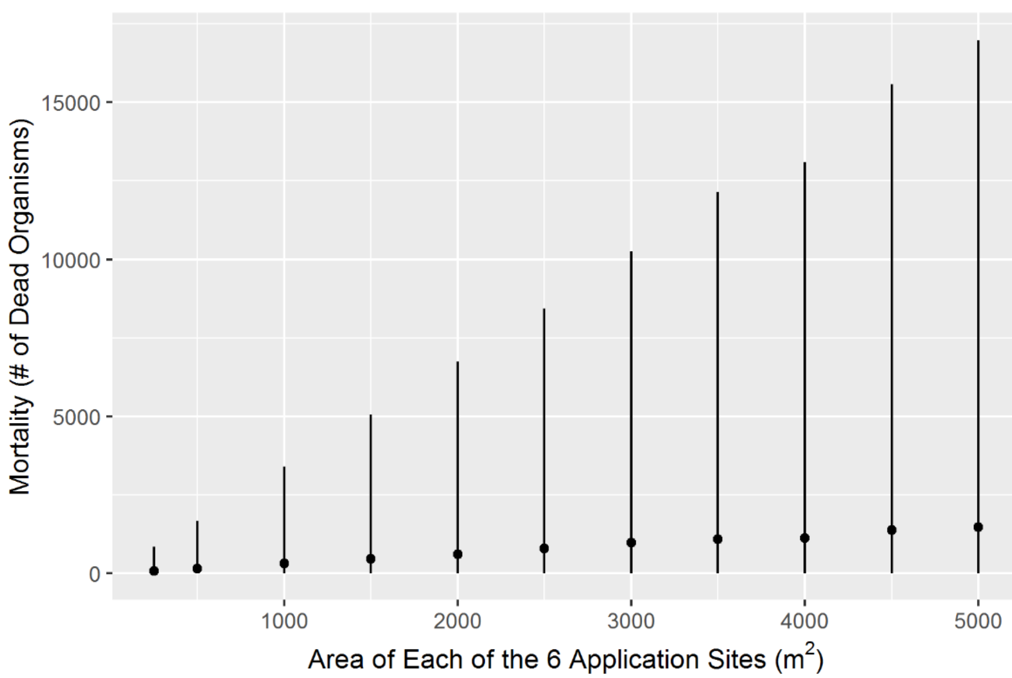

Figure B42. The influence of the size of six application sites on Northern Brook Lamprey and unidentified Ichthyomyzon *sp. mortality in the Thames River. Error bars represents the upper and lower 95th percentile.*

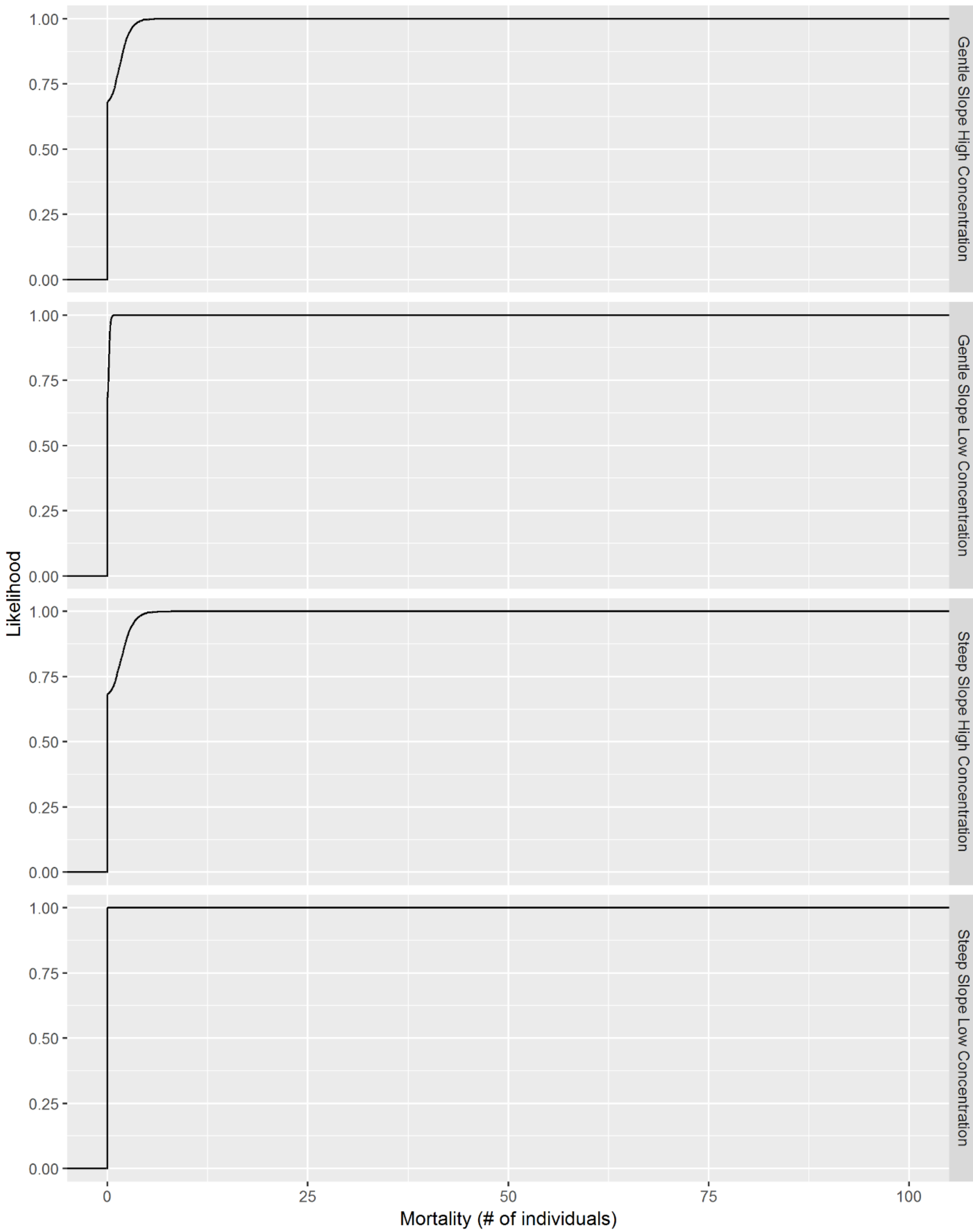

Figure B43. Cumulative likelihood results for Northern Madtom mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

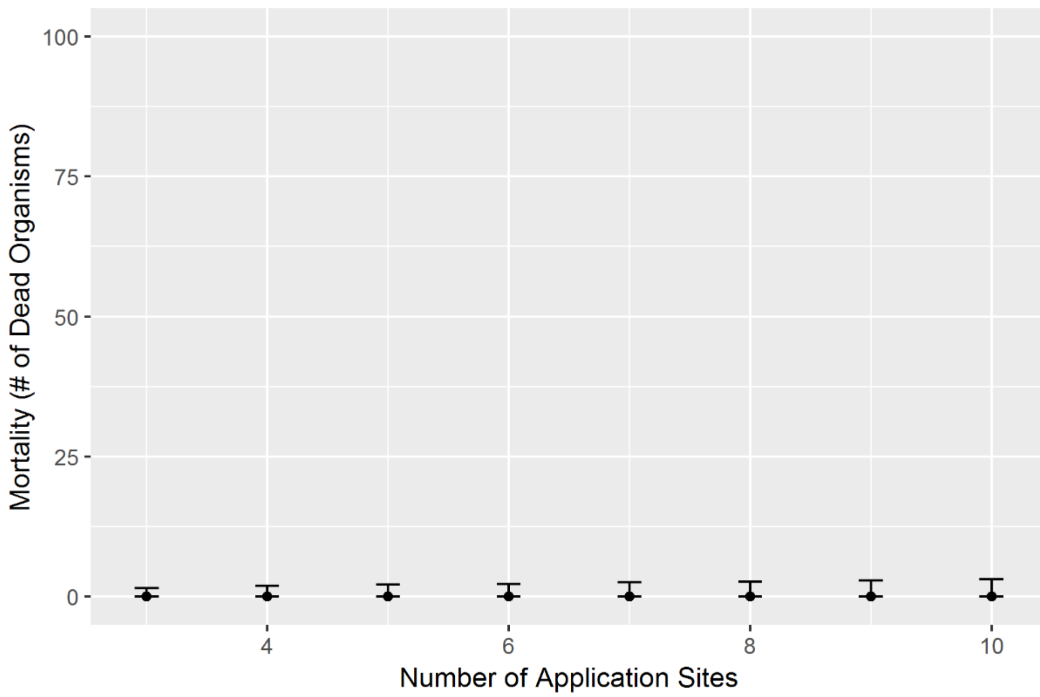

Figure B44. The influence of the number of 500 m² application sites on Northern Madtom mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

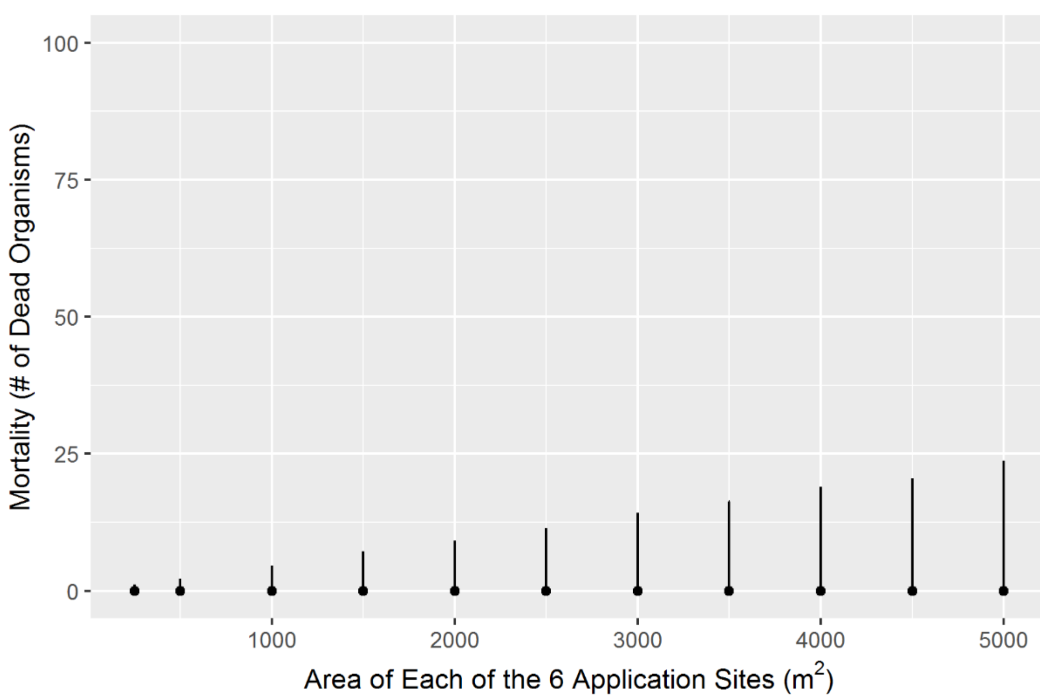

Figure B45. The influence of the size of six application sites on Northern Madtom mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

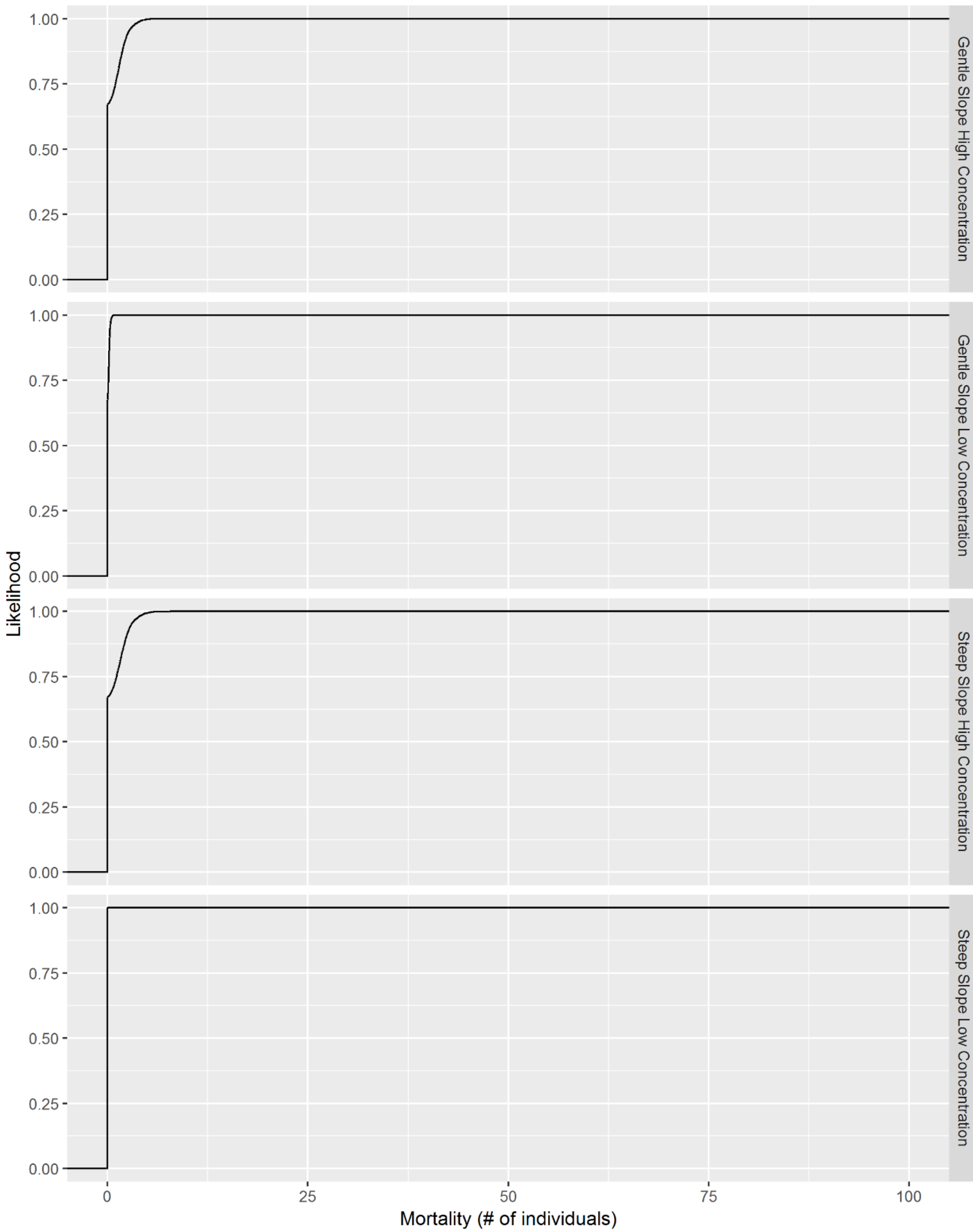

Figure B46. Cumulative likelihood results for Northern Madtom mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

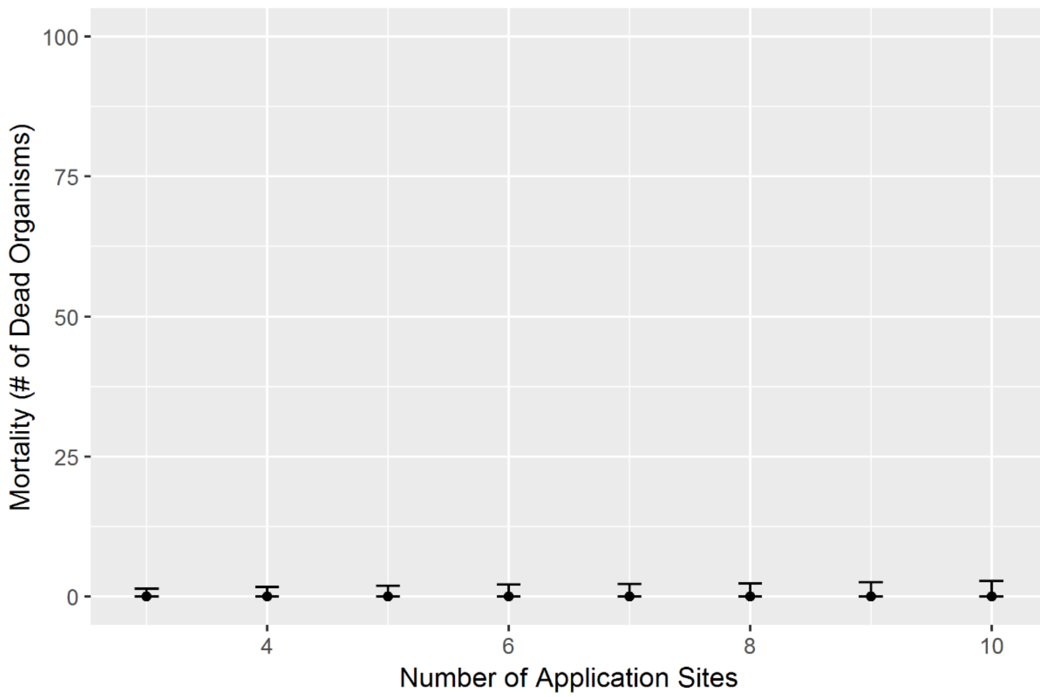

Figure B47. The influence of the number of 500 m² application sites on Northern Madtom mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

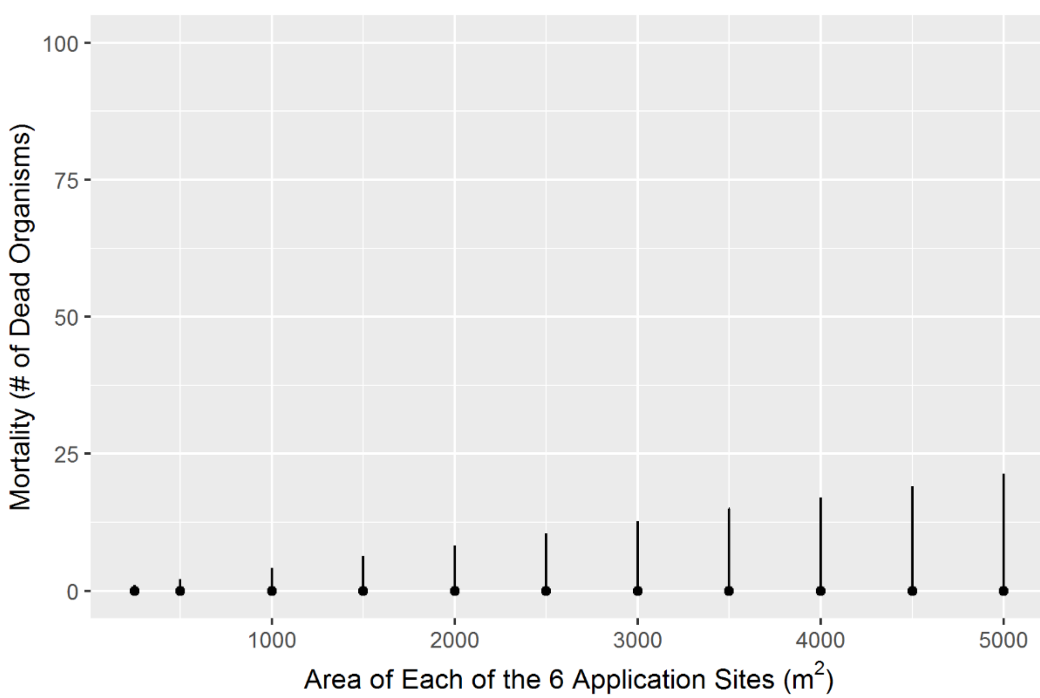

Figure B48. The influence of the size of six application sites on Northern Madtom mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

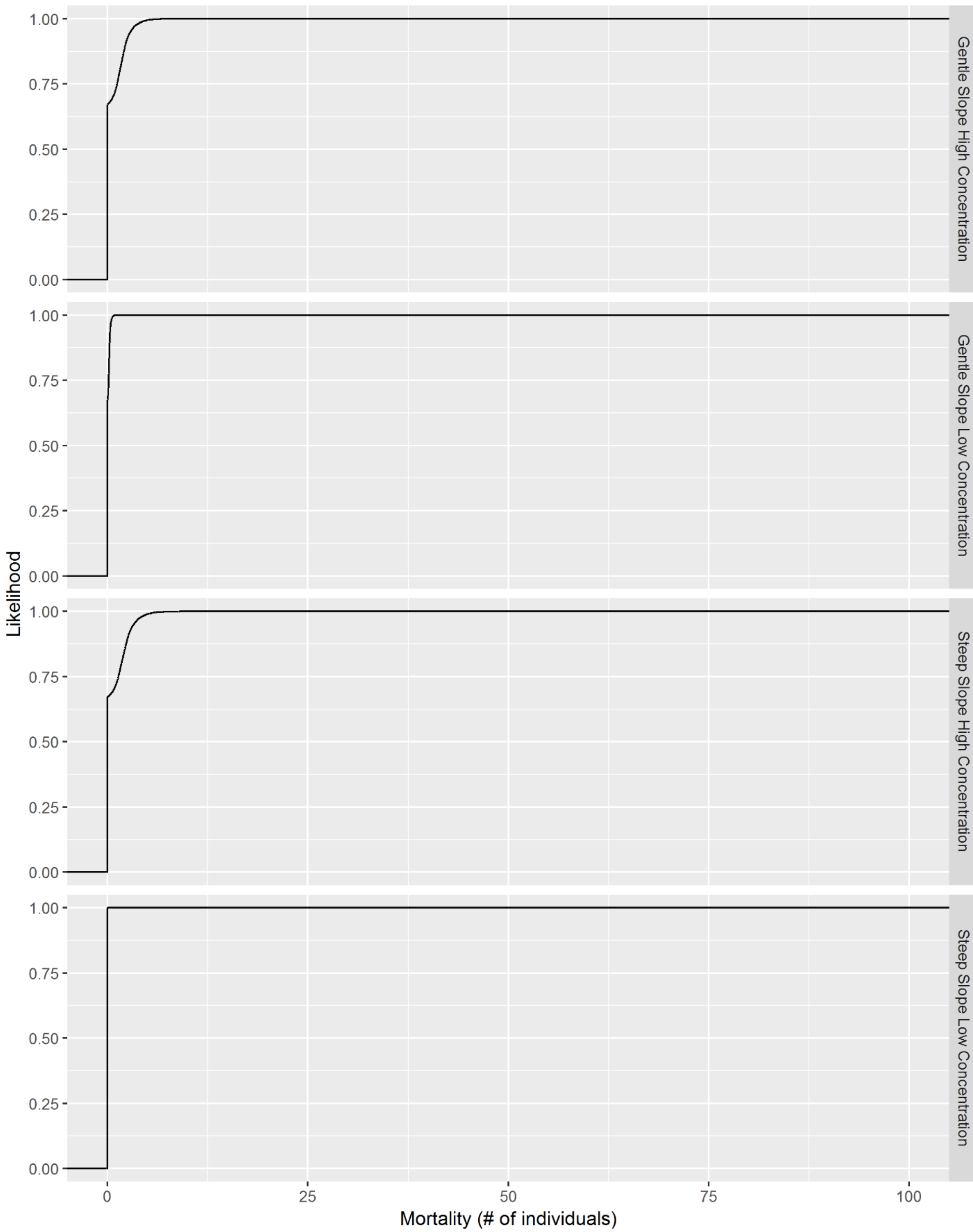

Figure B49. Cumulative likelihood results for Northern Madtom mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

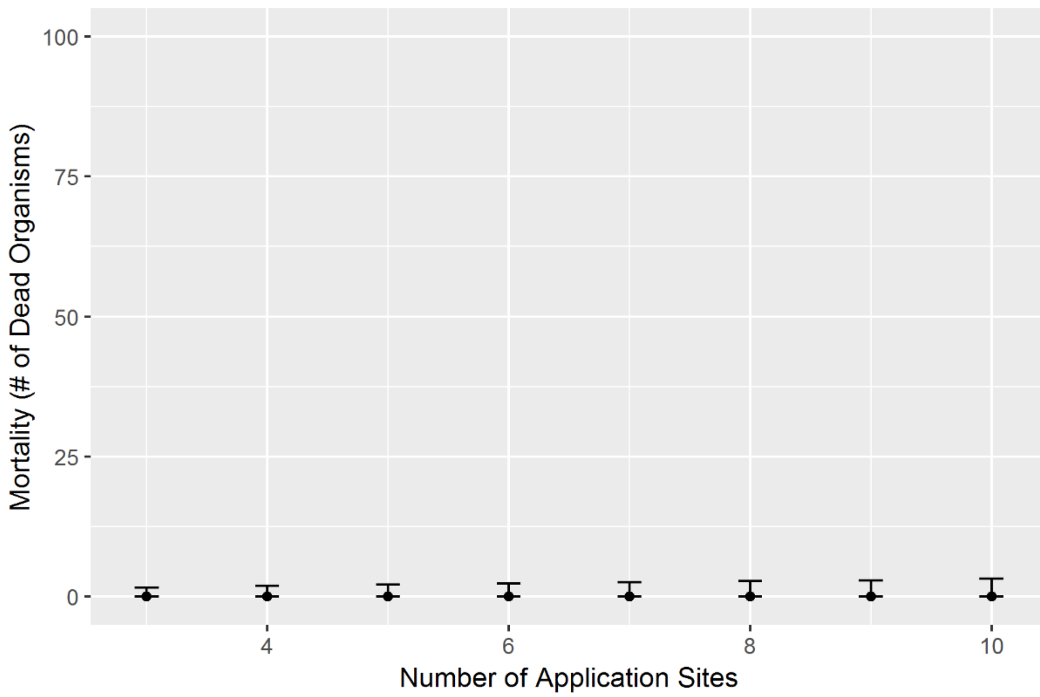

Figure B50. The influence of the number of 500 m^2 application sites on Northern Madtom mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

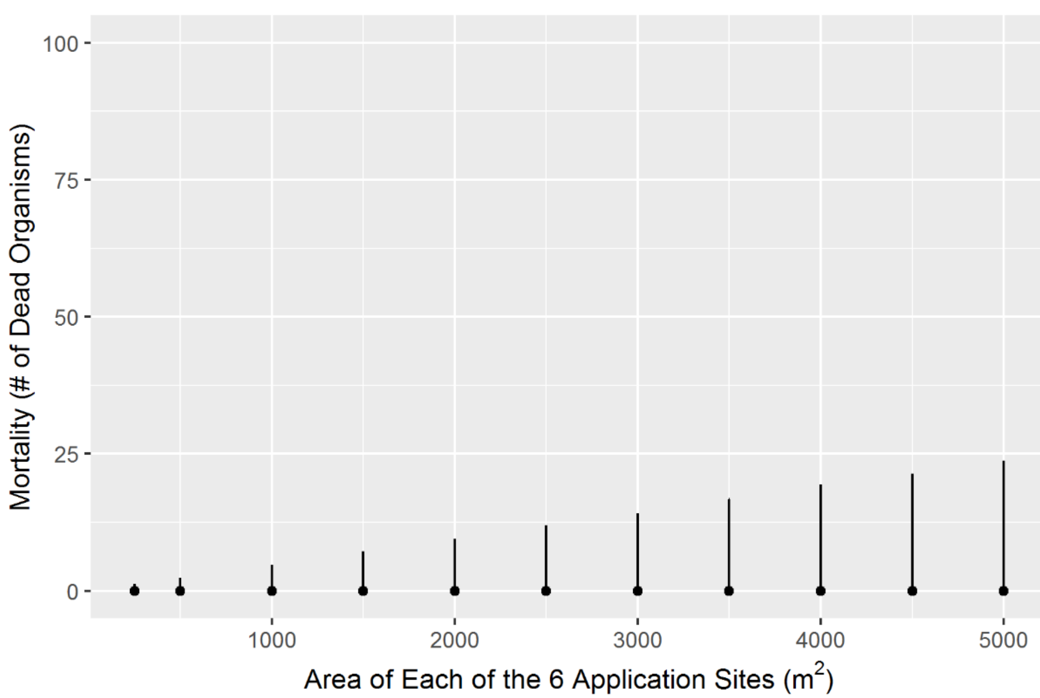

Figure B51. The influence of the size of six application sites on Northern Madtom mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

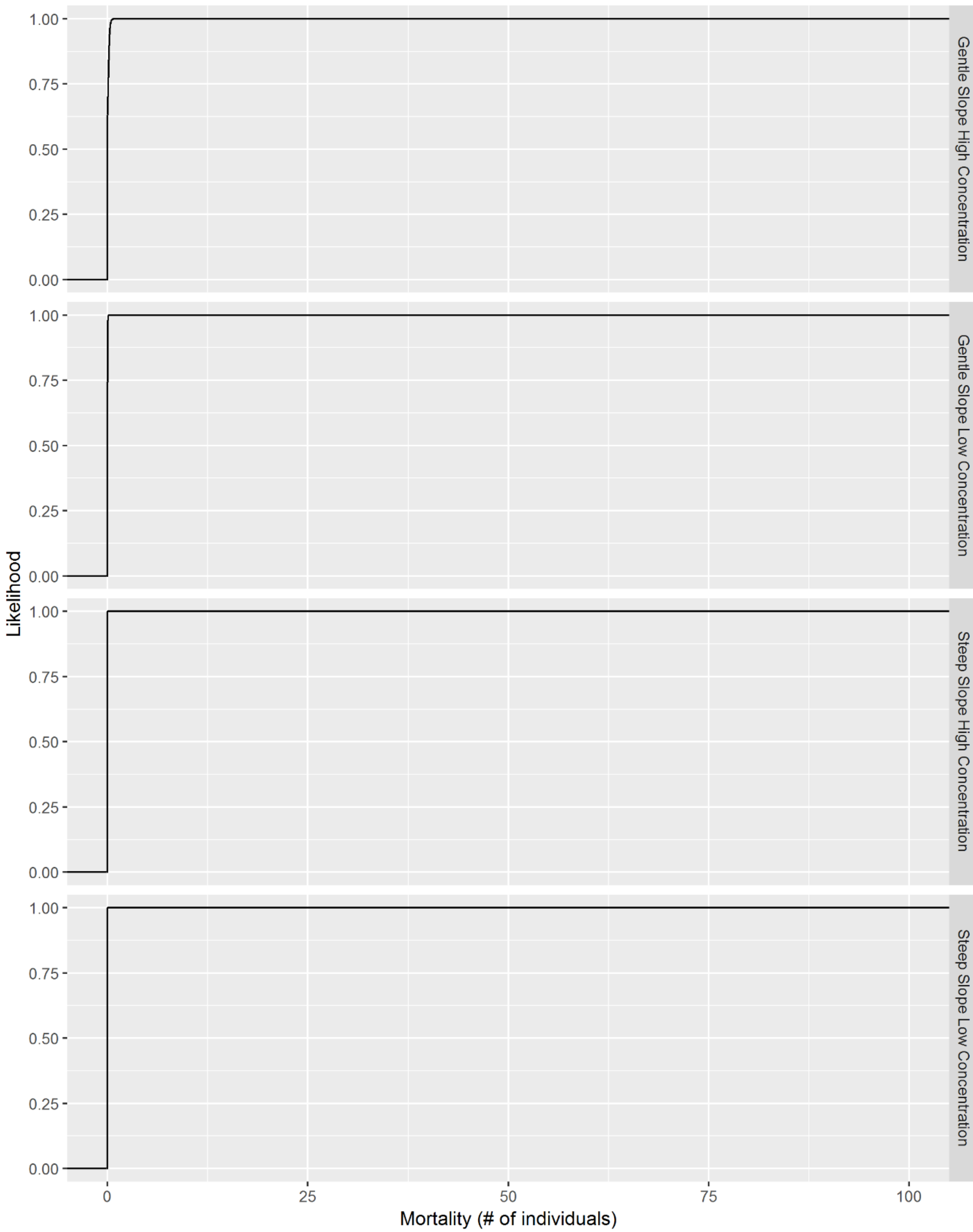

Figure B52. Cumulative likelihood results for Northern Sunfish mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

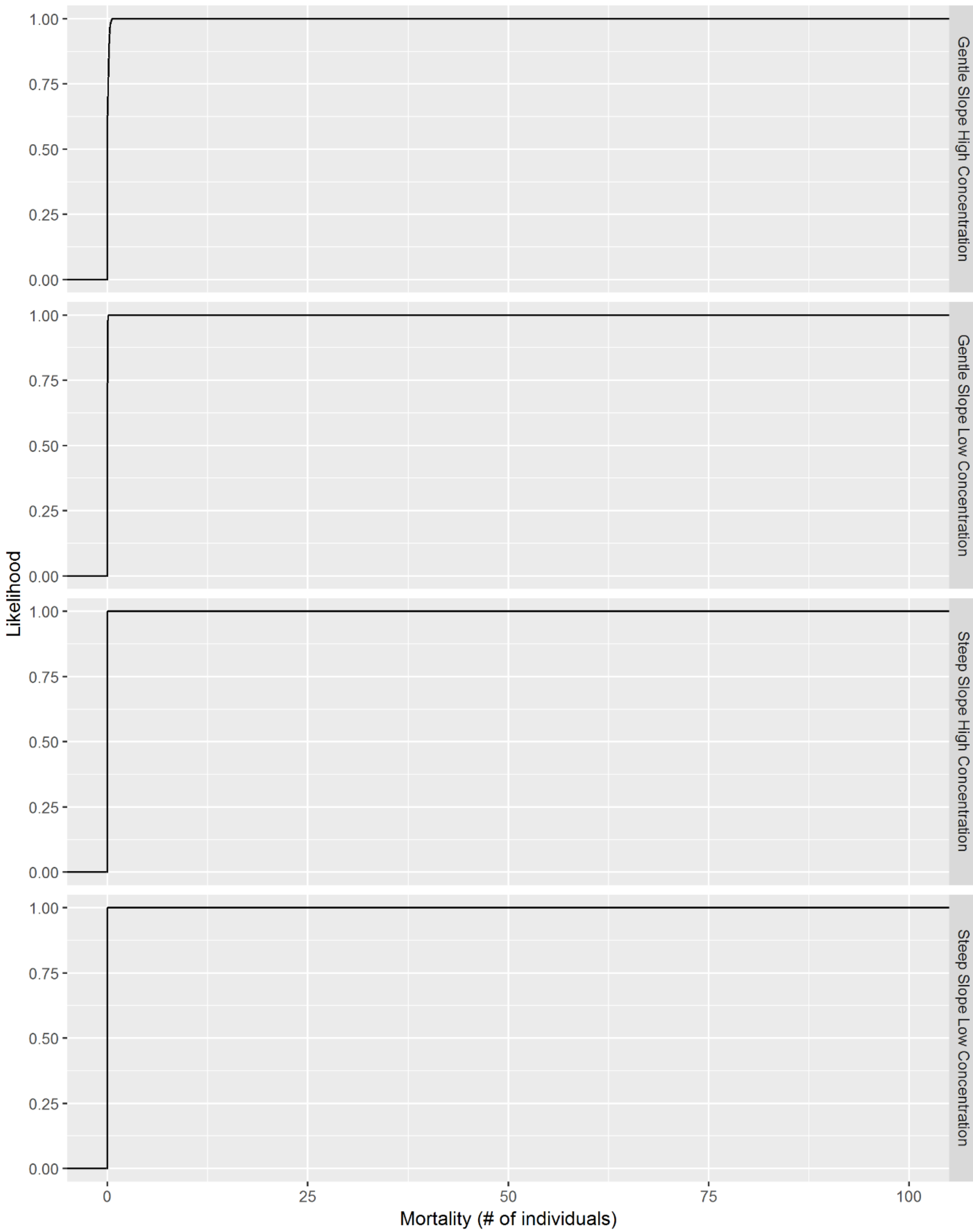

Figure B53. Cumulative likelihood results for Northern Sunfish mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

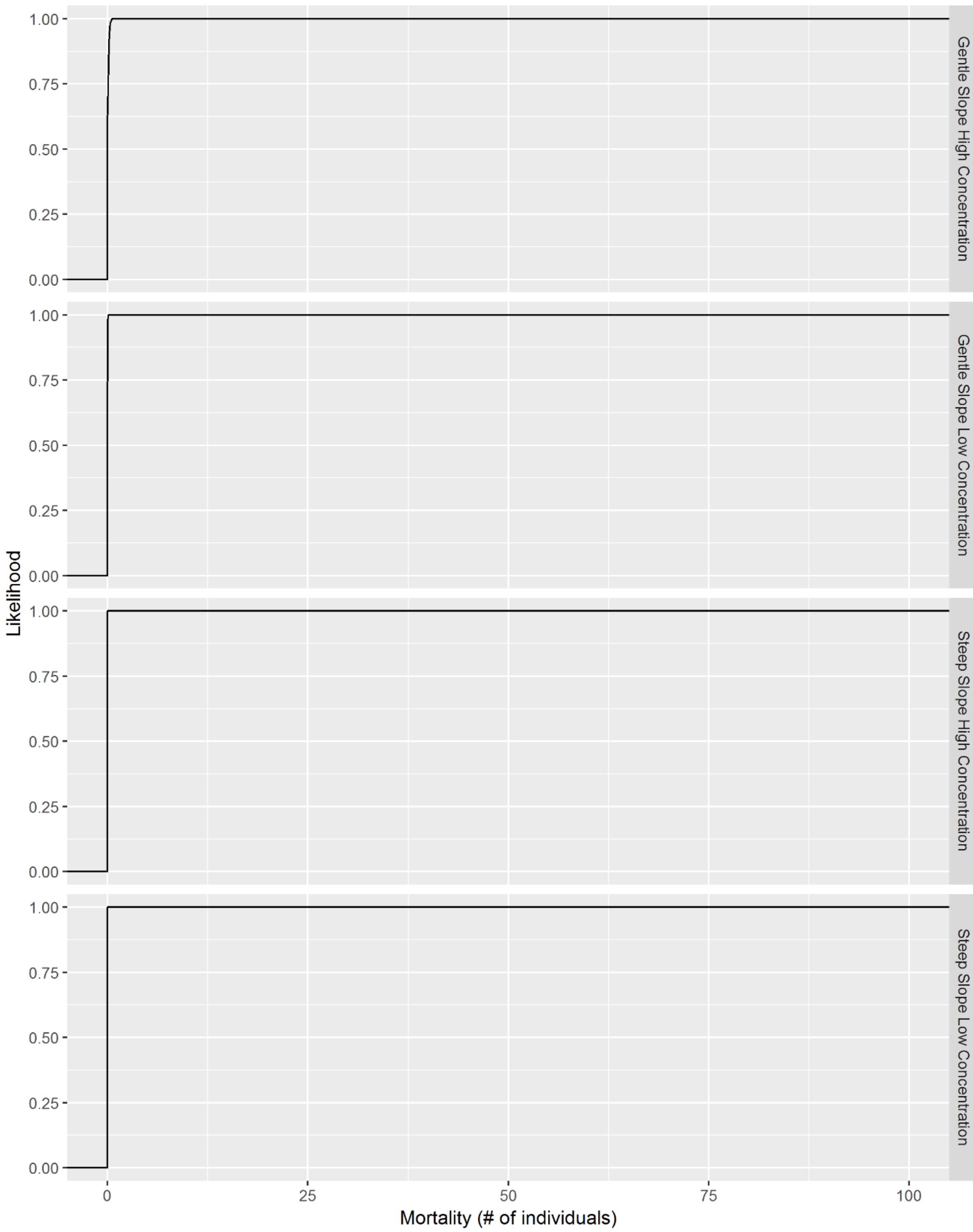

Figure B54. Cumulative likelihood results for Northern Sunfish mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

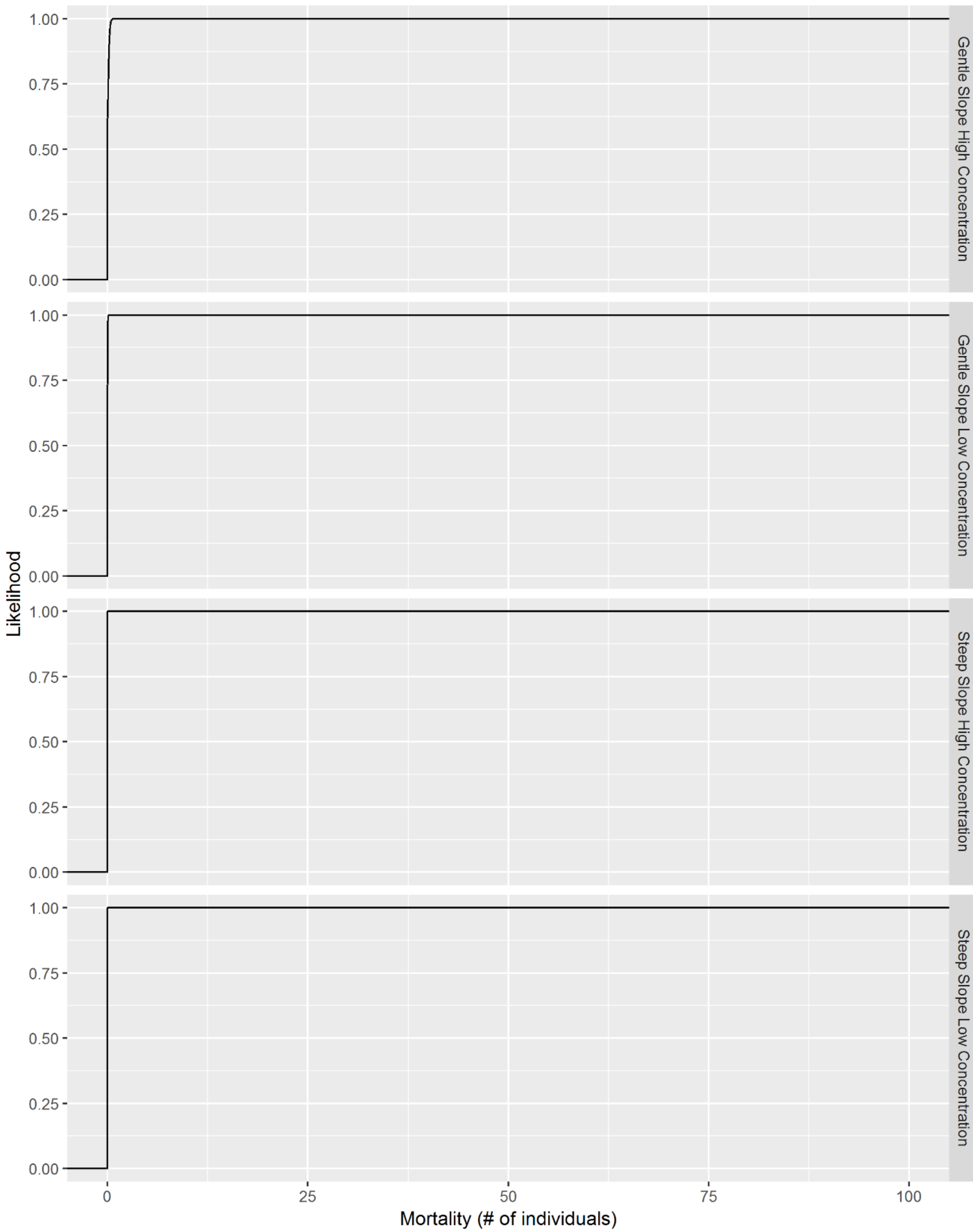

Figure B55. Cumulative likelihood results for Northern Sunfish mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

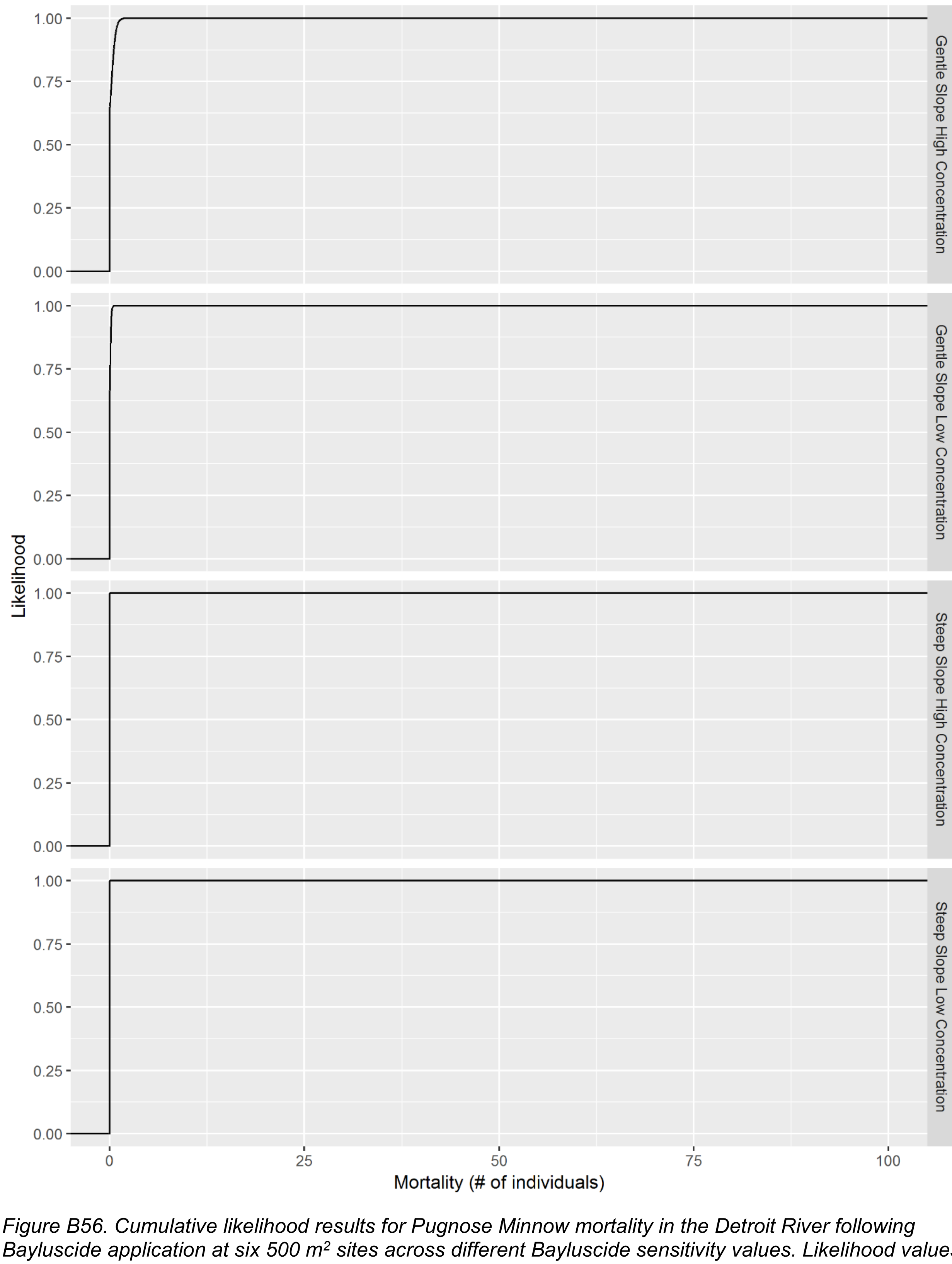

Figure B56. Cumulative likelihood results for Pugnose Minnow mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

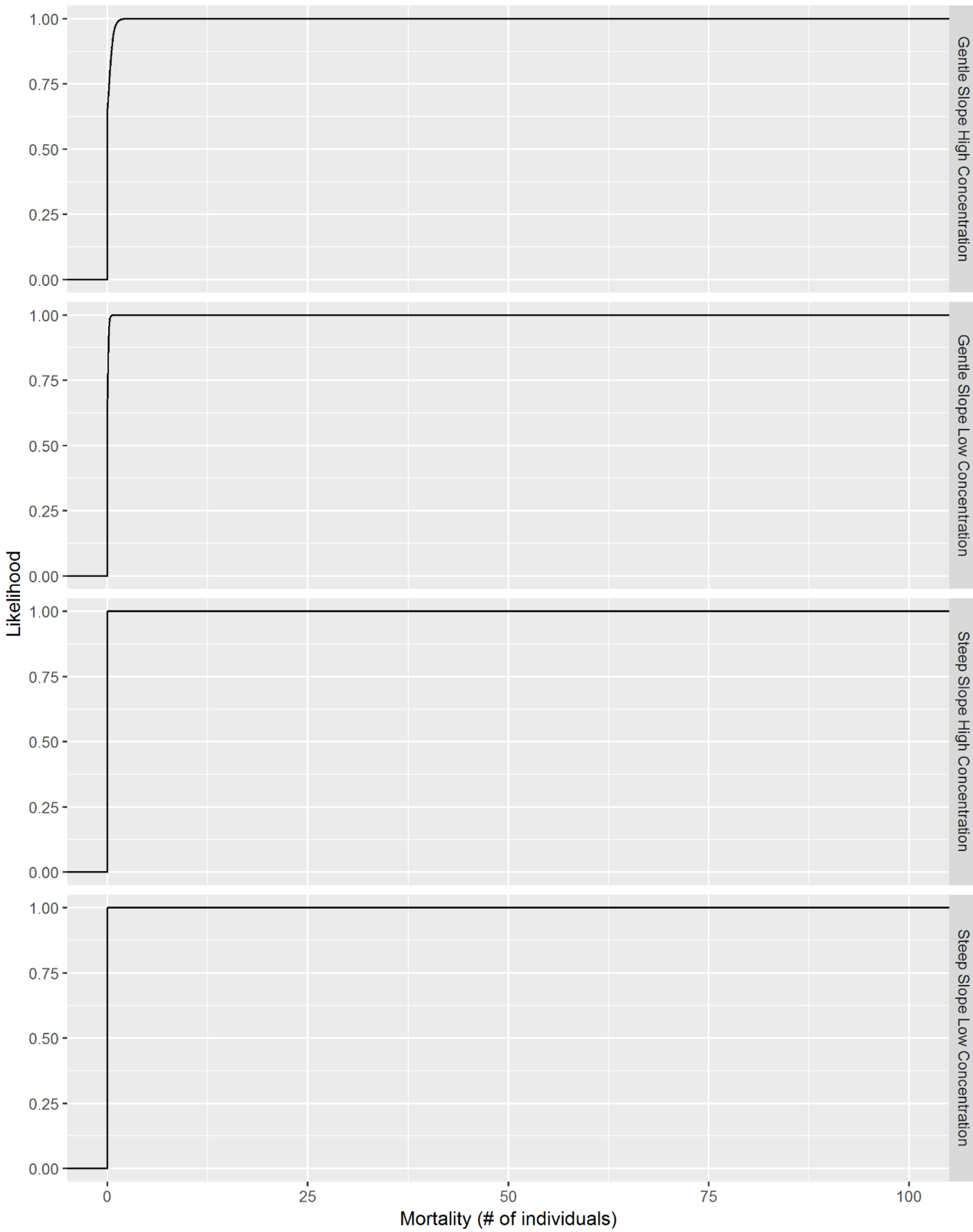

Figure B57. Cumulative likelihood results for Pugnose Minnow mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

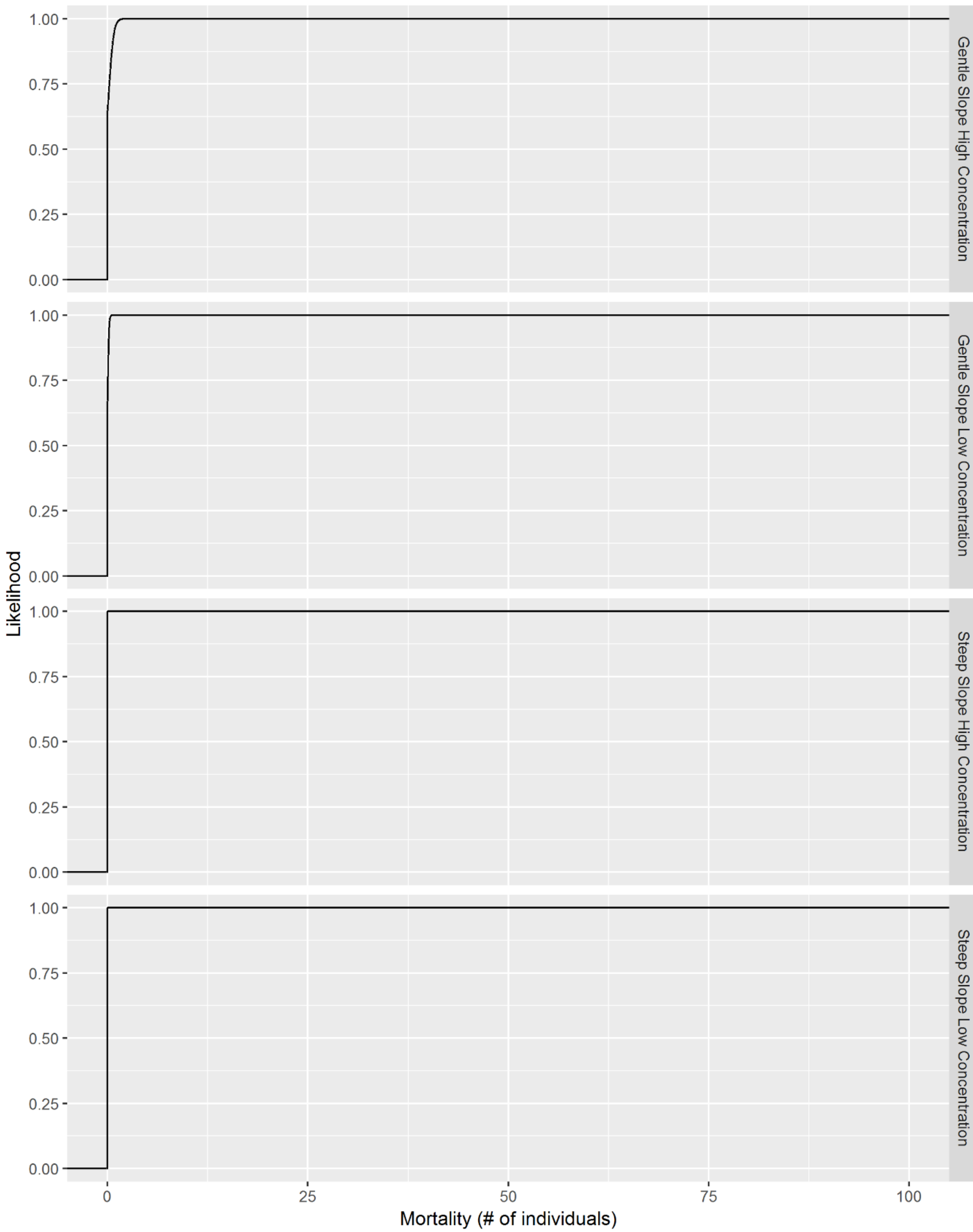

Figure B58. Cumulative likelihood results for Pugnose Minnow mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

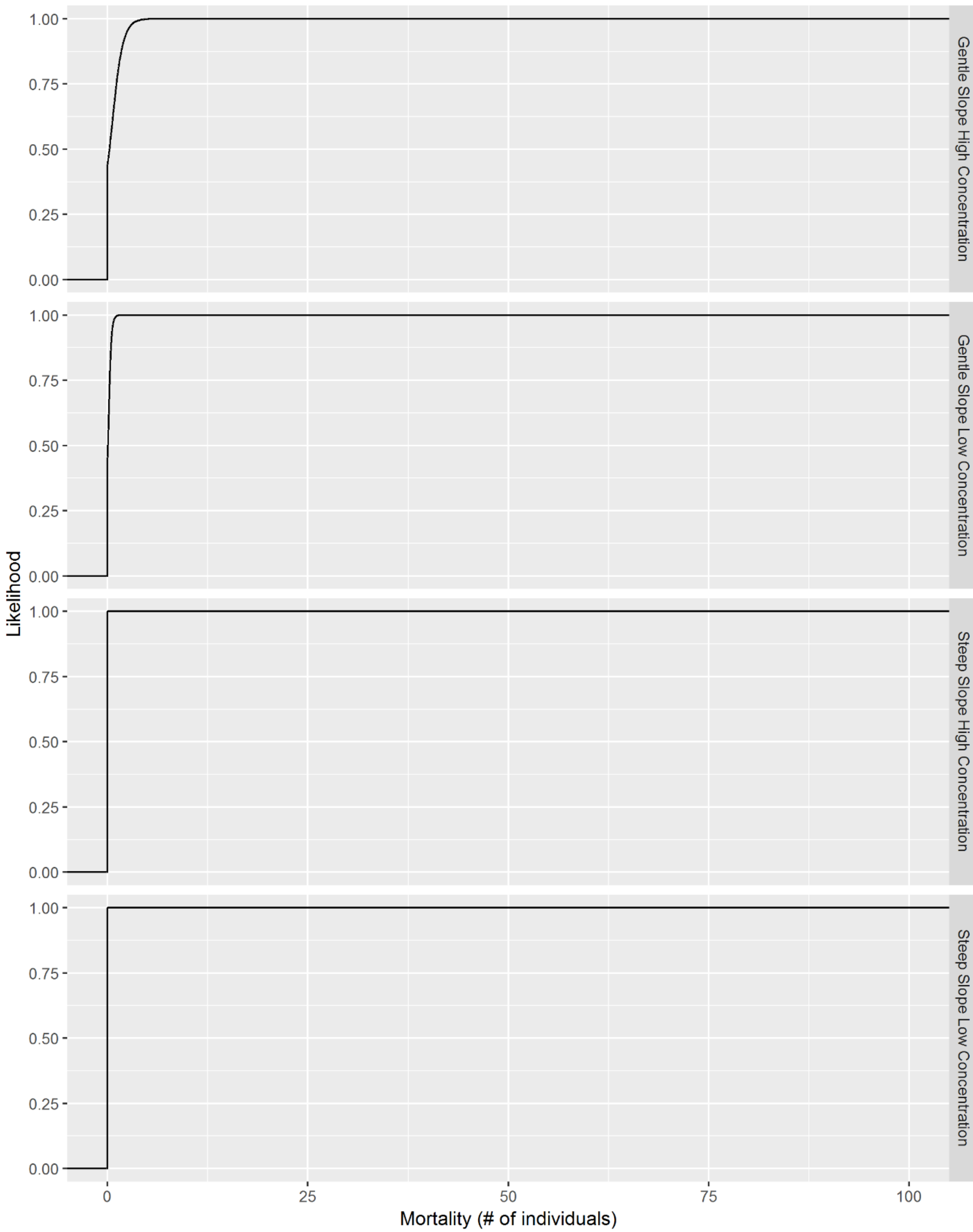

Figure B59. Cumulative likelihood results for Pugnose Shiner mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

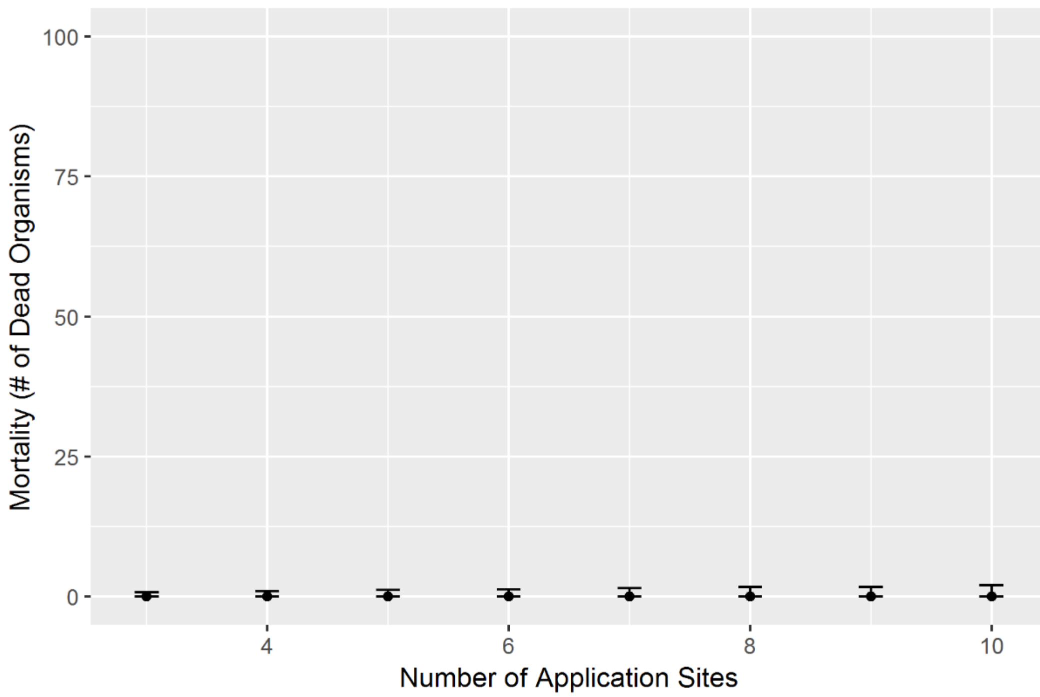

Figure B60. The influence of the number of 500 m² application sites on Pugnose Shiner mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

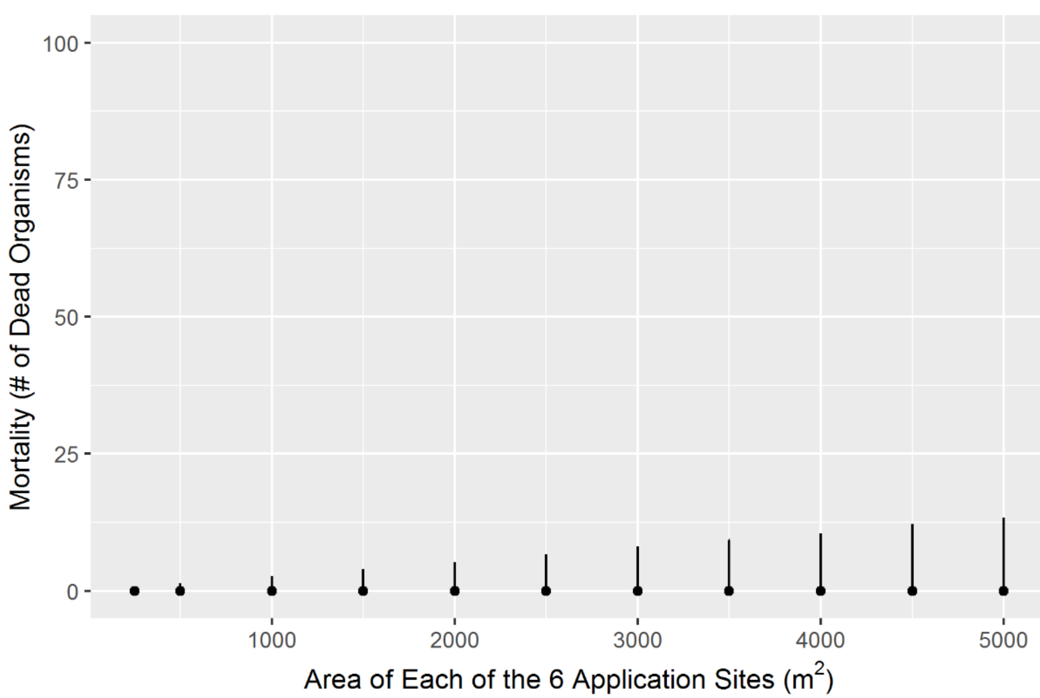

Figure B61. The influence of the size of six application sites on Pugnose Shiner mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.

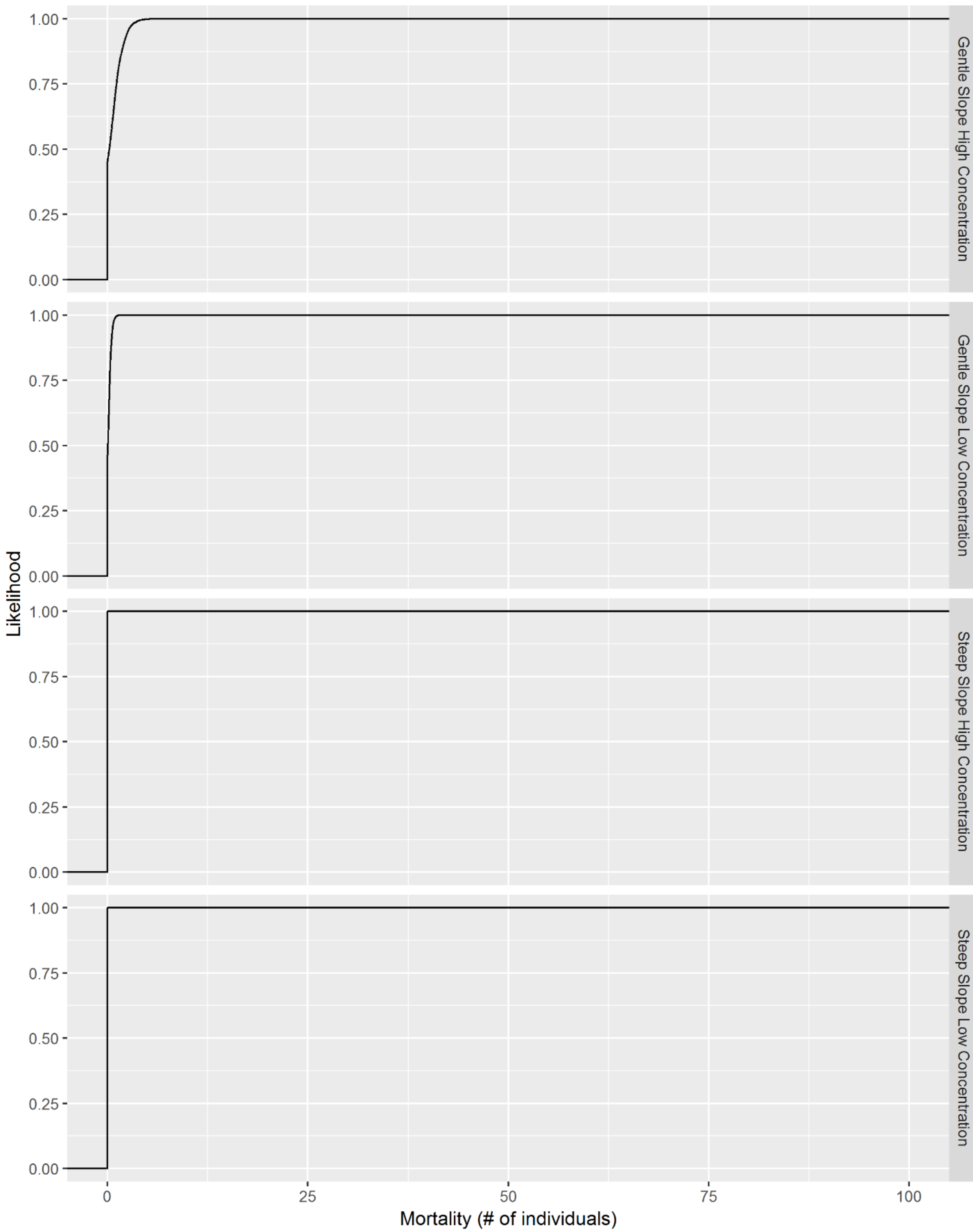

Figure B62. Cumulative likelihood results for Pugnose Shiner mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

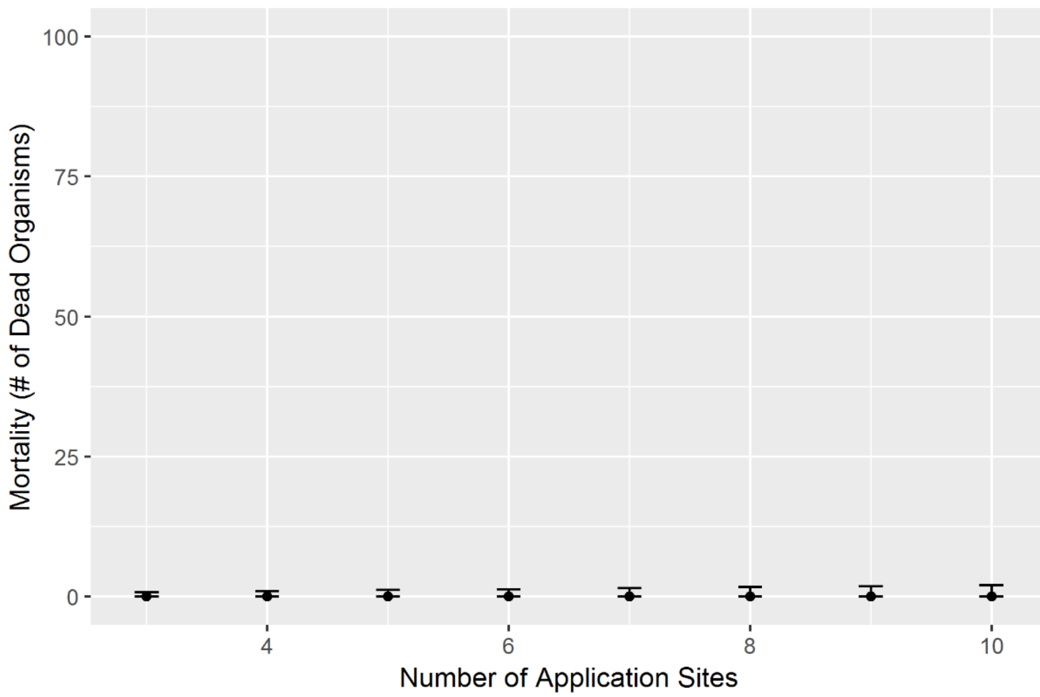

Figure B63. The influence of the number of 500 m² application sites on Pugnose Shiner mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

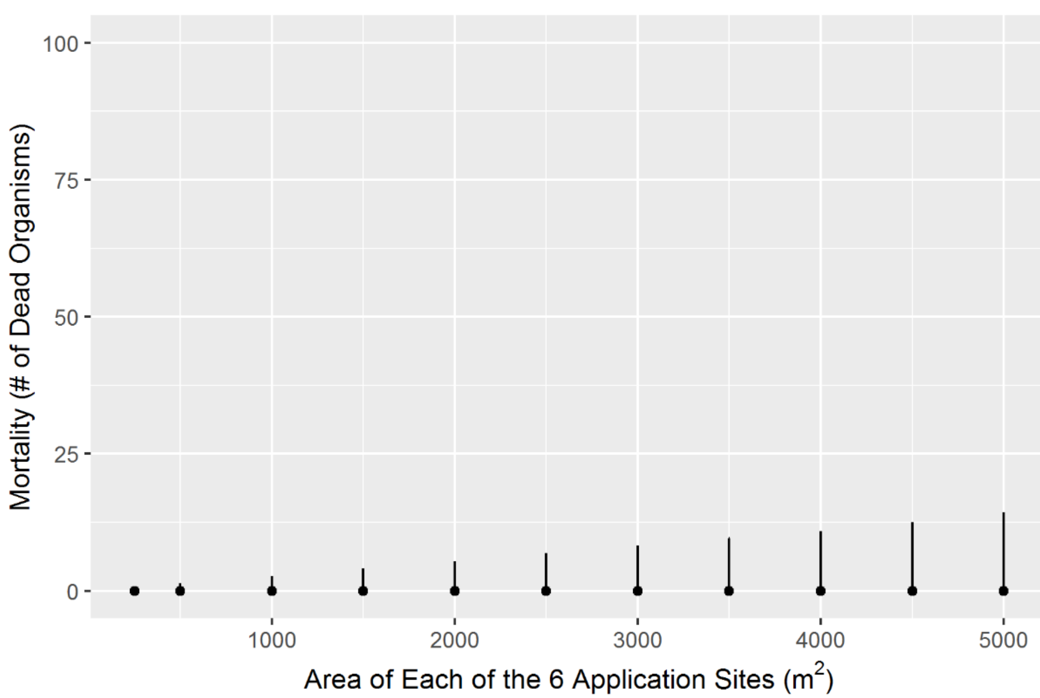

Figure B64. The influence of the size of six application sites on Pugnose Shiner mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.

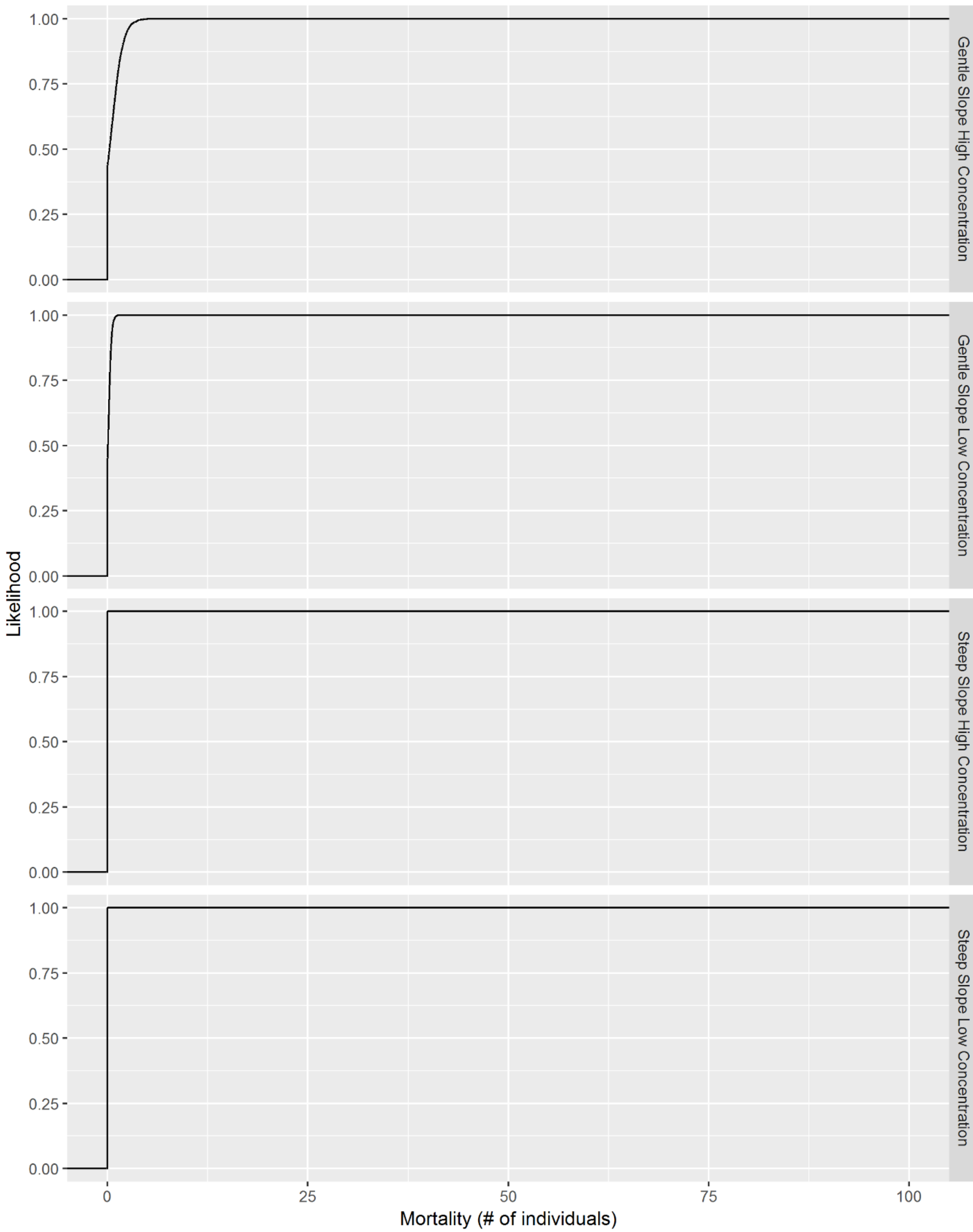

Figure B65. Cumulative likelihood results for Pugnose Shiner mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

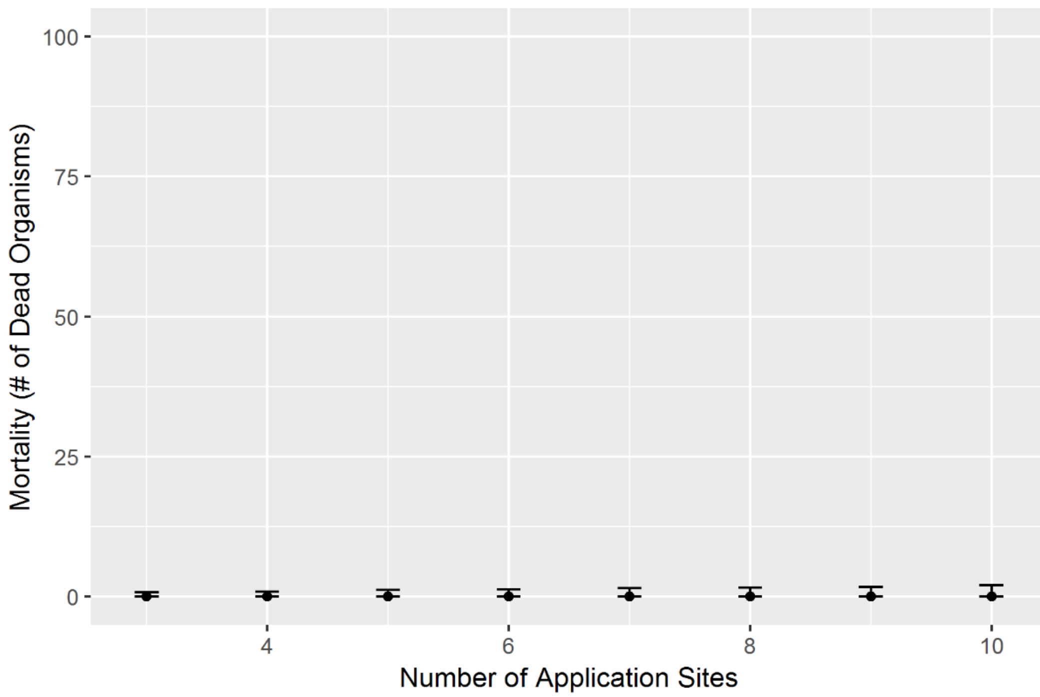

Figure B66. The influence of the number of 500 m² application sites on Pugnose Shiner mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

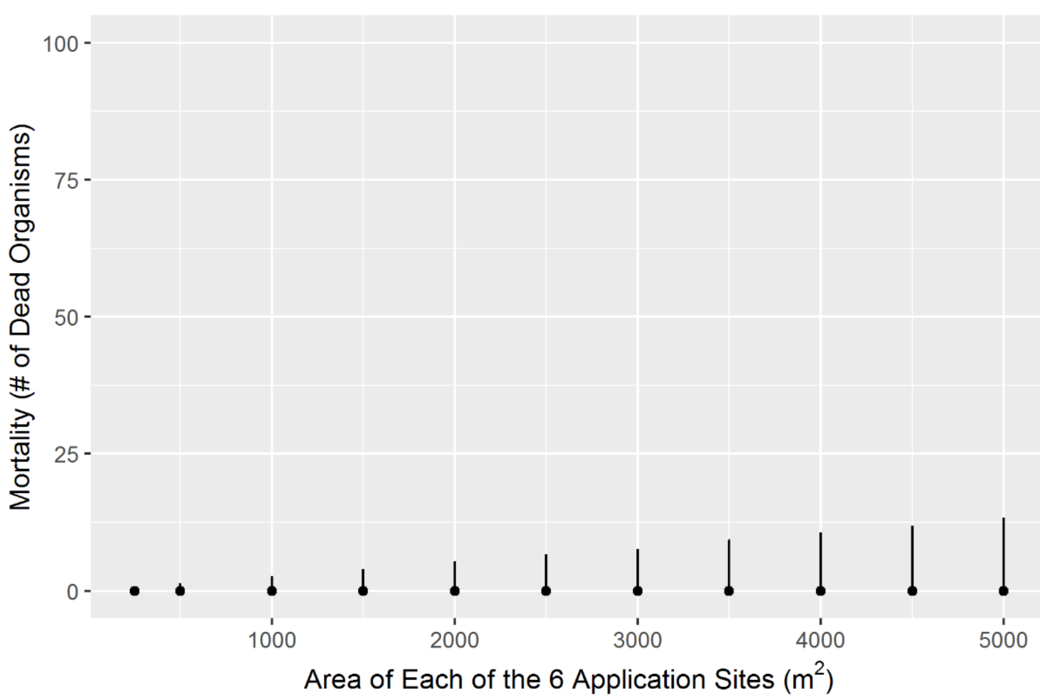

Figure B67. The influence of the size of six application sites on Pugnose Shiner mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

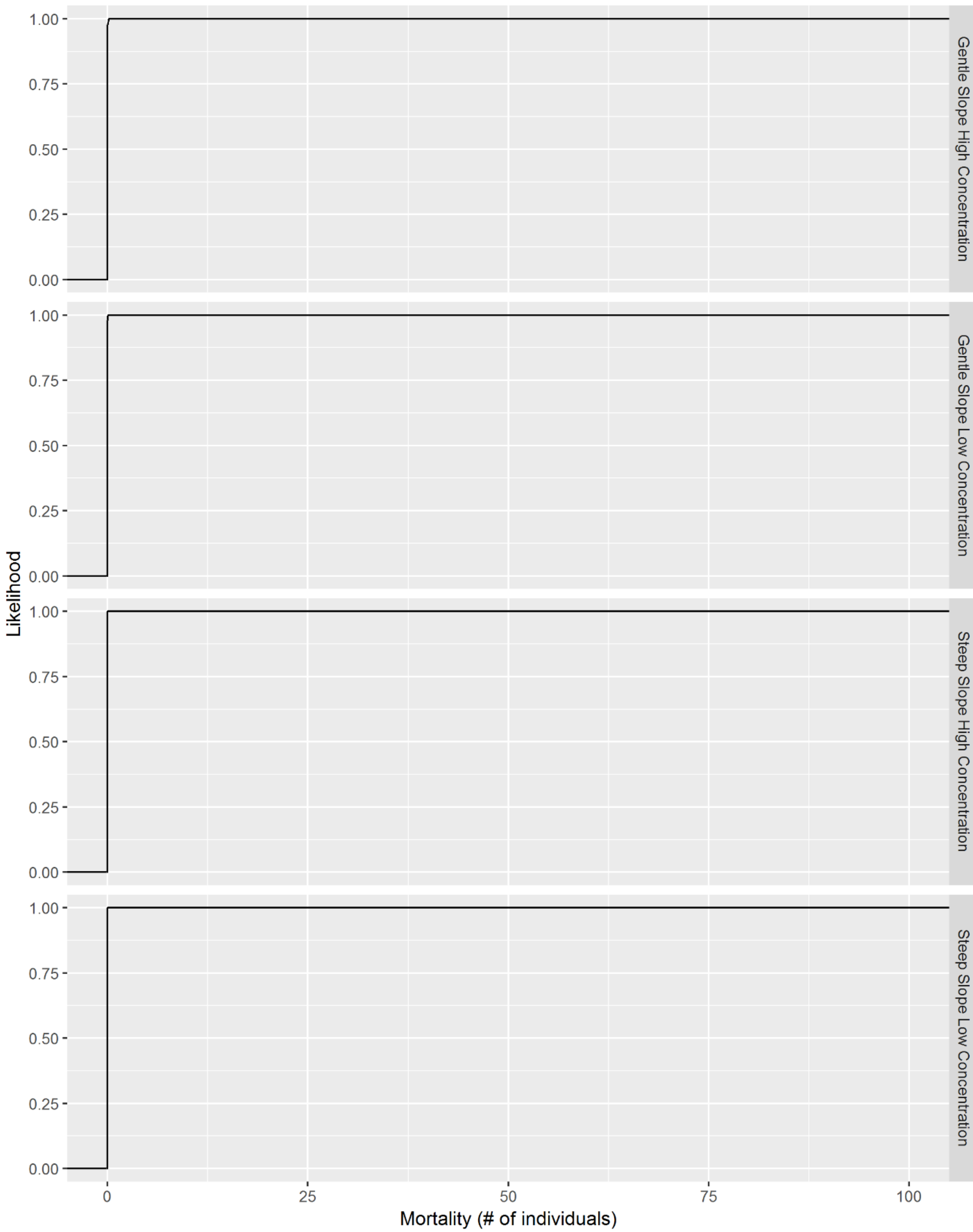

Figure B68. Cumulative likelihood results for River Darter mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

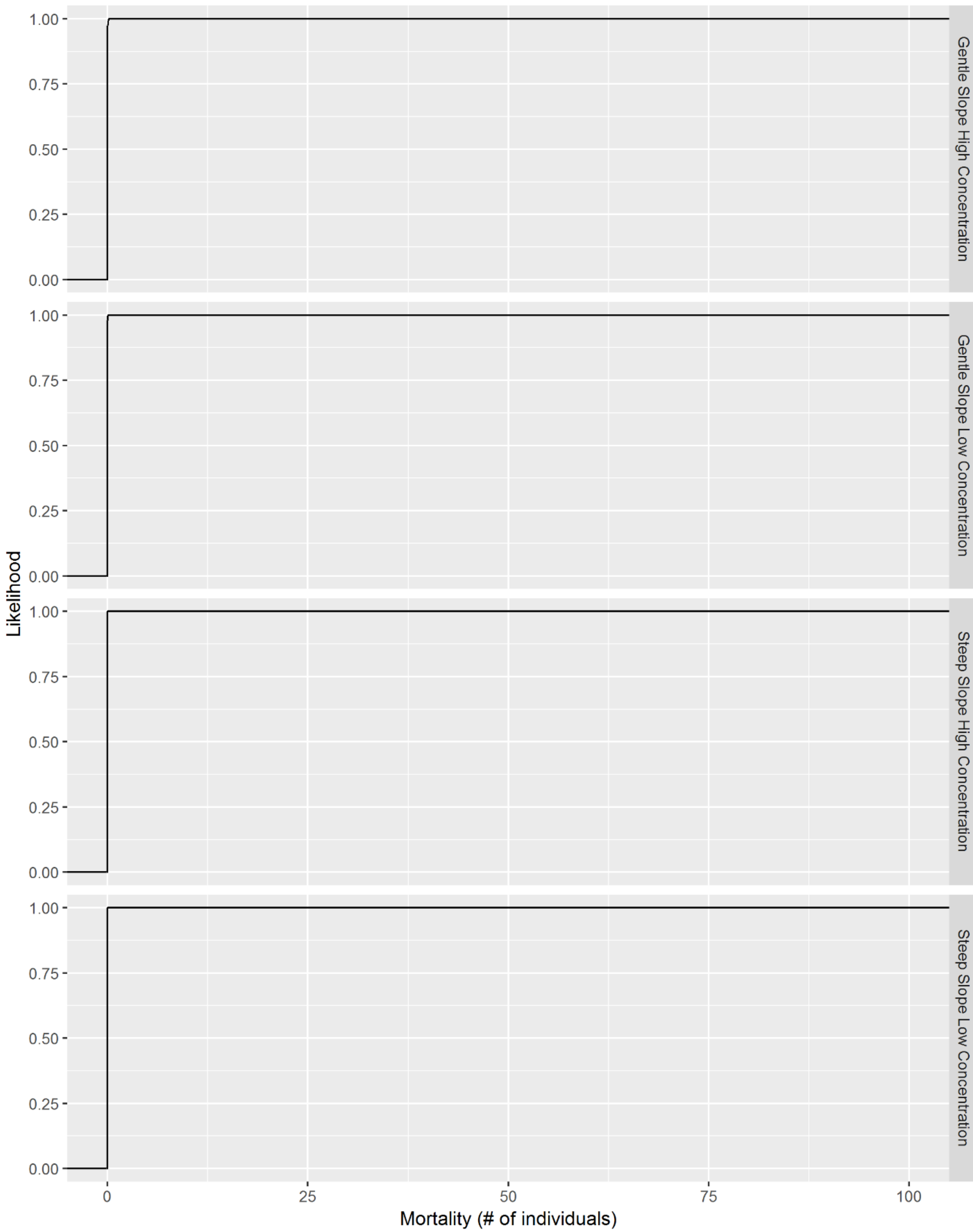

Figure B69. Cumulative likelihood results for River Darter mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

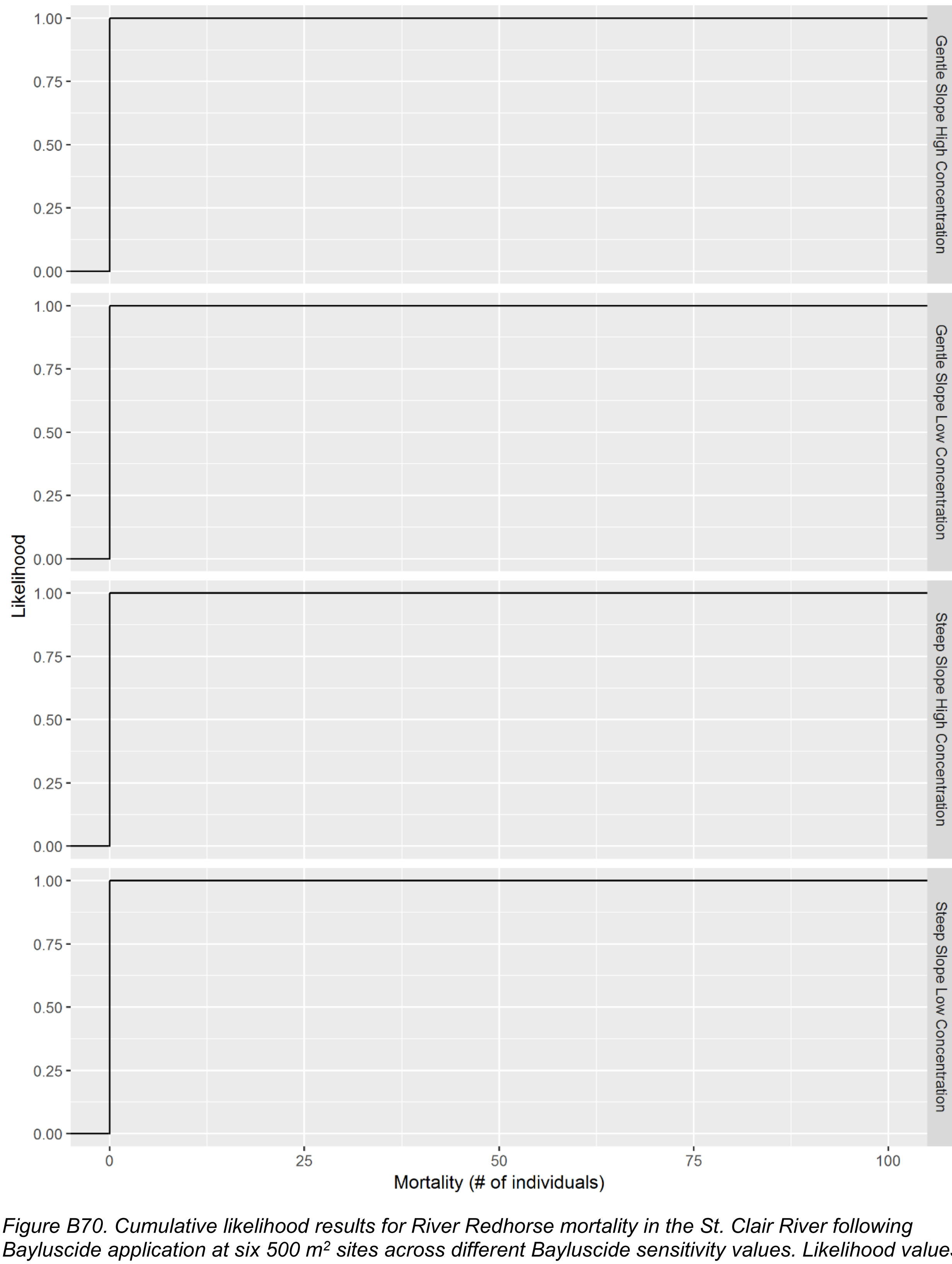

Figure B70. Cumulative likelihood results for River Redhorse mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

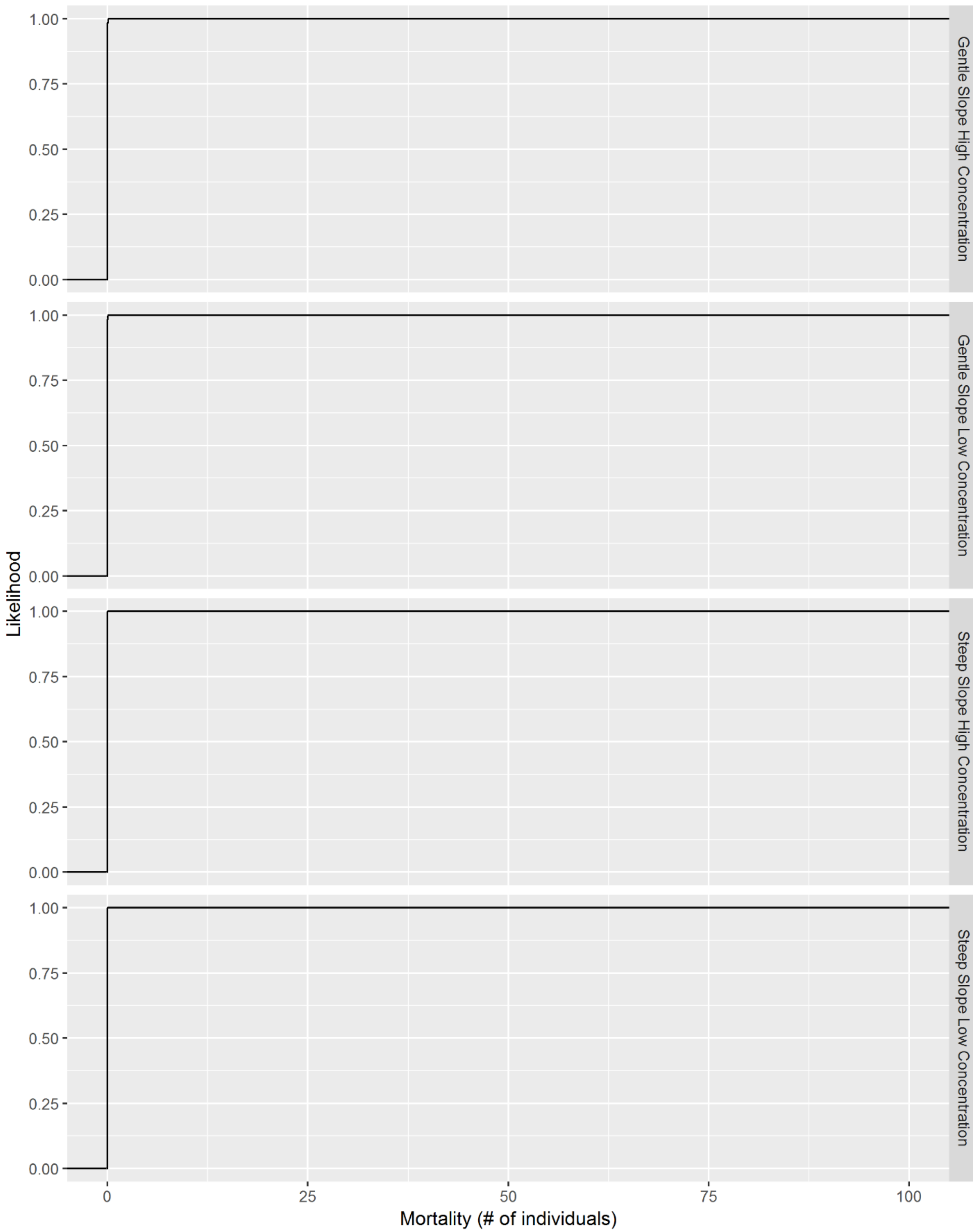

Figure B71. Cumulative likelihood results for Silver Chub mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

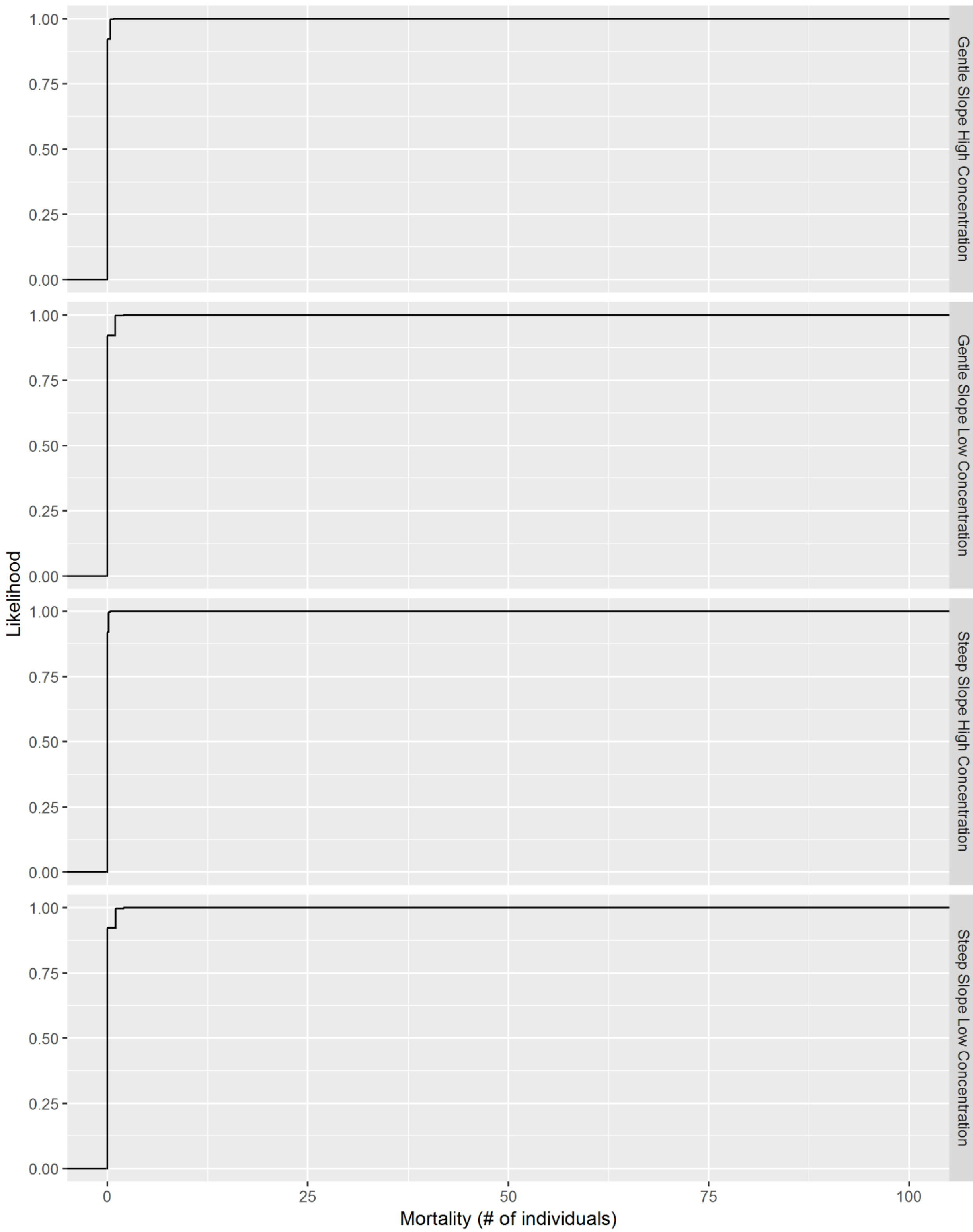

Figure B72. Cumulative likelihood results for Silver Lamprey mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

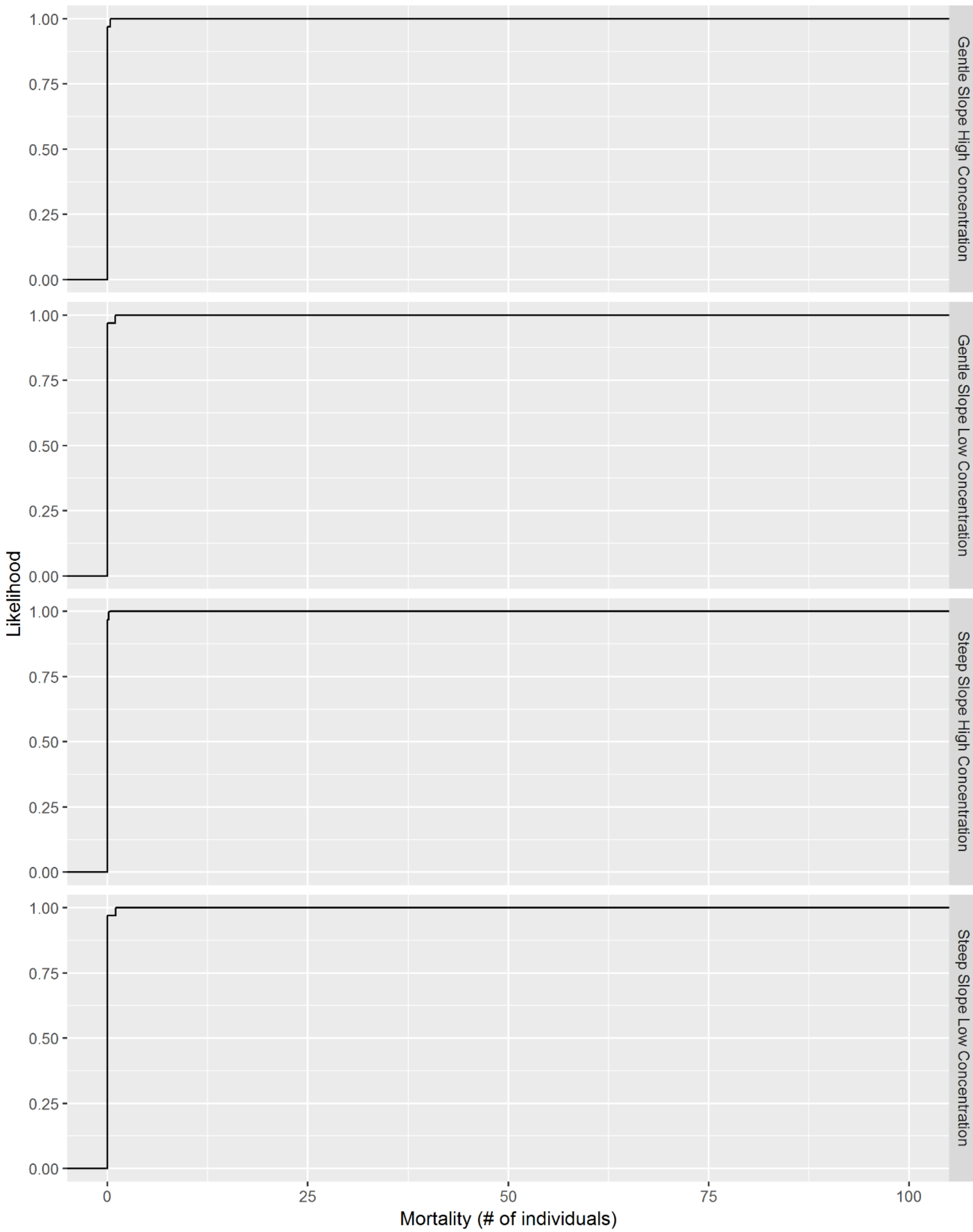

Figure B73. Cumulative likelihood results for Silver Lamprey mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

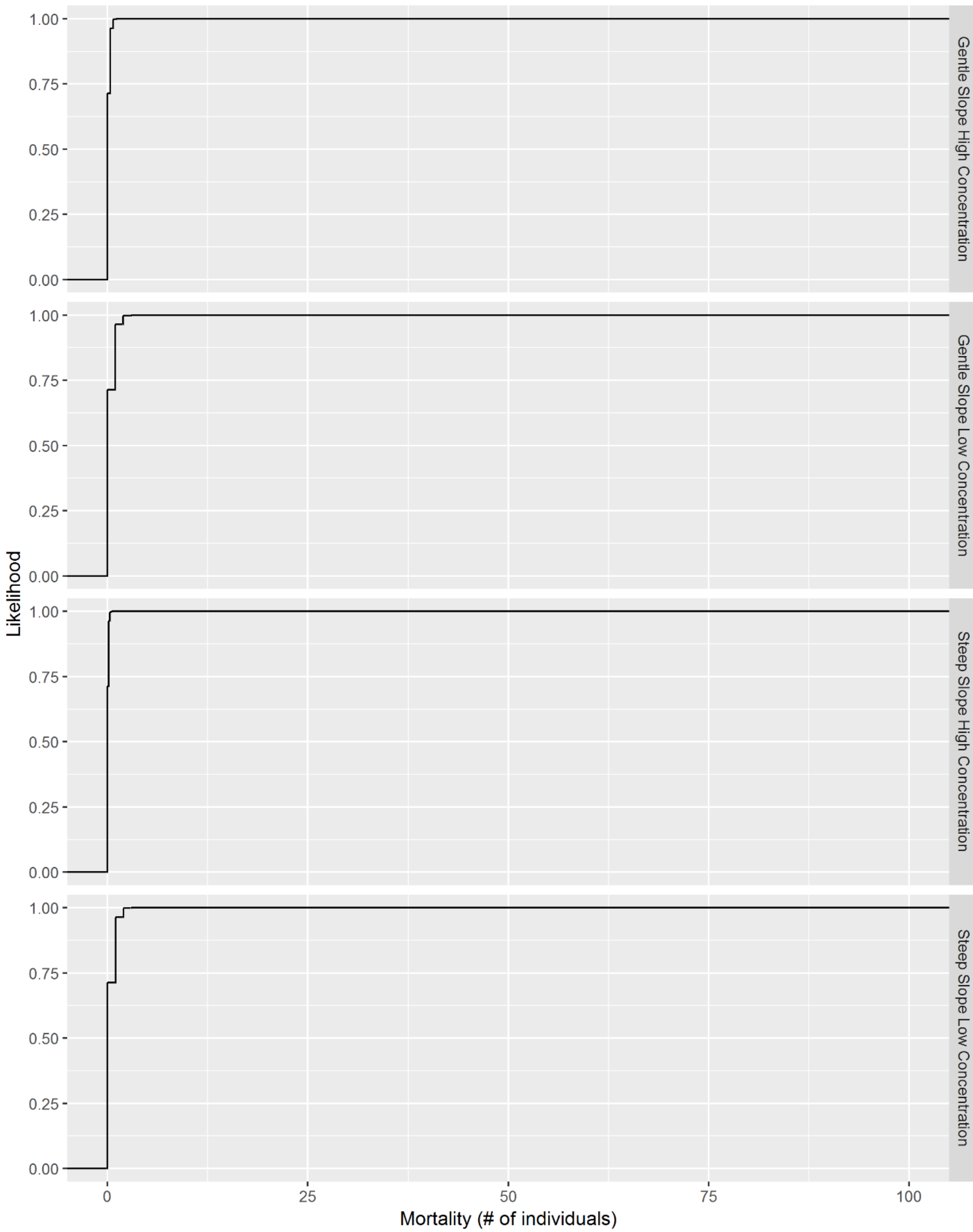

Figure B74. Cumulative likelihood results for Silver Lamprey mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

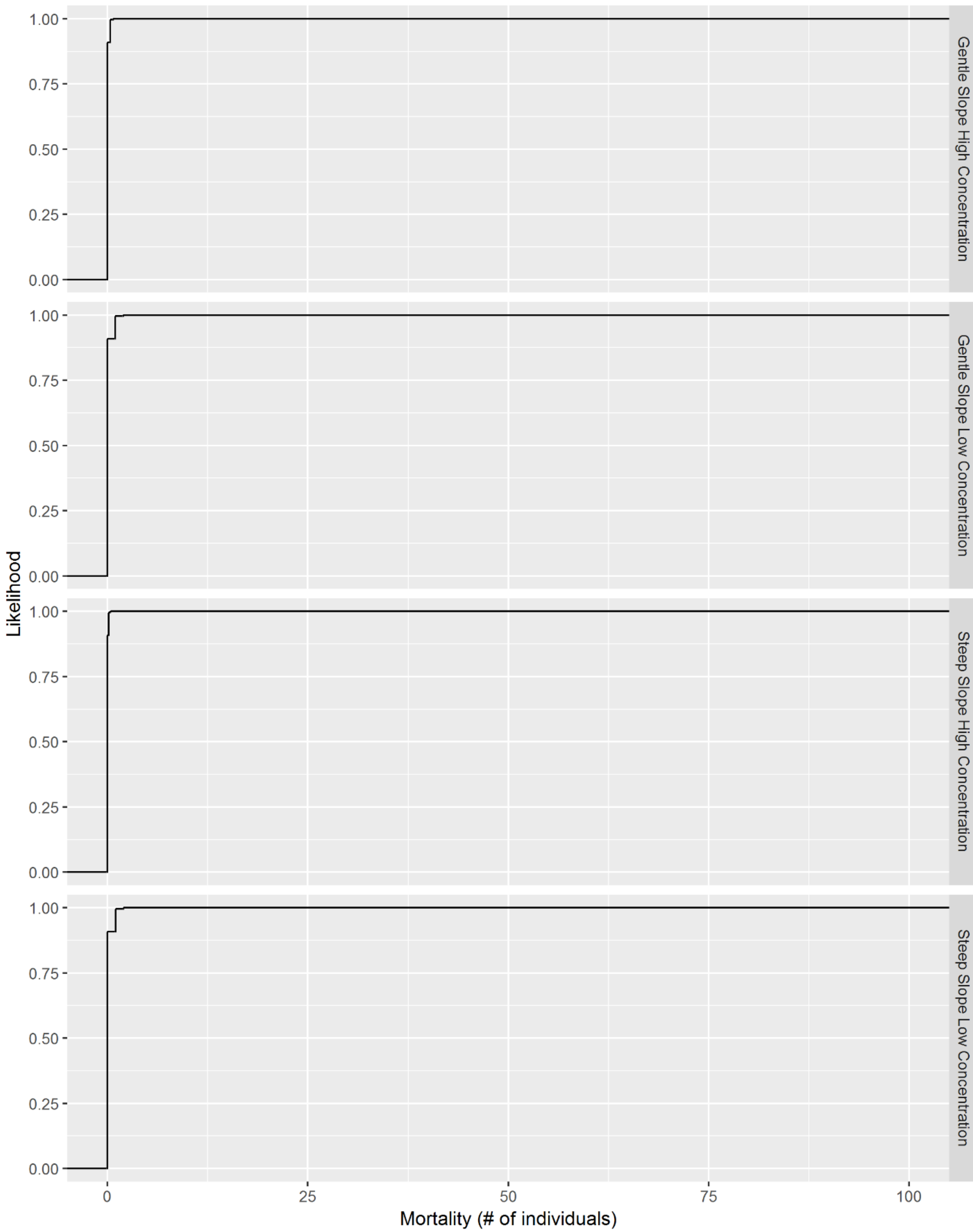

Figure B75. Cumulative likelihood results for Silver Lamprey mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

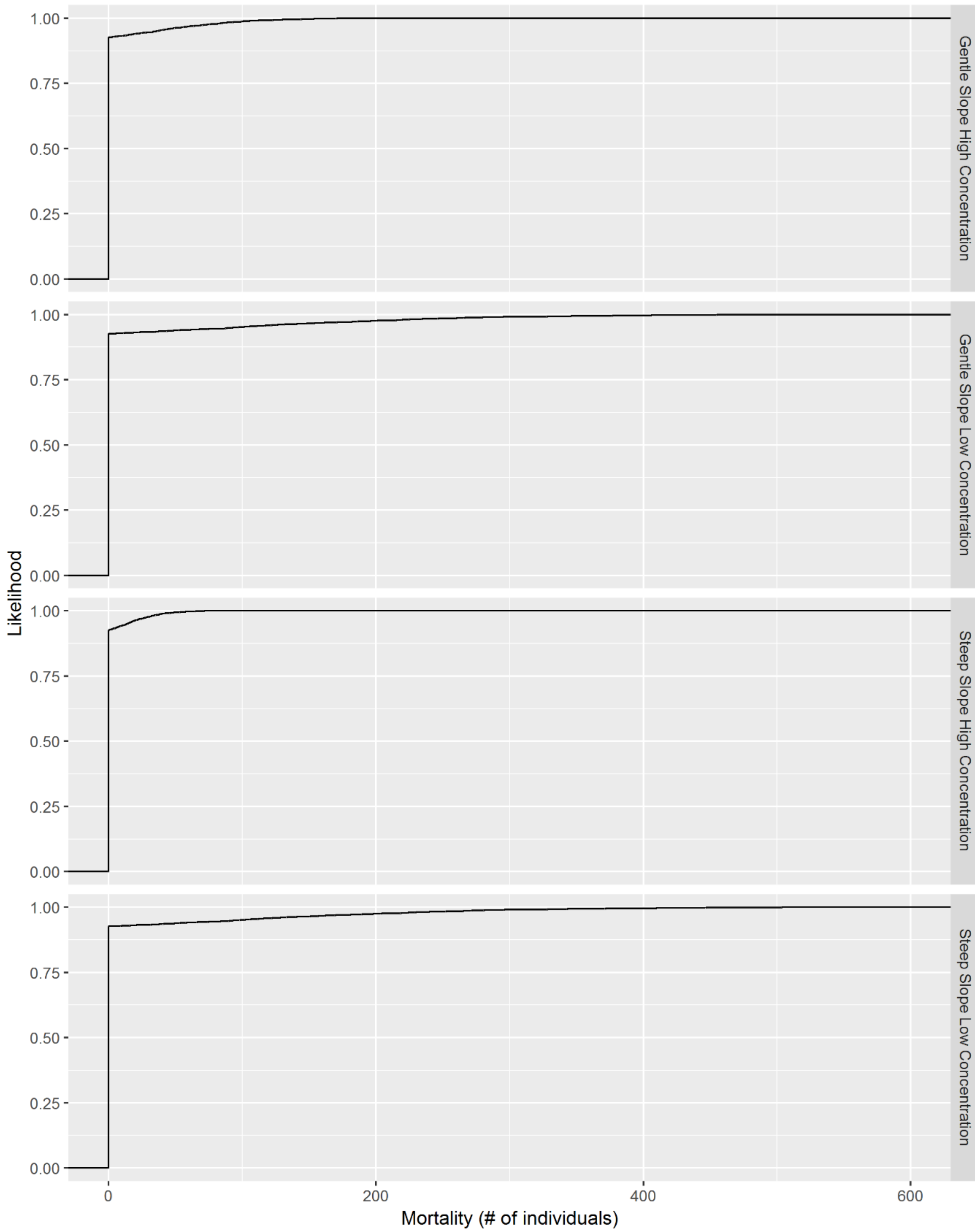

Figure B76. Cumulative likelihood results for Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

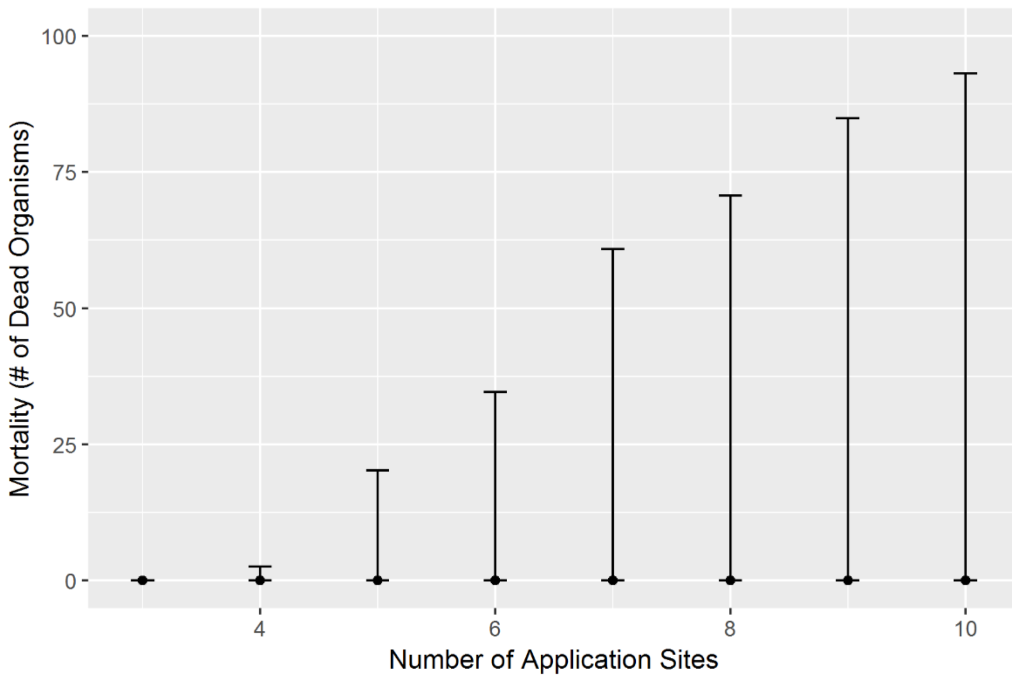

Figure B77. The influence of the number of 500 m² application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.*

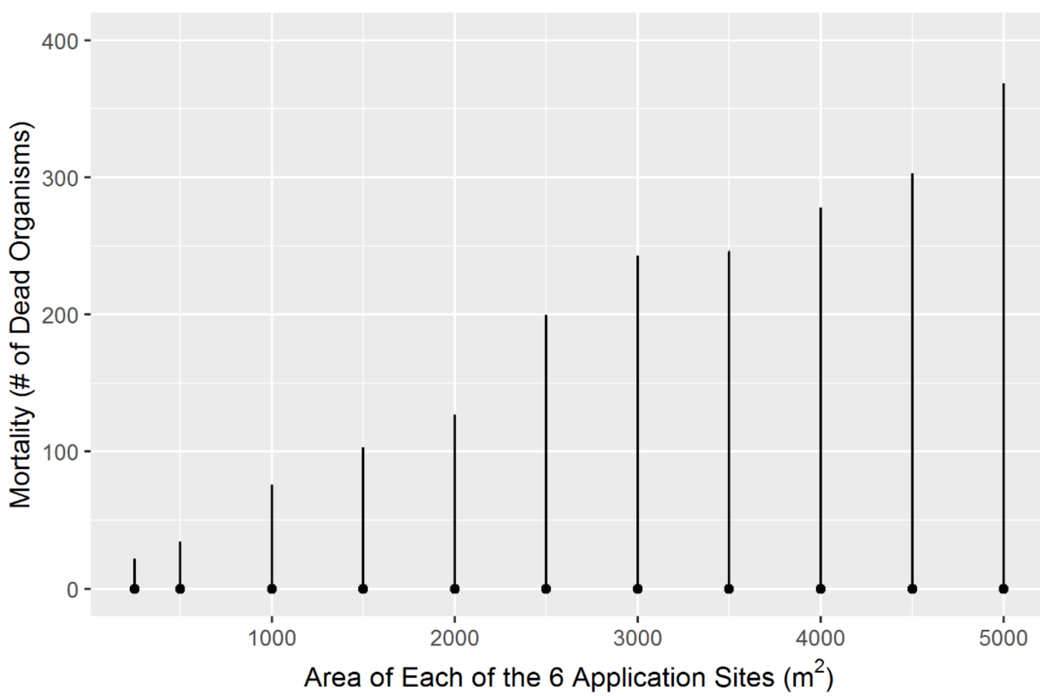

Figure B78. The influence of the size of six application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.*

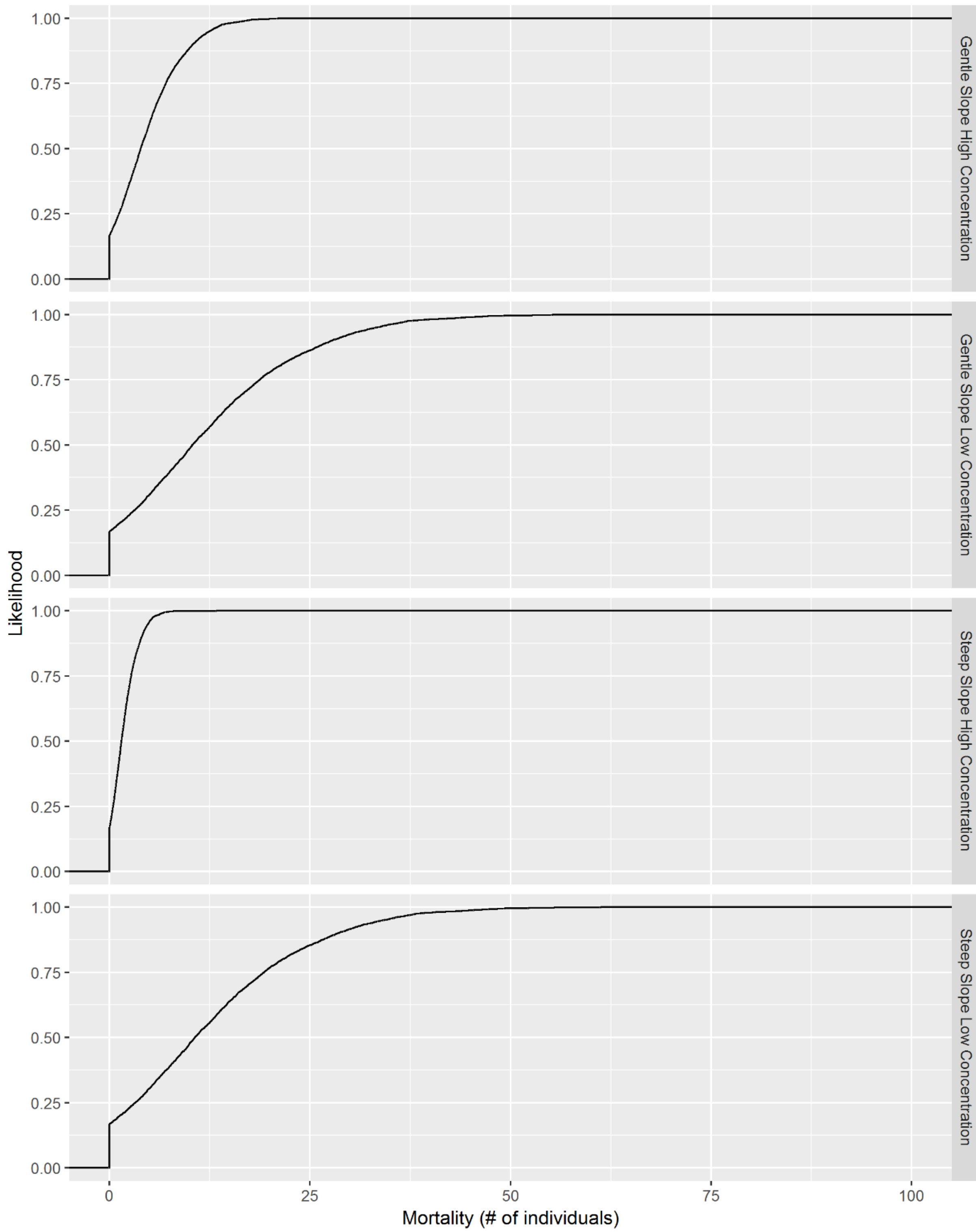

Figure B79. Cumulative likelihood results for Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

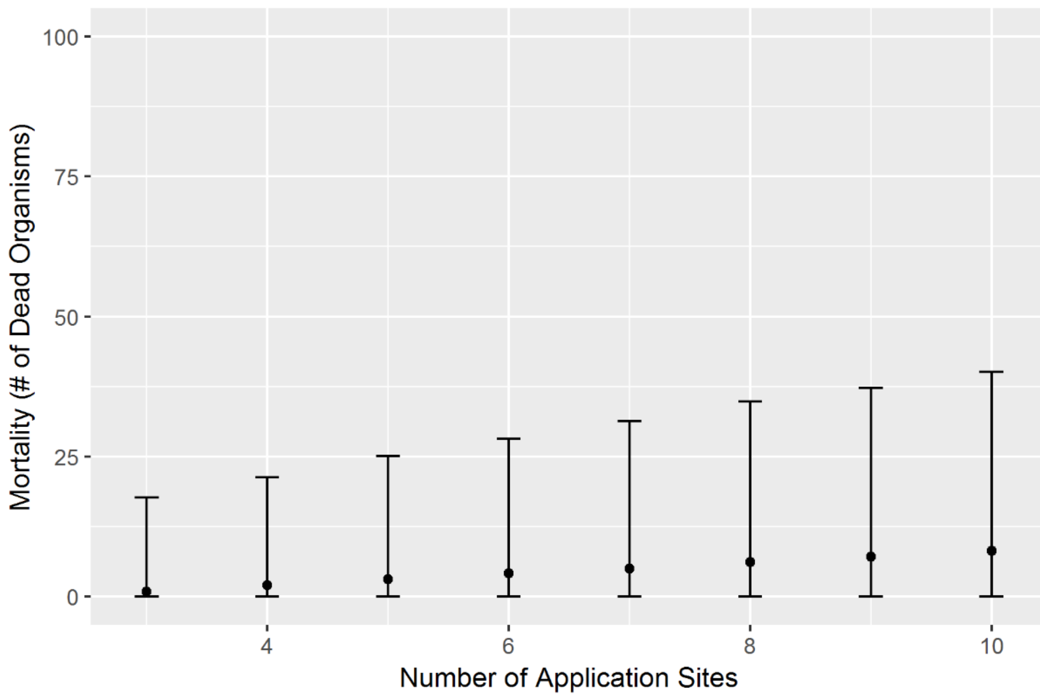

Figure B80. The influence of the number of 500 m² application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.*

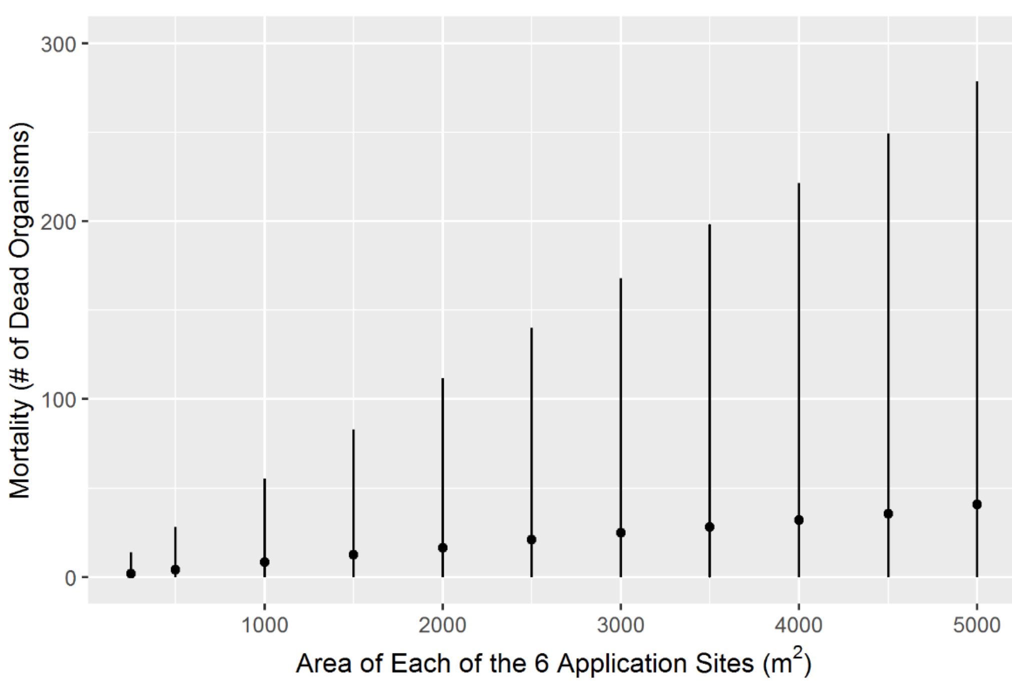

Figure B81. The influence of the size of six application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.*

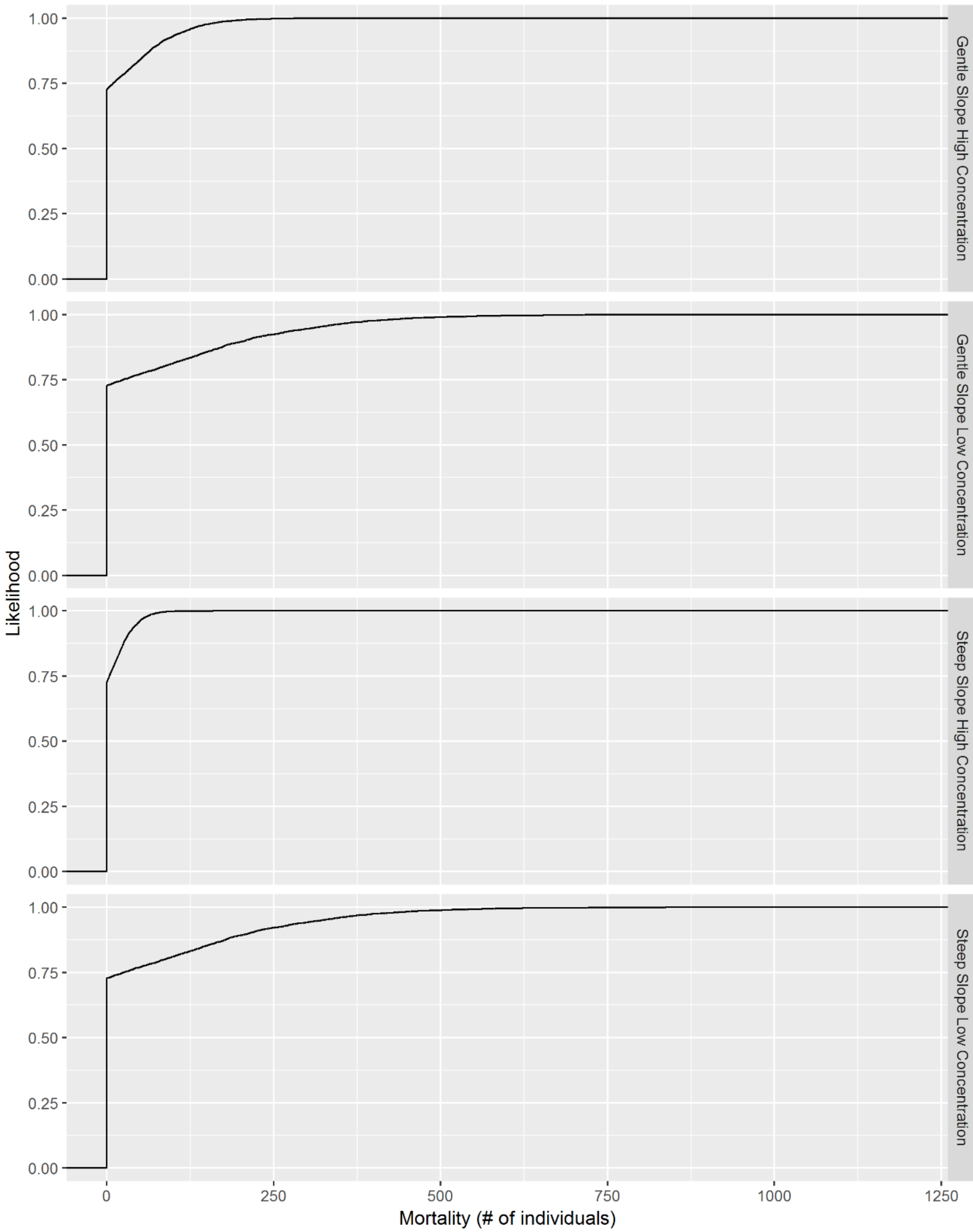

Figure B82. Cumulative likelihood results for Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

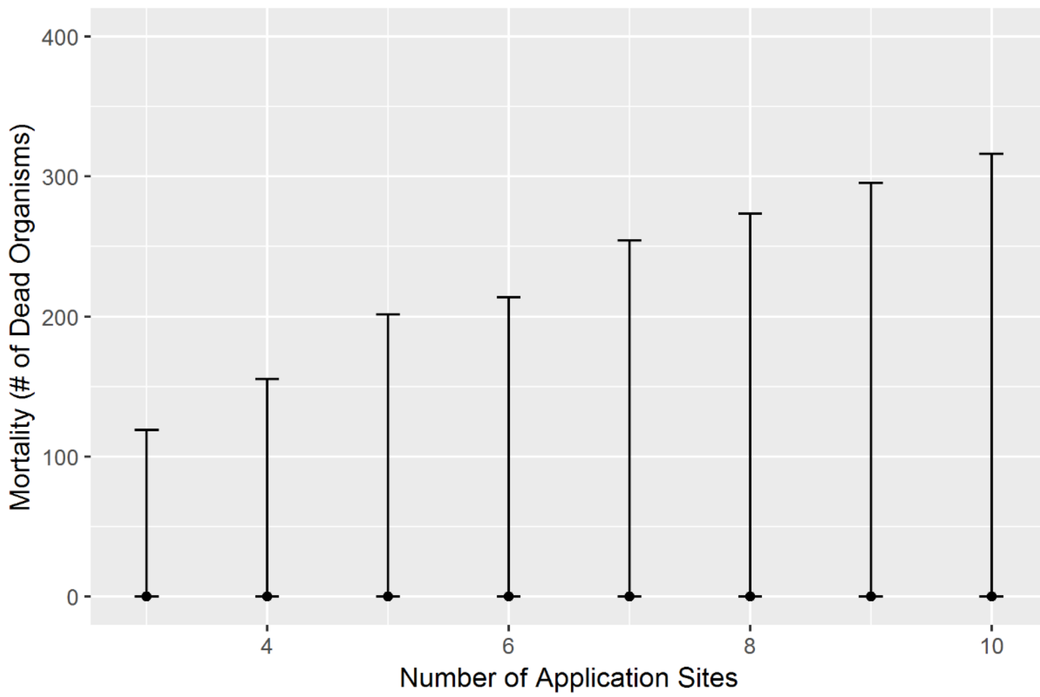

Figure B83. The influence of the number of 500 m² application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.*

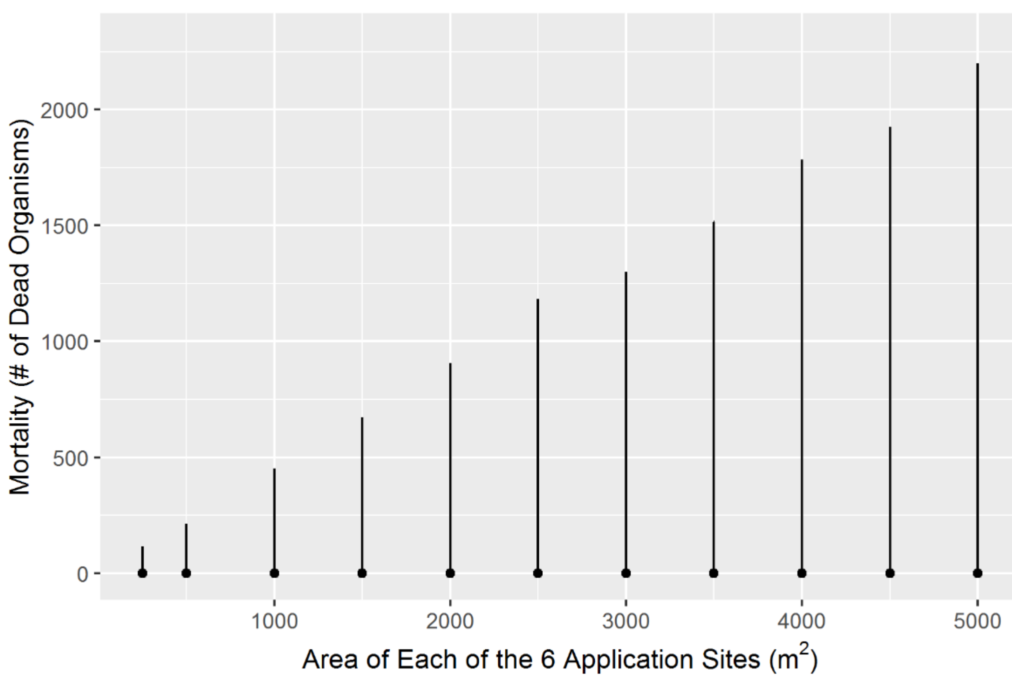

Figure B84. The influence of the size of six application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Detroit River. Error bars represents the upper and lower 95th percentile.*

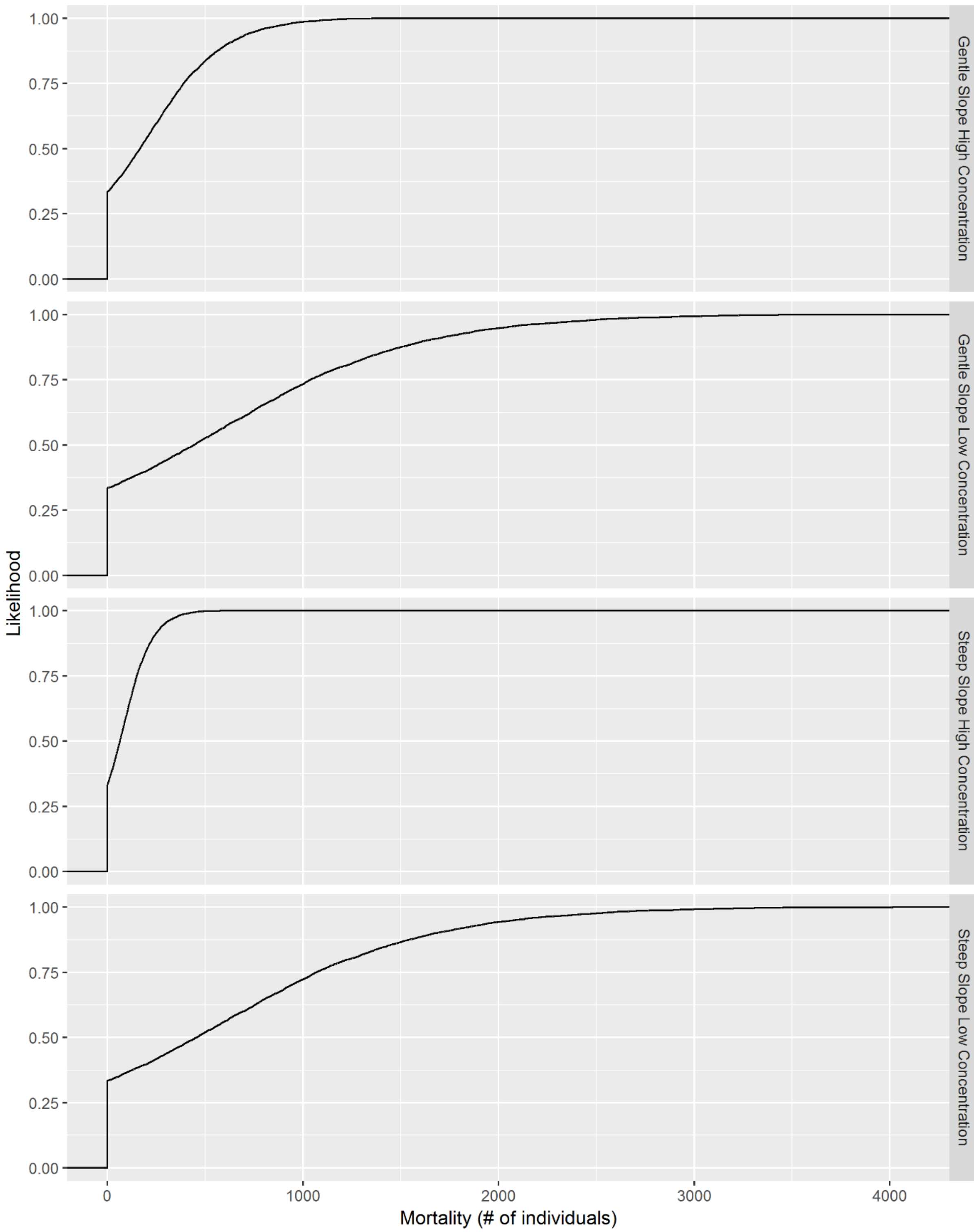

Figure B85. Cumulative likelihood results for Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.*

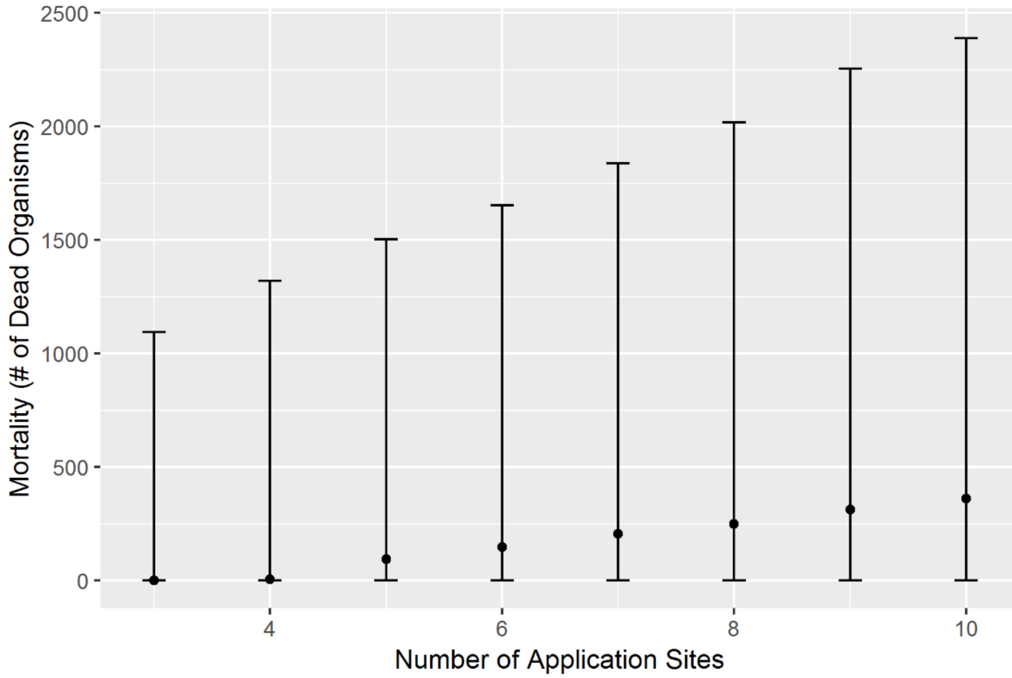

Figure B86. The influence of the number of 500 m² application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the Thames River. Error bars represents the upper and lower 95th percentile.*

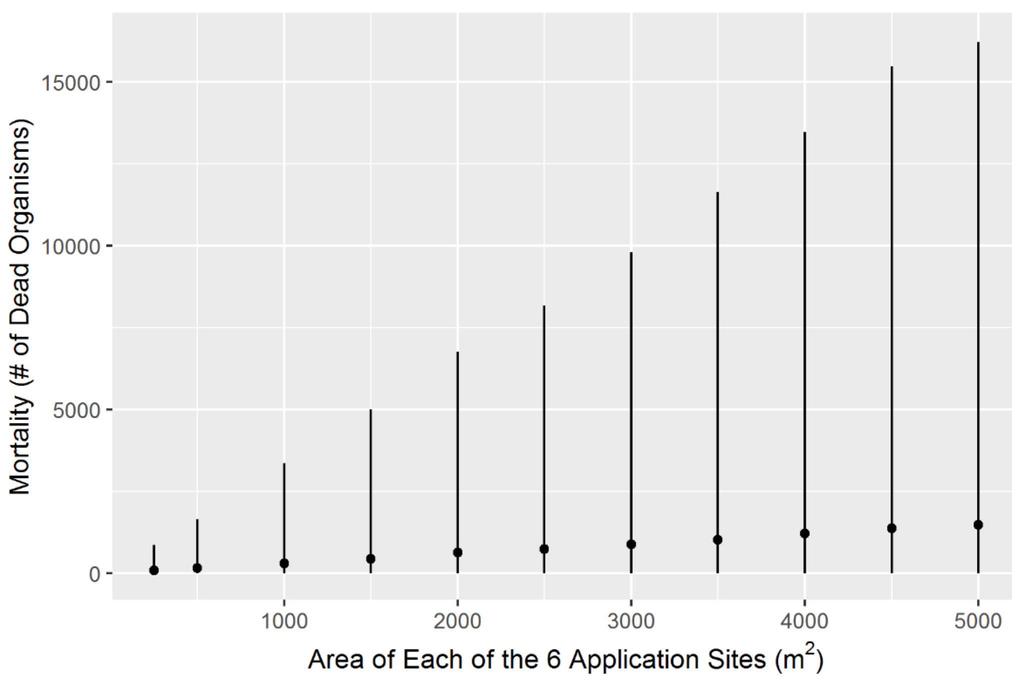

Figure B87. The influence of the size of six application sites on Silver Lamprey and unidentified Ichthyomyzon *sp. mortality in the St. Clair River. Error bars represents the upper and lower 95th percentile.*

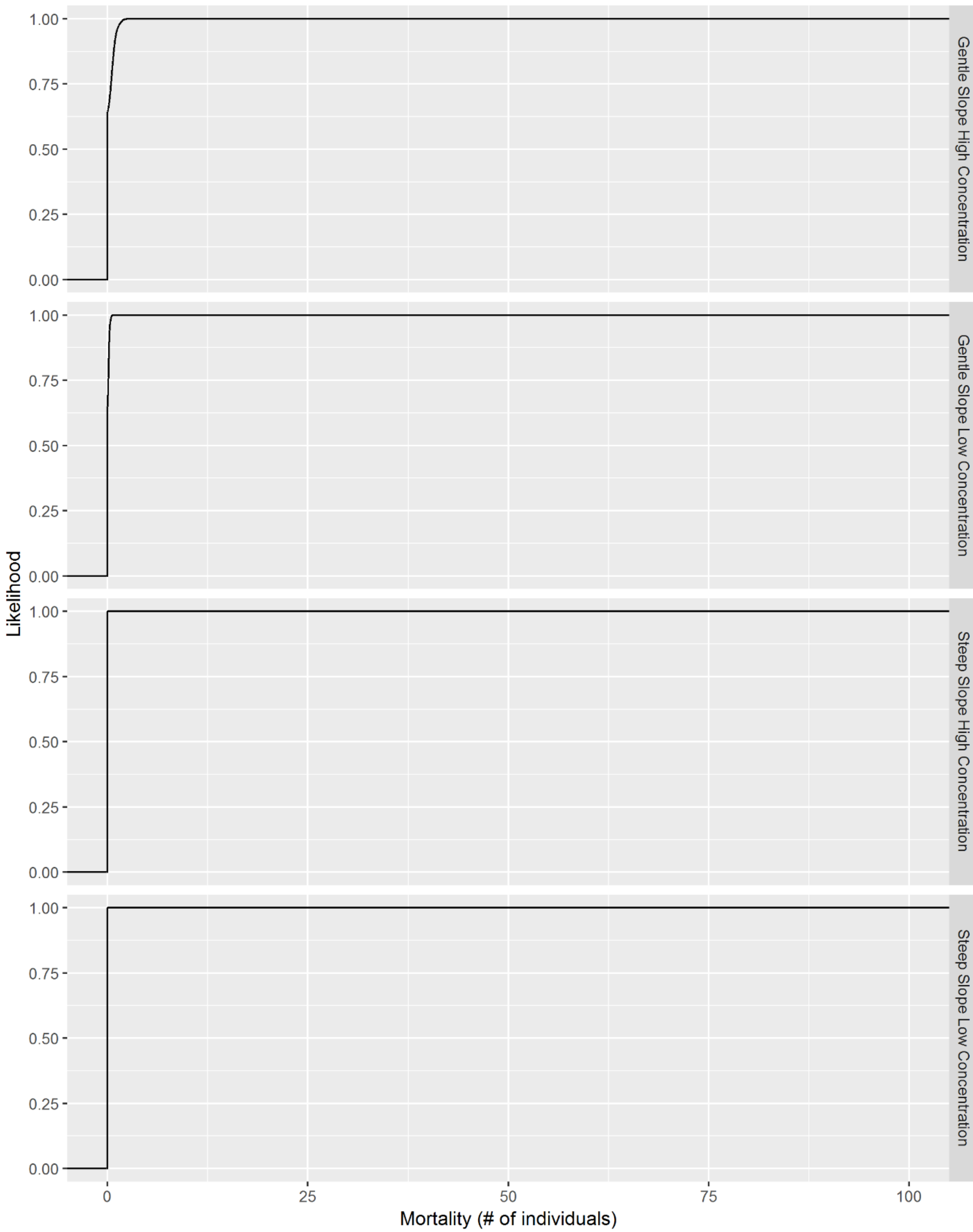

Figure B88. Cumulative likelihood results for Silver Shiner mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

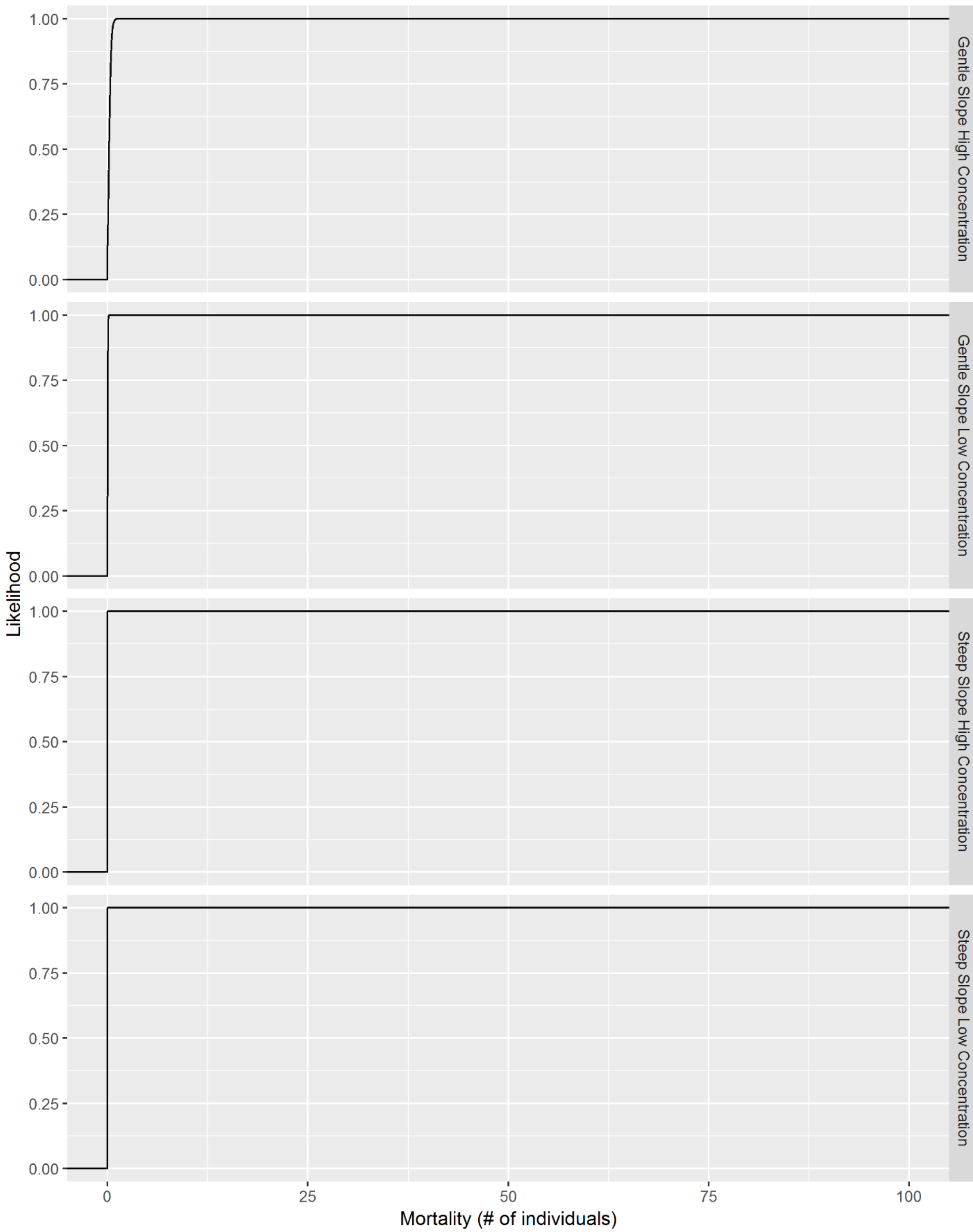

Figure B89. Cumulative likelihood results for Spotted Sucker mortality in the Detroit River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

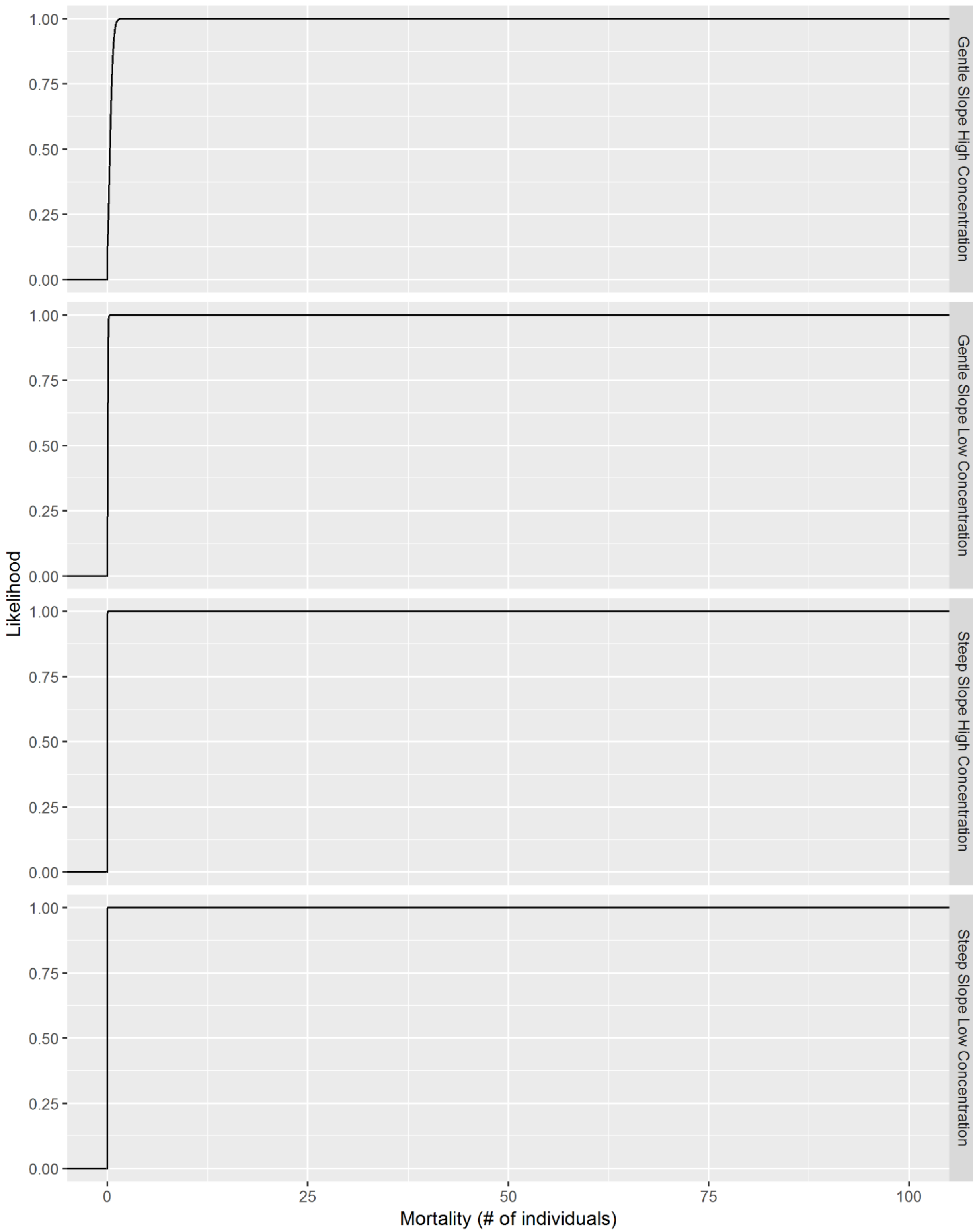

Figure B90. Cumulative likelihood results for Spotted Sucker mortality in the St. Clair River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

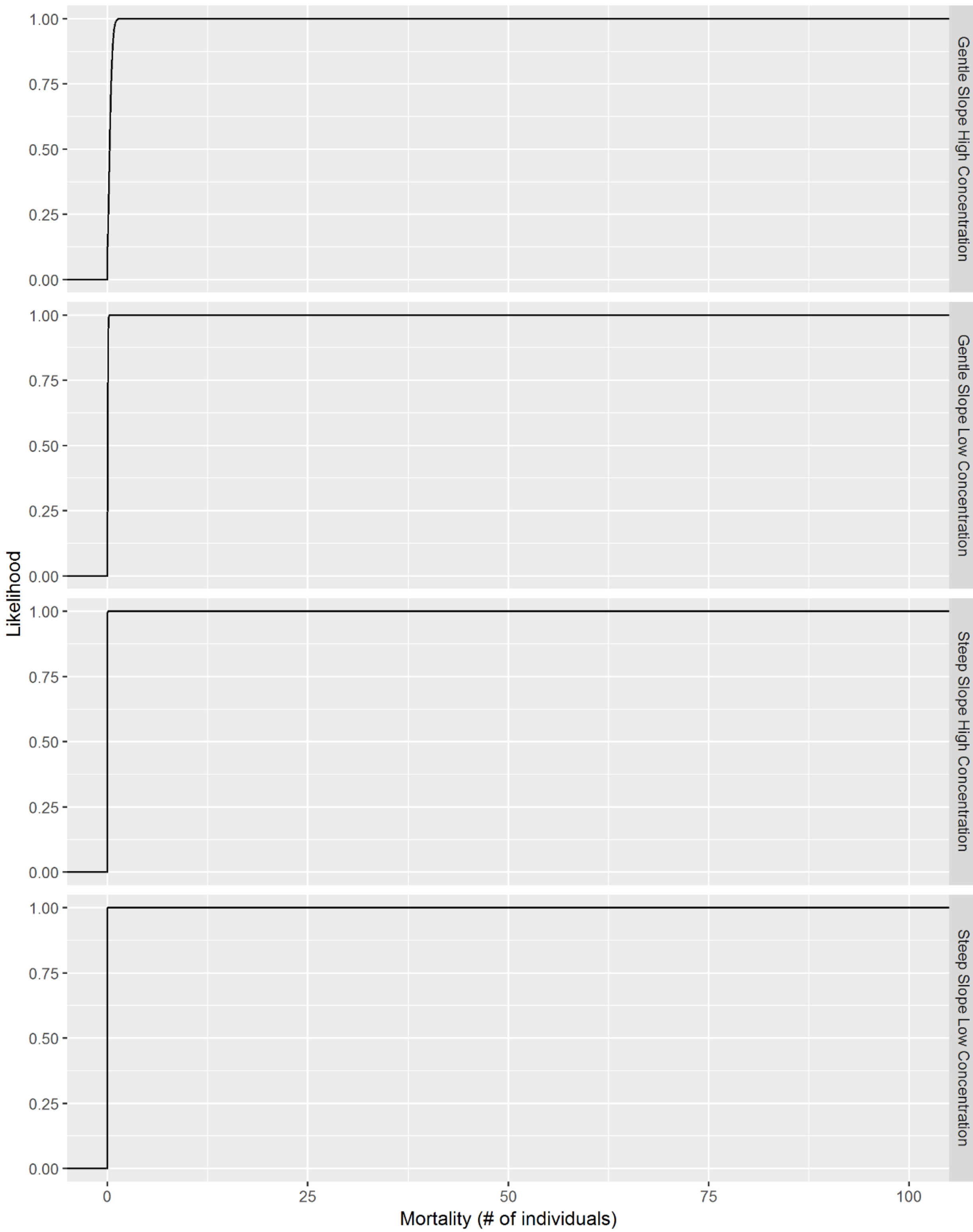

Figure B91. Cumulative likelihood results for Spotted Sucker mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

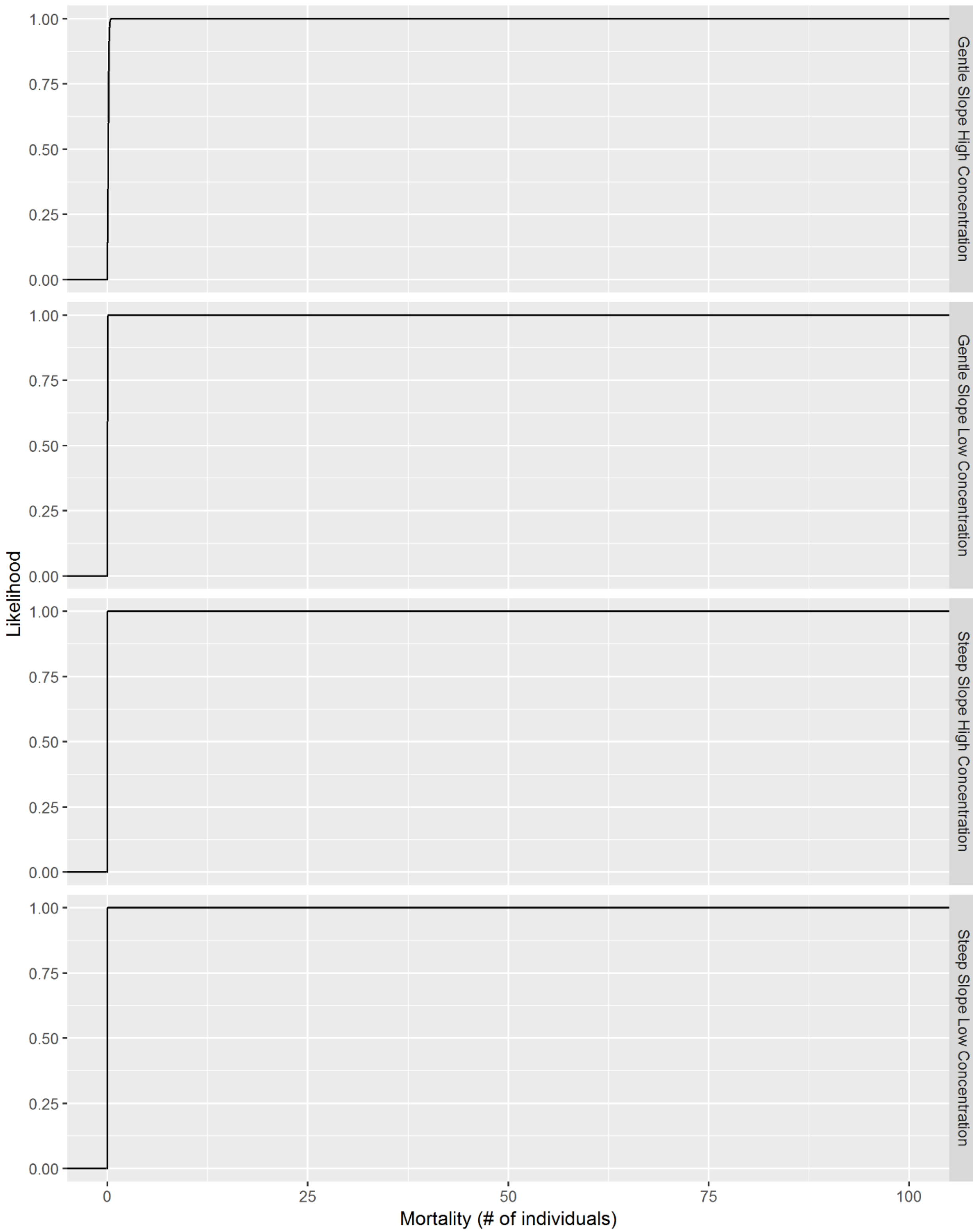

Figure B92. Cumulative likelihood results for Spotted Sucker mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

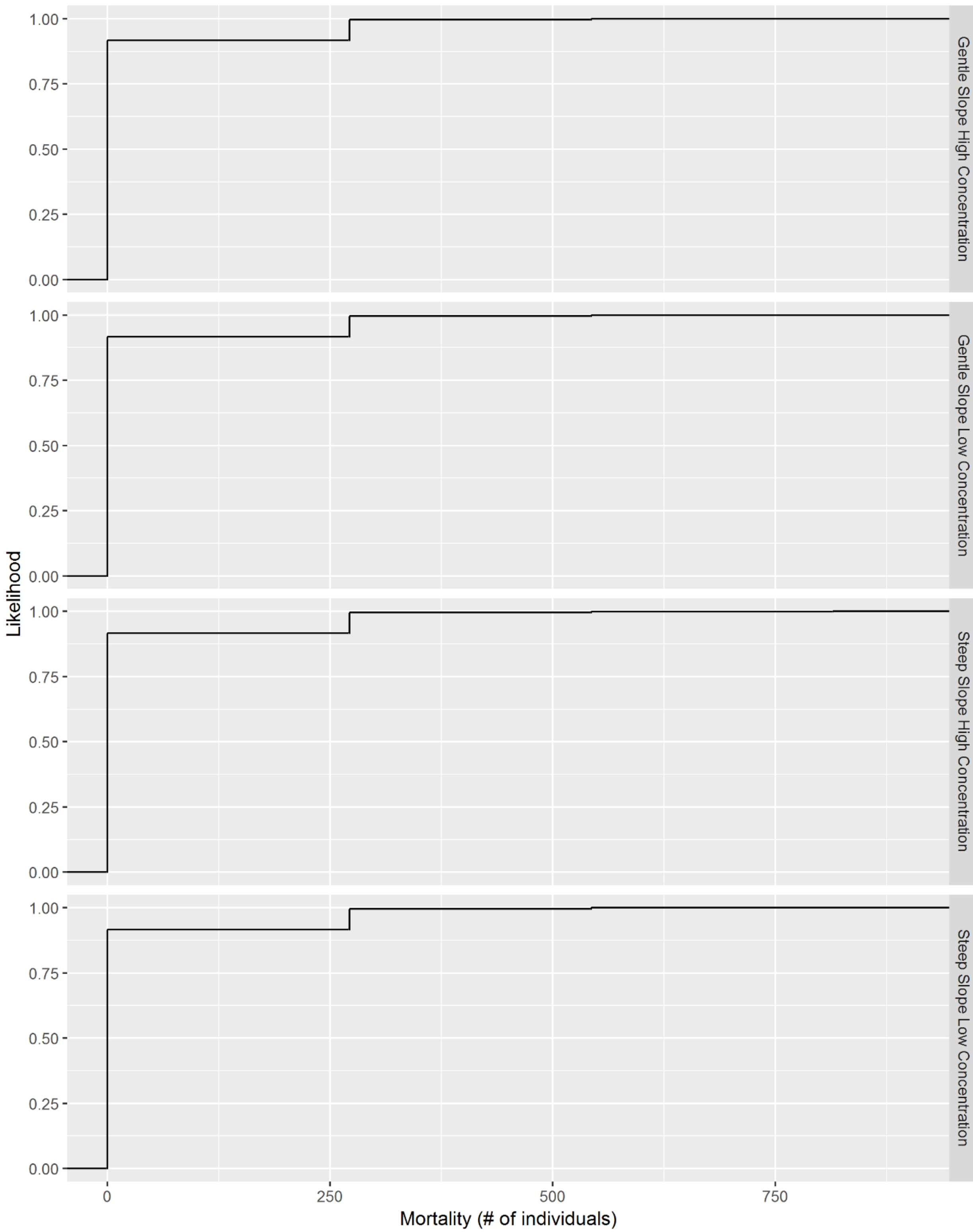

Figure B93. Cumulative likelihood results for Fawnsfoot mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

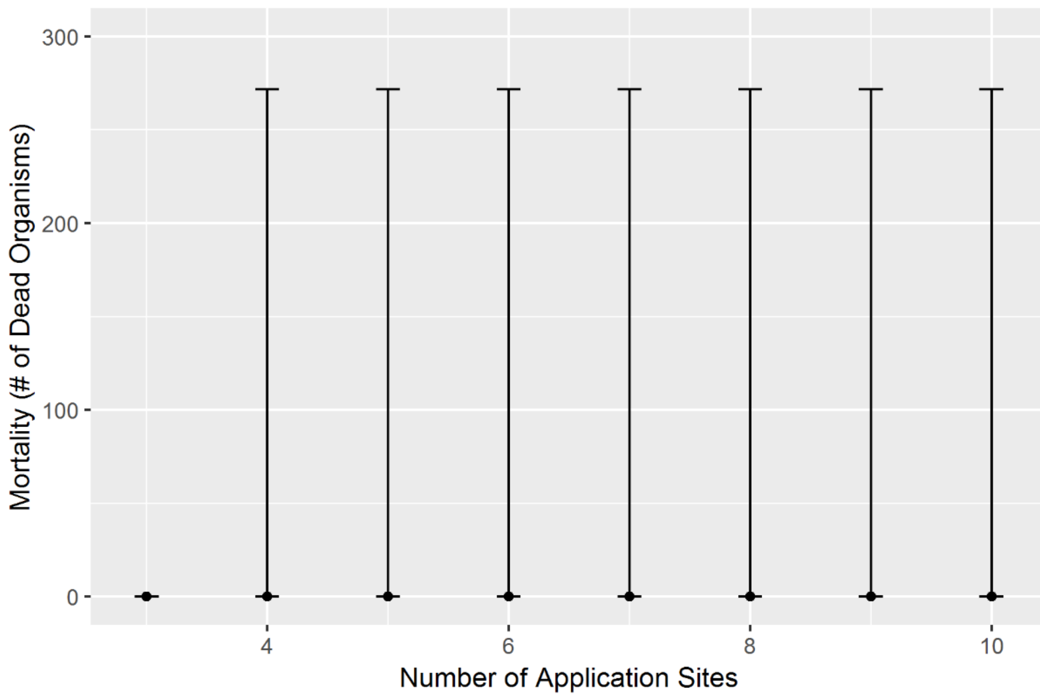

Figure B94. The influence of the number of 500 m² application sites on Fawnsfoot mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

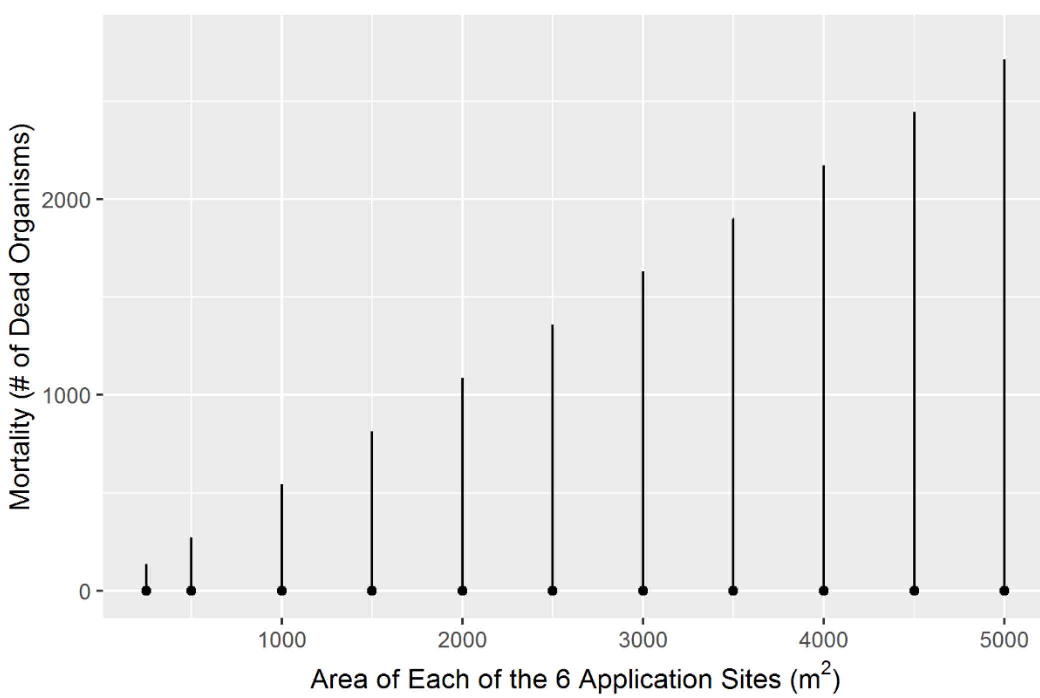

Figure B95. The influence of the size of six application sites on Fawnsfoot mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

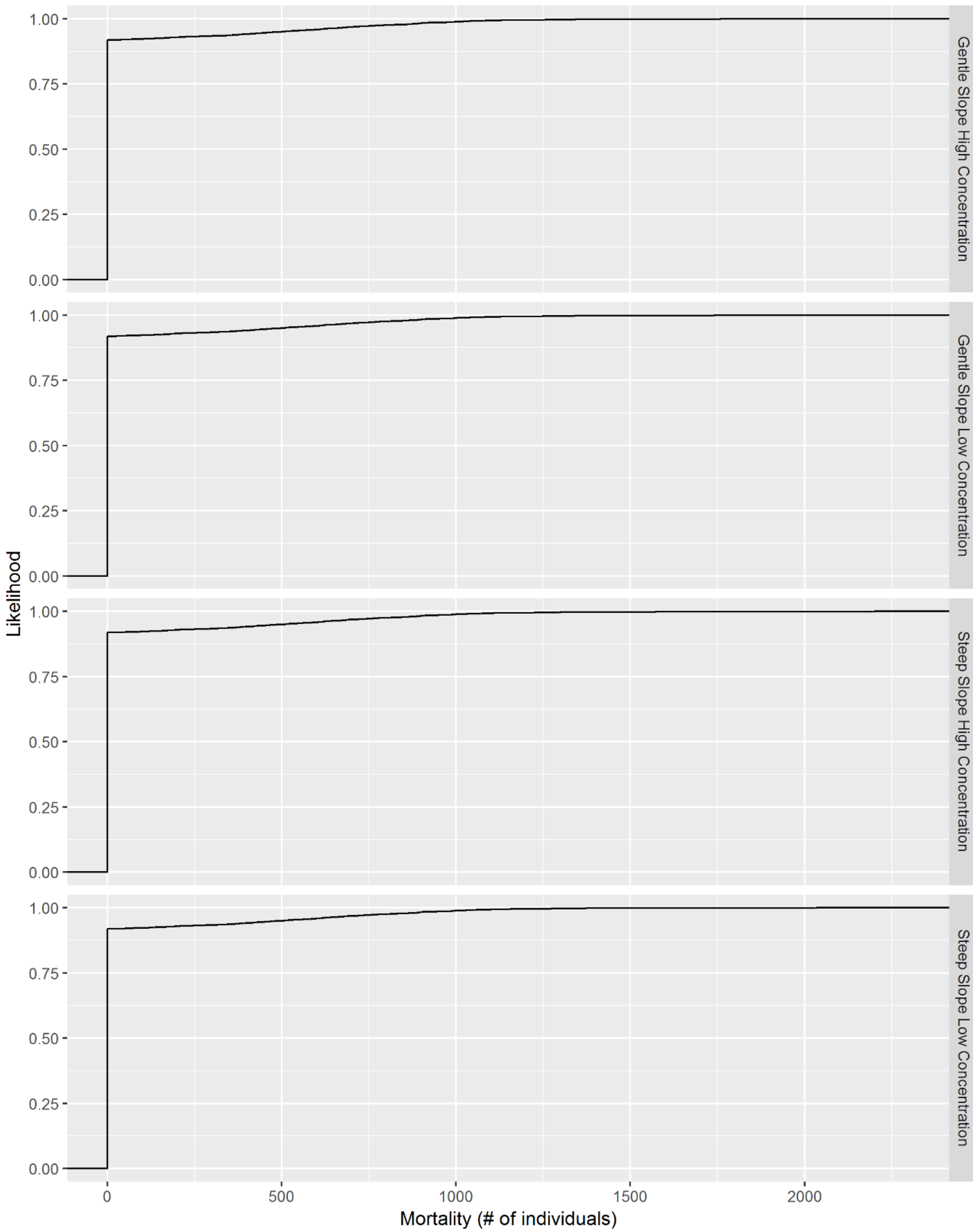

Figure B96. Cumulative likelihood results for Fawnsfoot mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

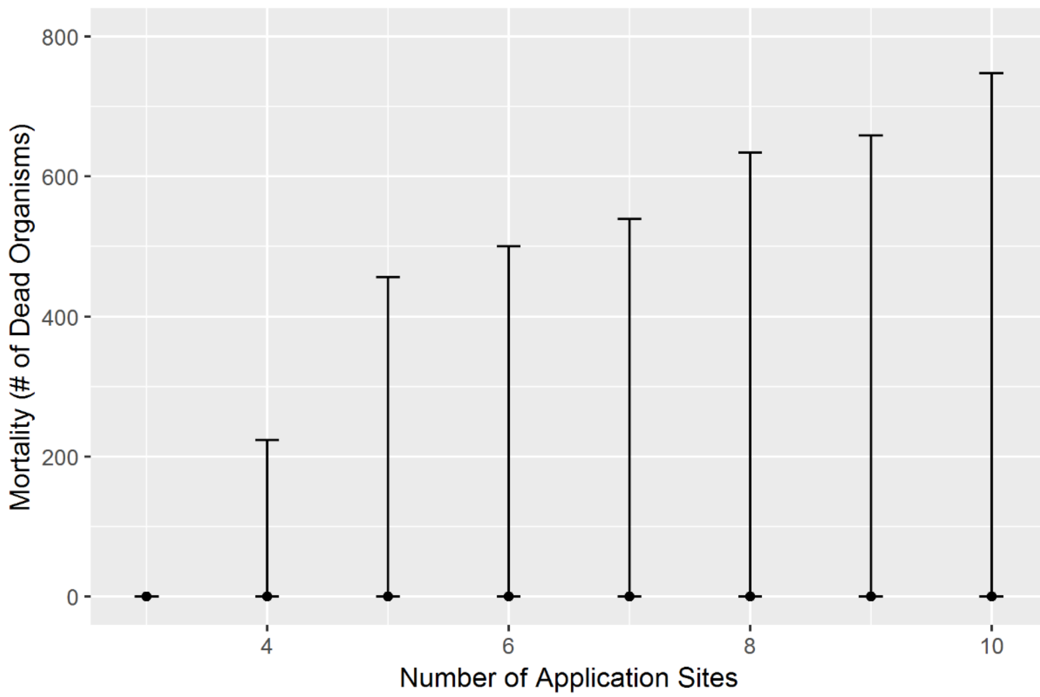

Figure B97. The influence of the number of 500 m^2 application sites on Fawnsfoot mortality in the Thames River. Error bars represents the upper and lower 95^{th} percentile.

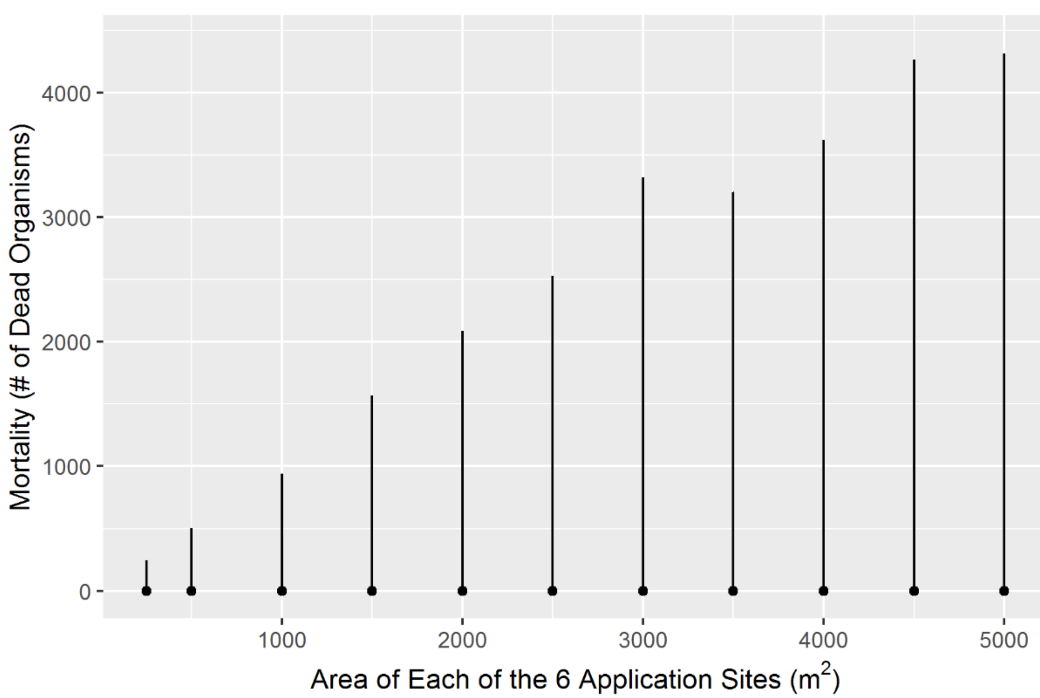

Figure B98. The influence of the size of six application sites on Fawnsfoot mortality in the Thames River. Error bars represents the upper and lower 95^{th} percentile.

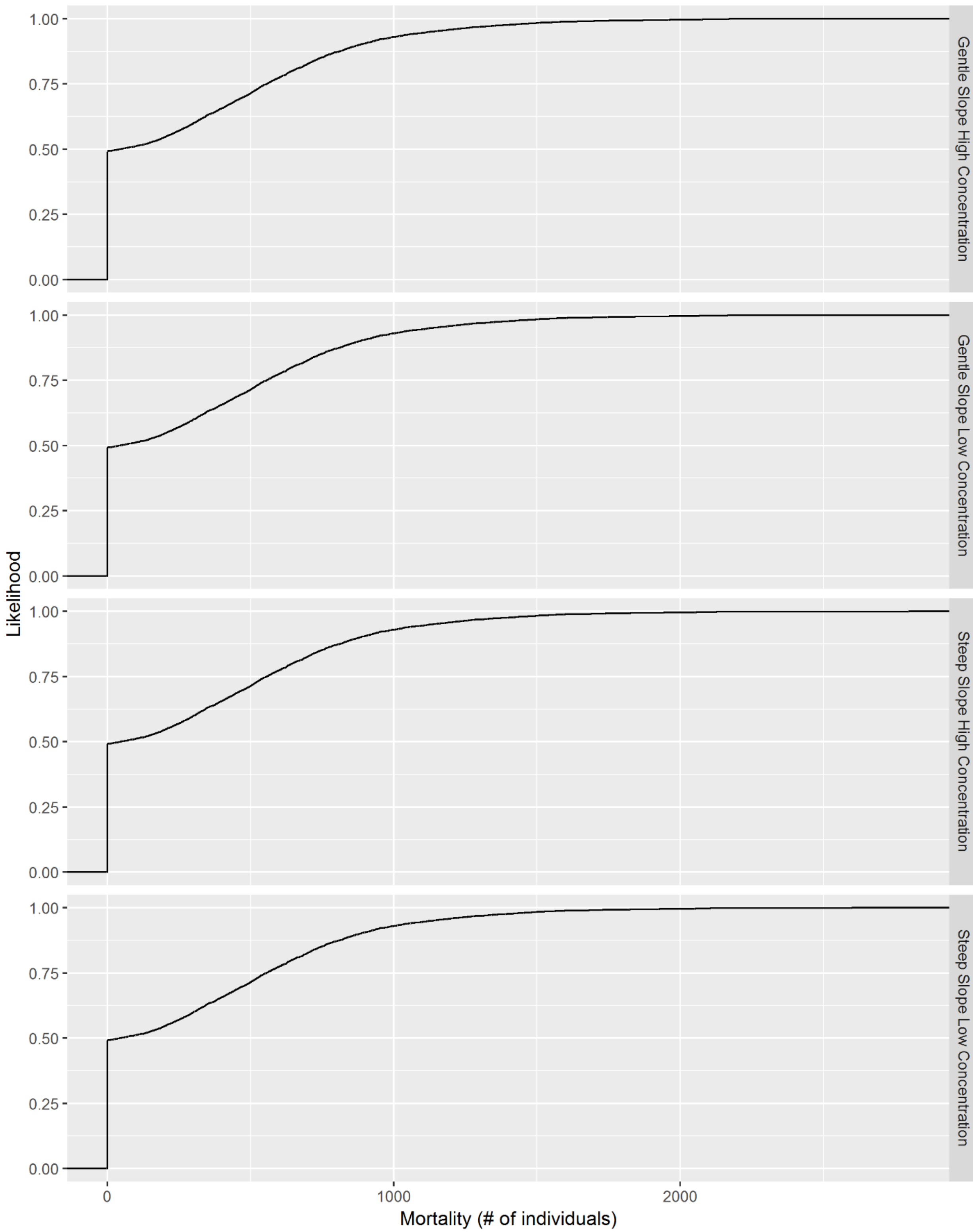

Figure B99. Cumulative likelihood results for Kidneyshell mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

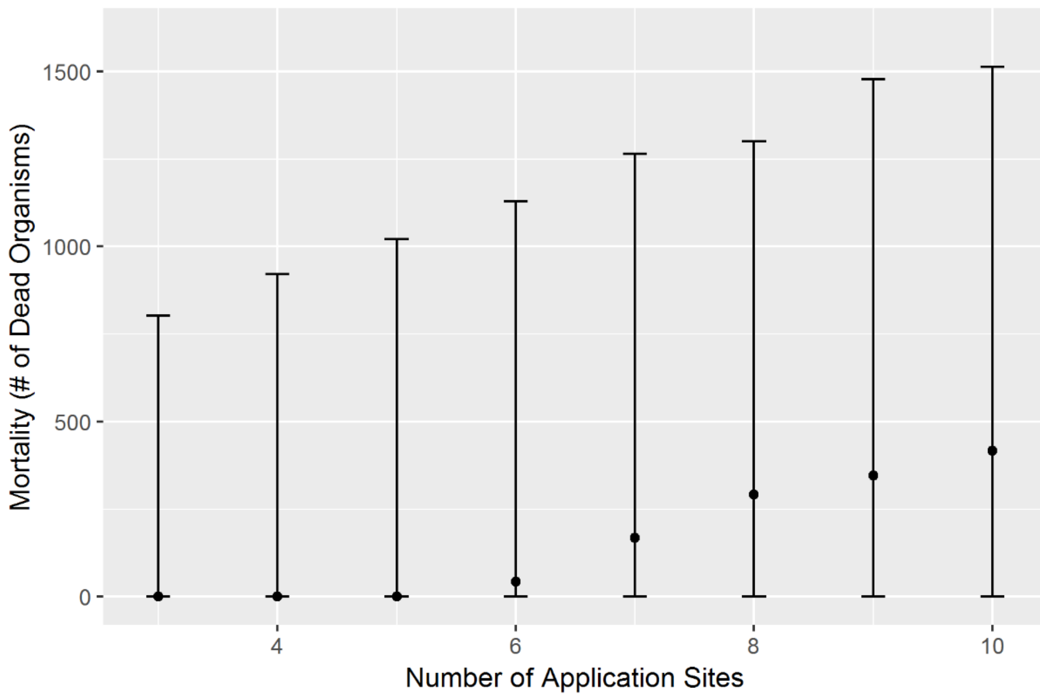

Figure B100. The influence of the number of 500 m² application sites on Kidneyshell mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

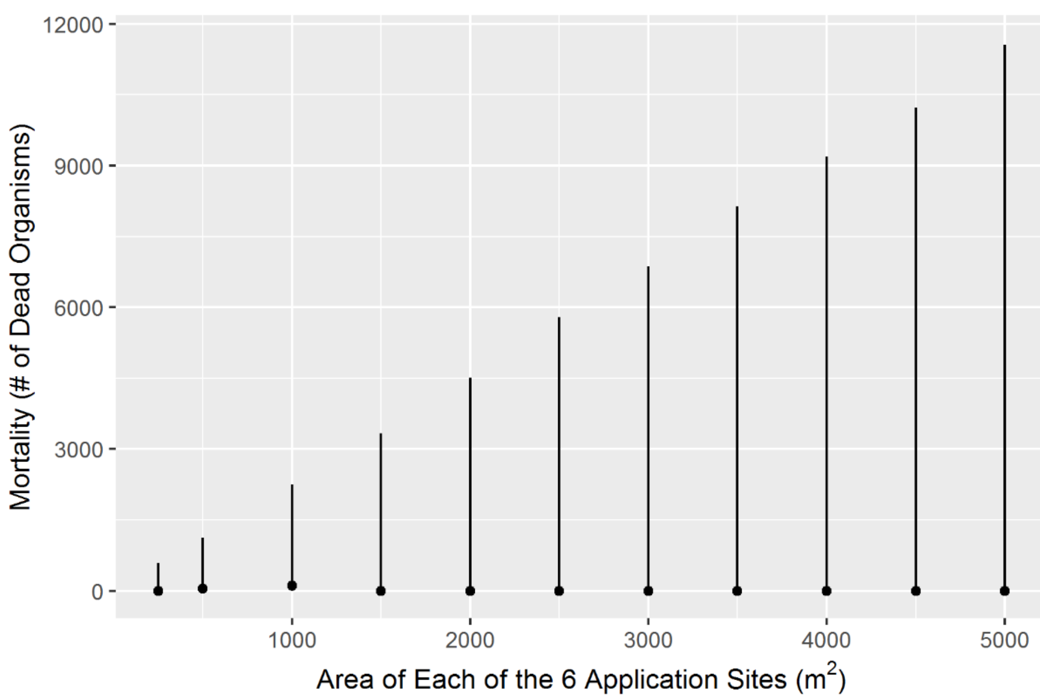

Figure B101. The influence of the size of six application sites on Kidneyshell mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

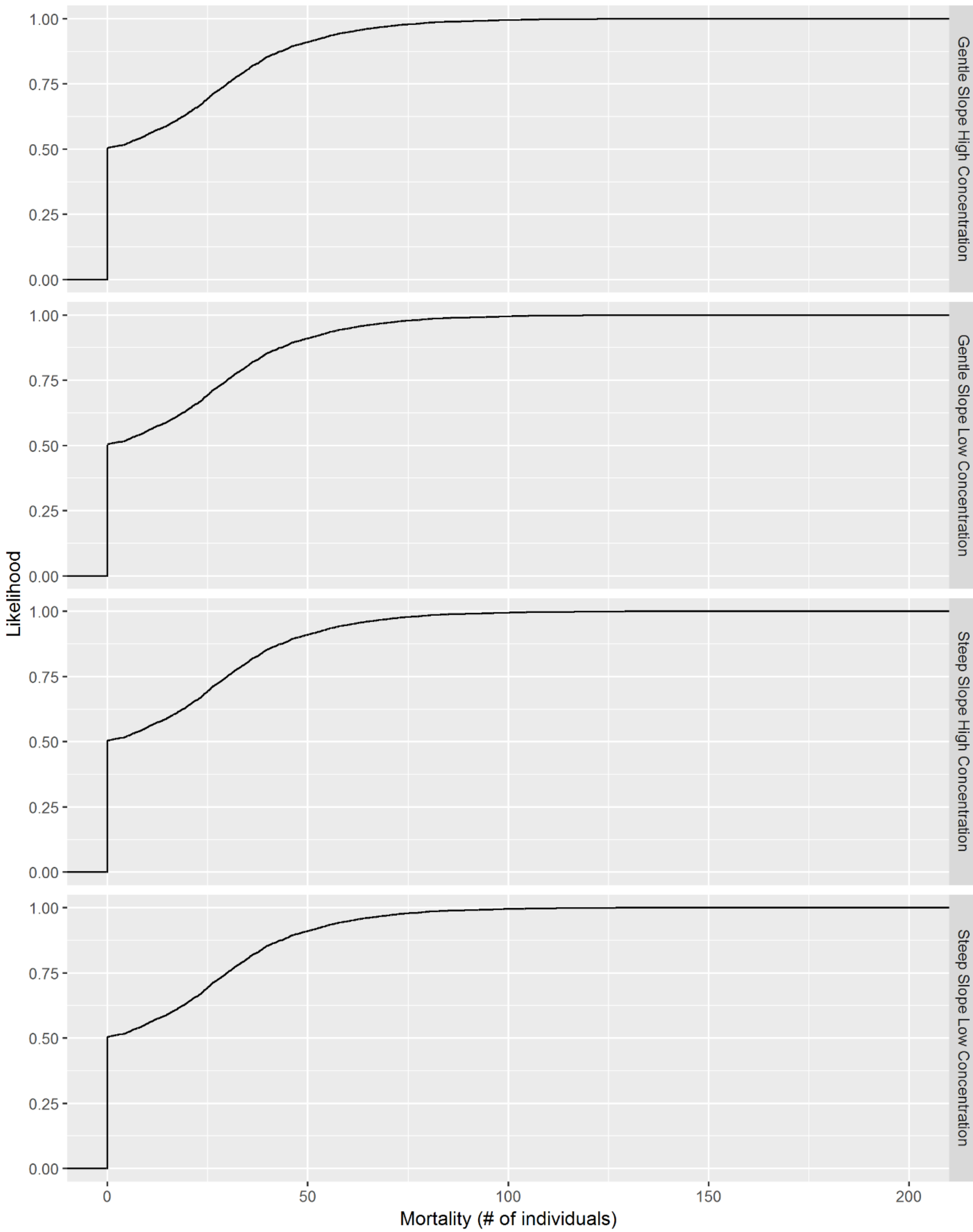

Figure B102. Cumulative likelihood results for Mapleleaf mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

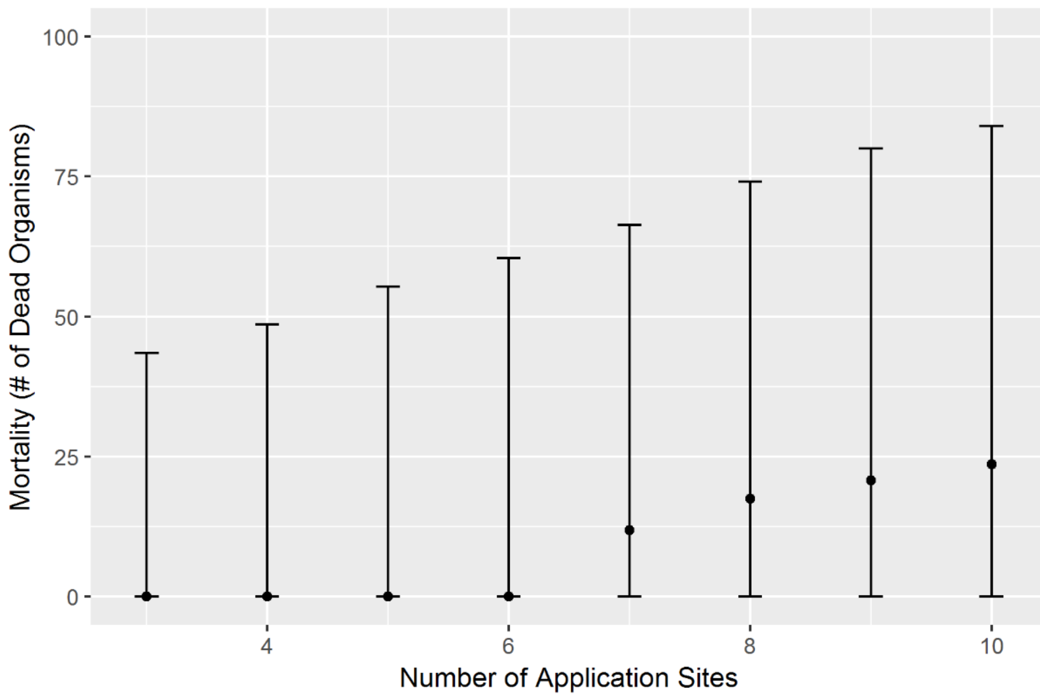

Figure B103. The influence of the number of 500 m^2 application sites on Mapleleaf mortality in the Sydenham River. Error bars represents the upper and lower 95^{th} percentile.

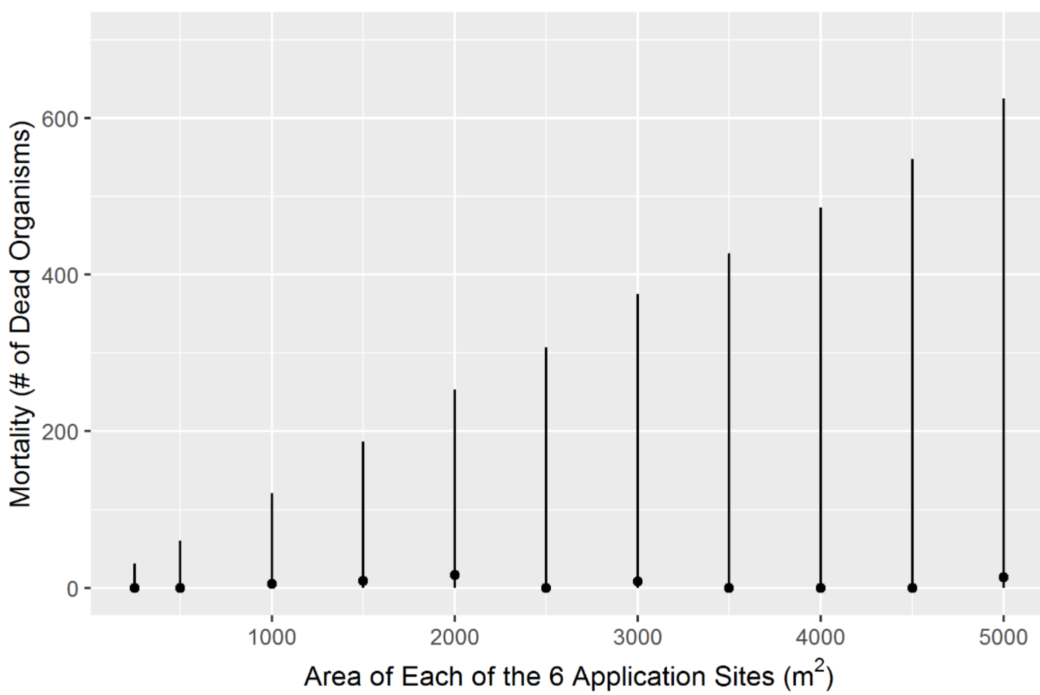

Figure B104. The influence of the size of six application sites on Mapleleaf mortality in the Sydenham River. Error bars represents the upper and lower 95^{th} percentile.

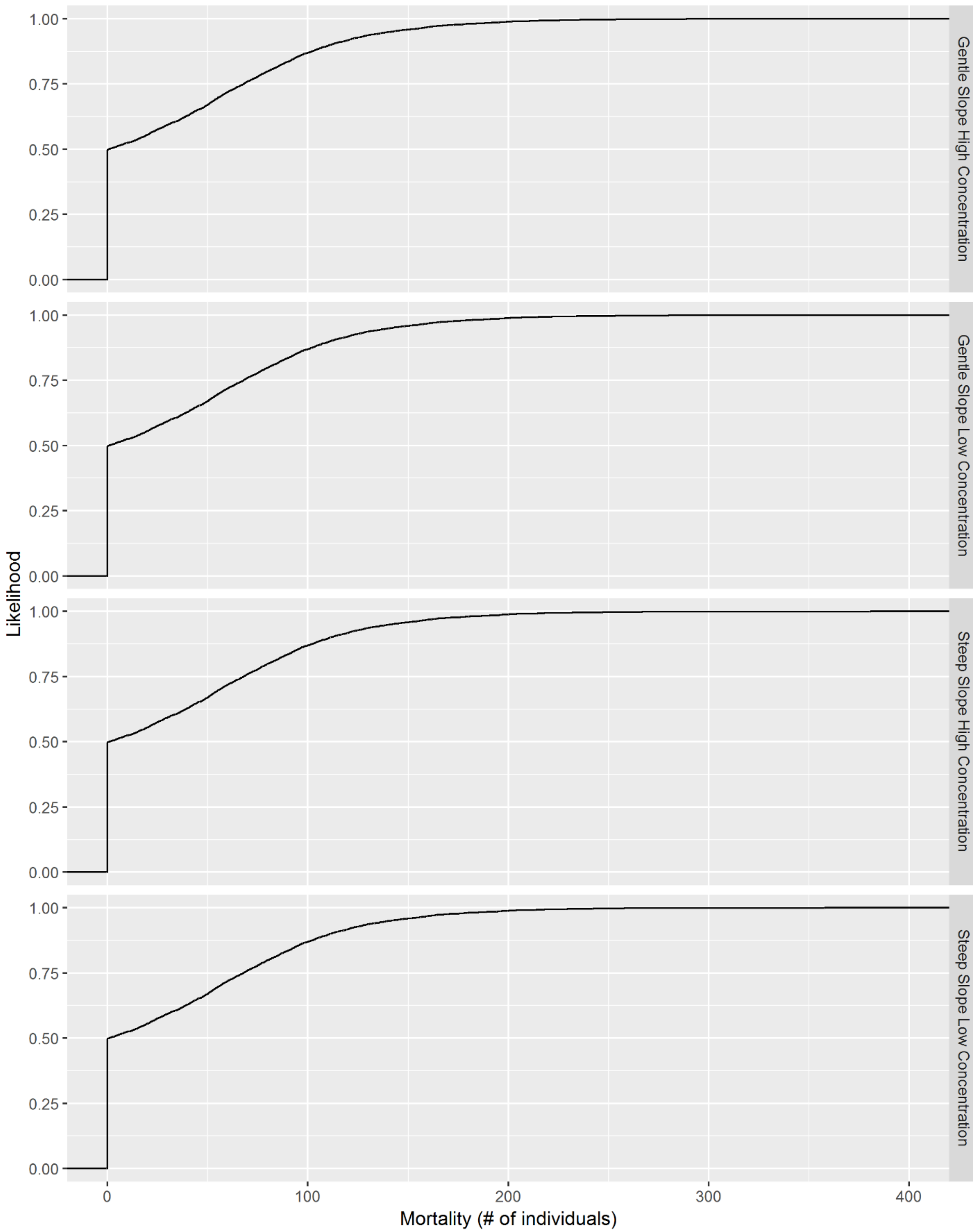

Figure B105. Cumulative likelihood results for Mapleleaf mortality in the Thames River following Bayluscide application at six 500 m2 sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

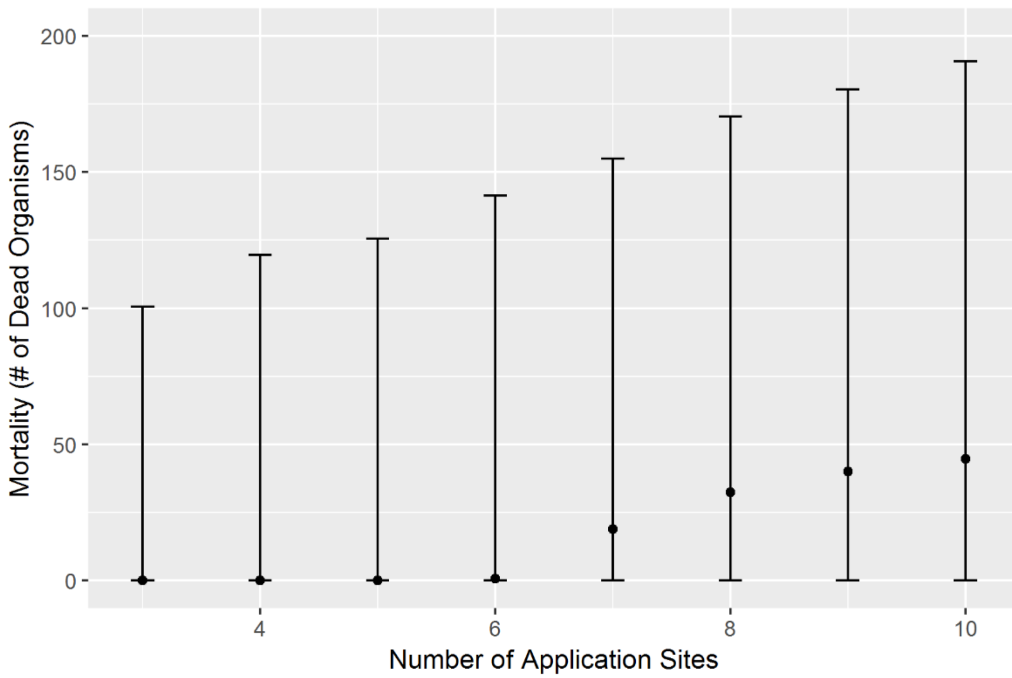

Figure B106. The influence of the number of 500 m^2 application sites on Mapleleaf mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

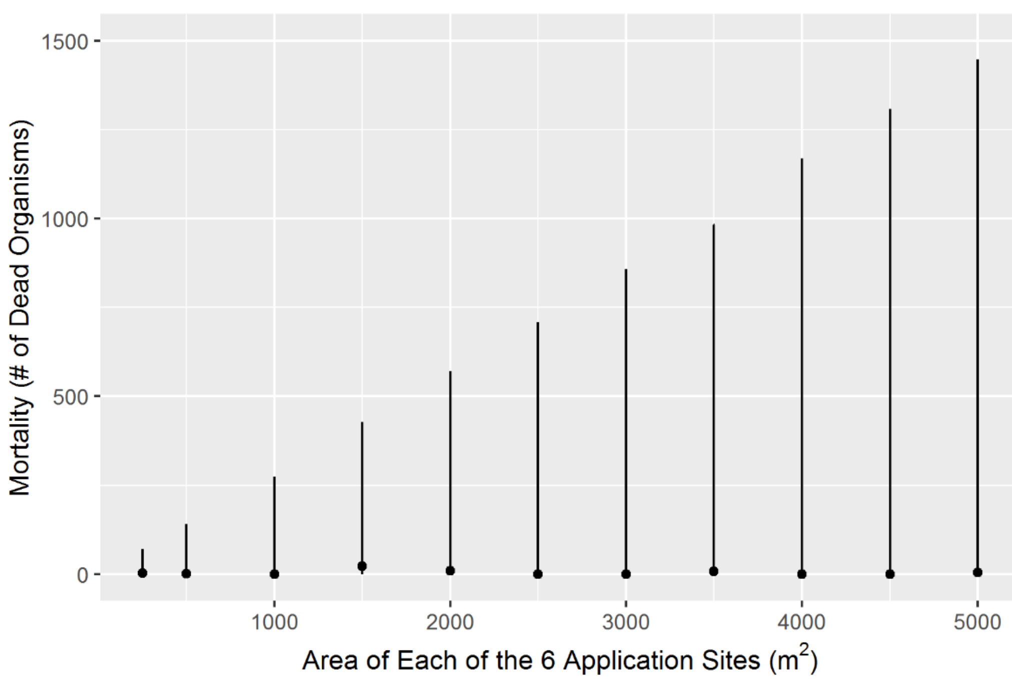

Figure B107. The influence of the size of six application sites on Mapleleaf mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

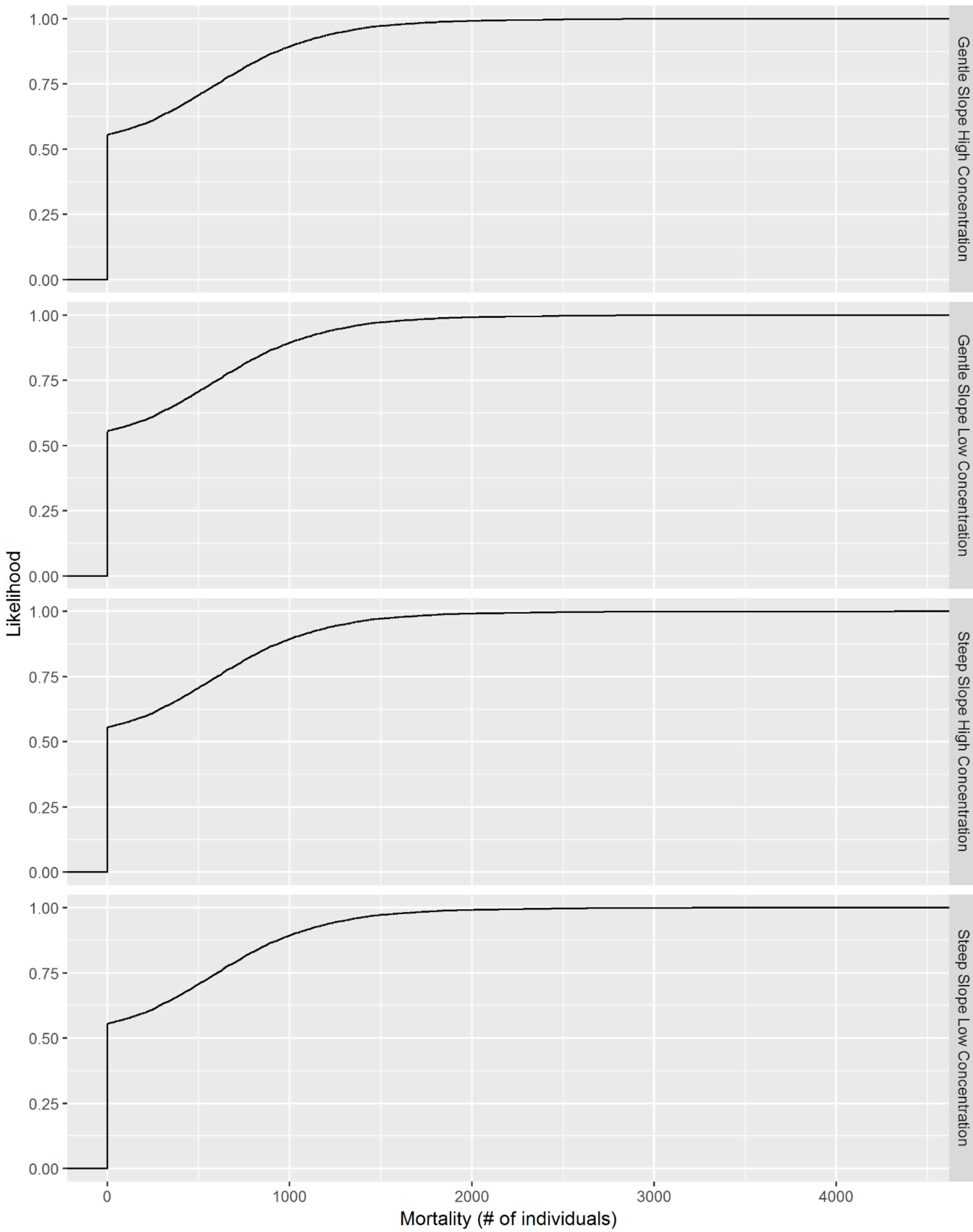

Figure B108. Cumulative likelihood results for Northern Riffleshell mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

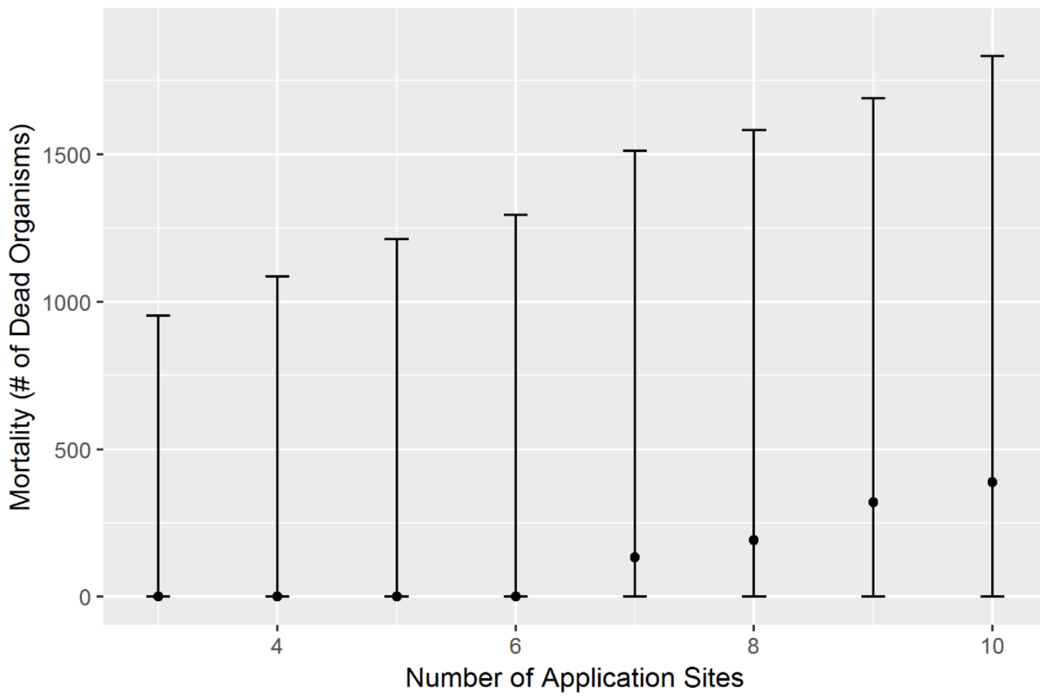

Figure B109. The influence of the number of 500 m^2 application sites on Northern Riffleshell mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

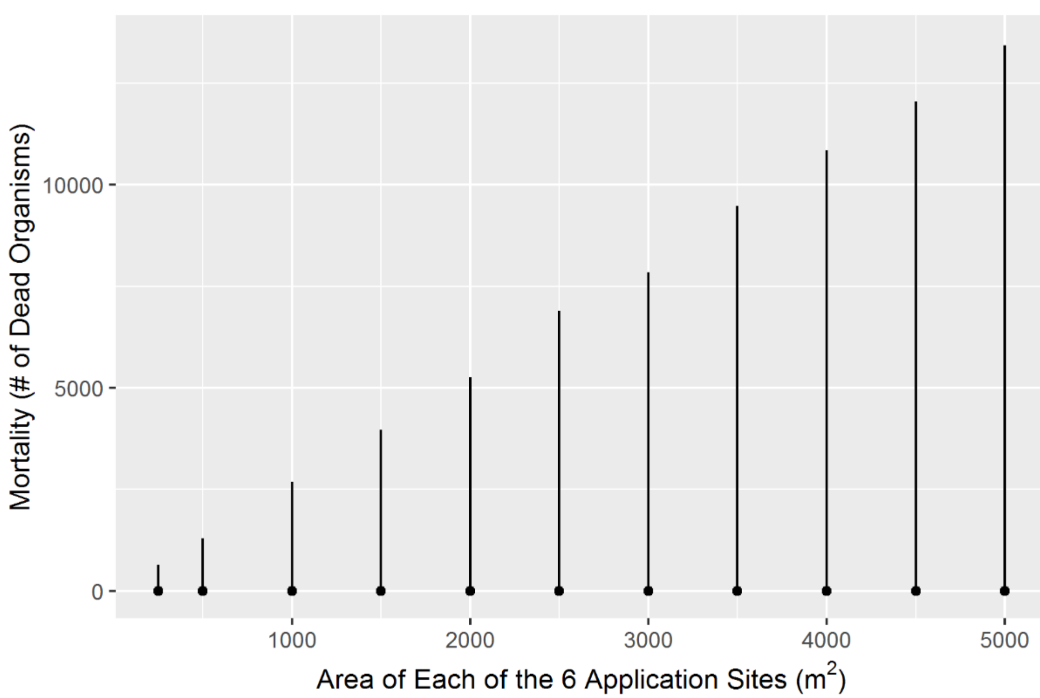

Figure B110. The influence of the size of six application sites on Northern Riffleshell mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

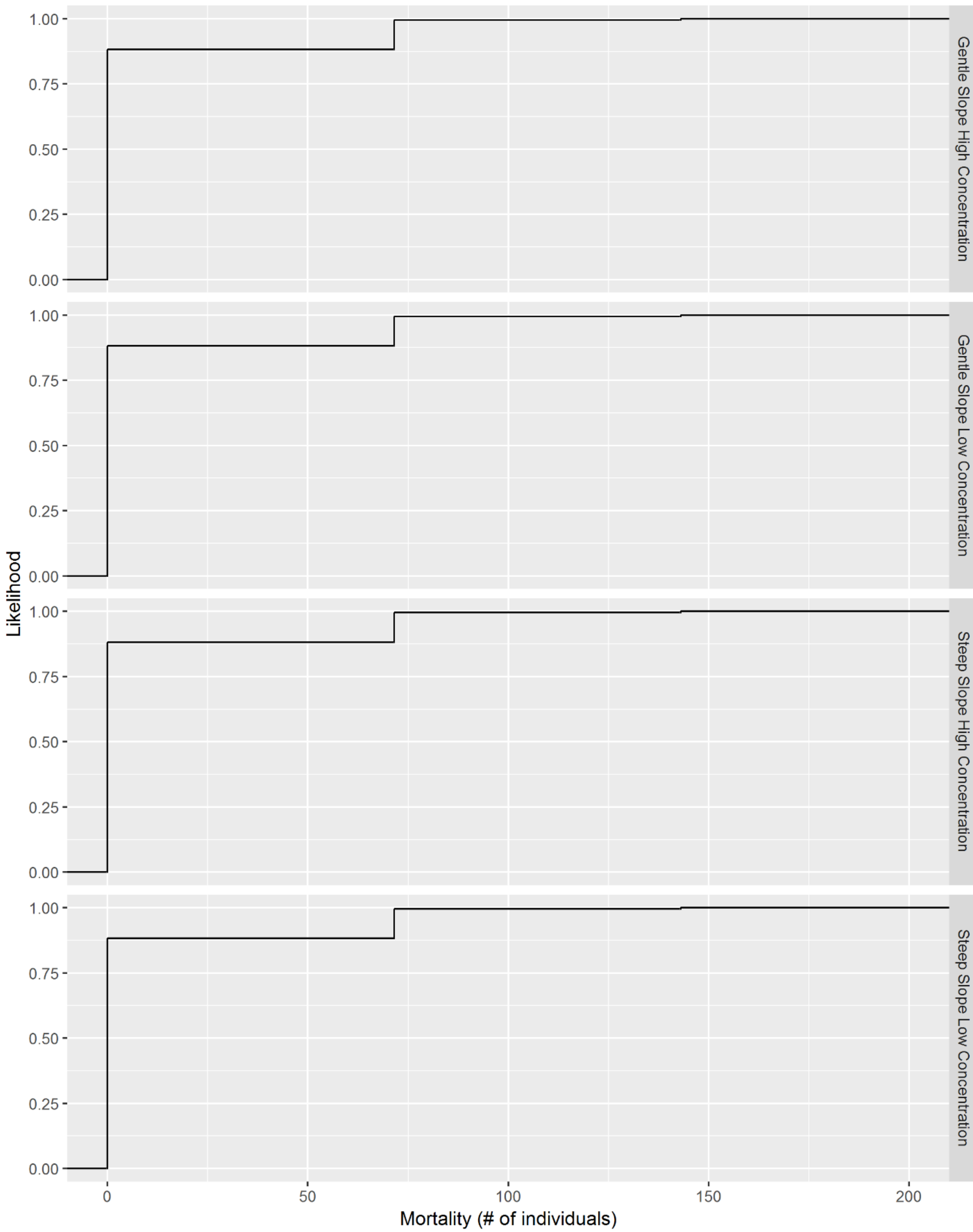

Figure B111. Cumulative likelihood results for Rainbow mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

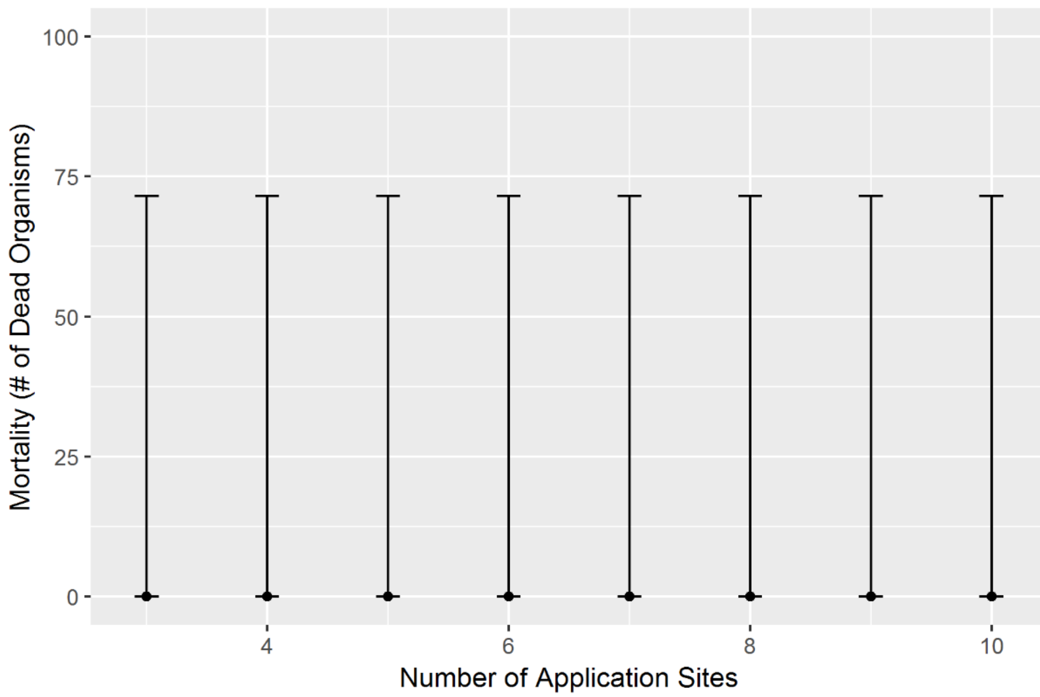

Figure B112. The influence of the number of 500 m^2 application sites on Rainbow mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

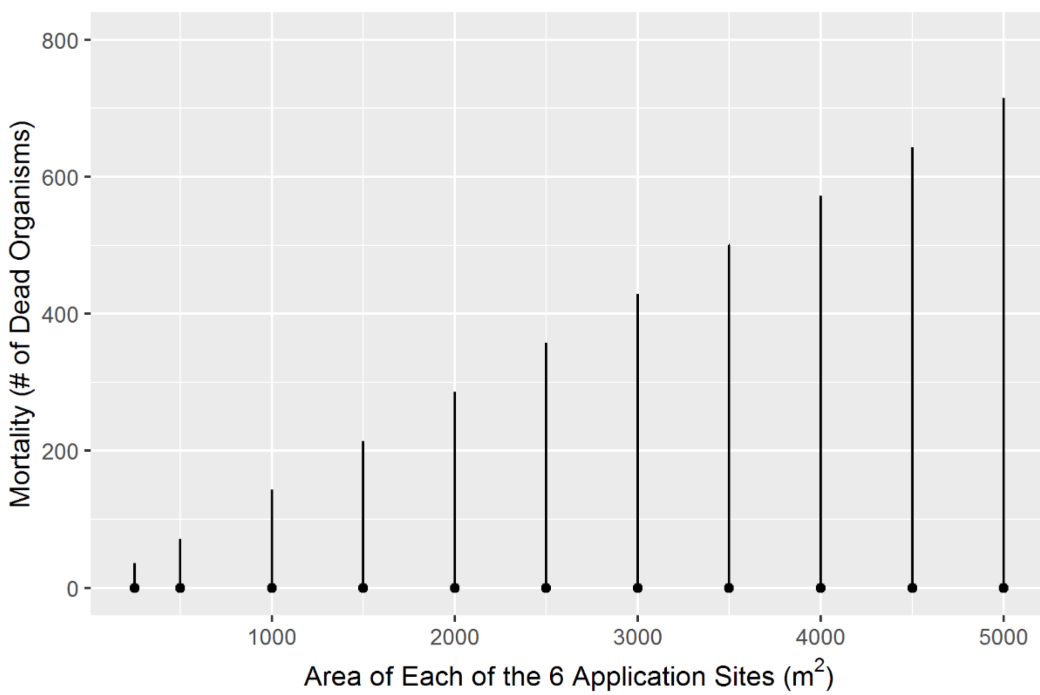

Figure B113. The influence of the size of six application sites on Rainbow mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

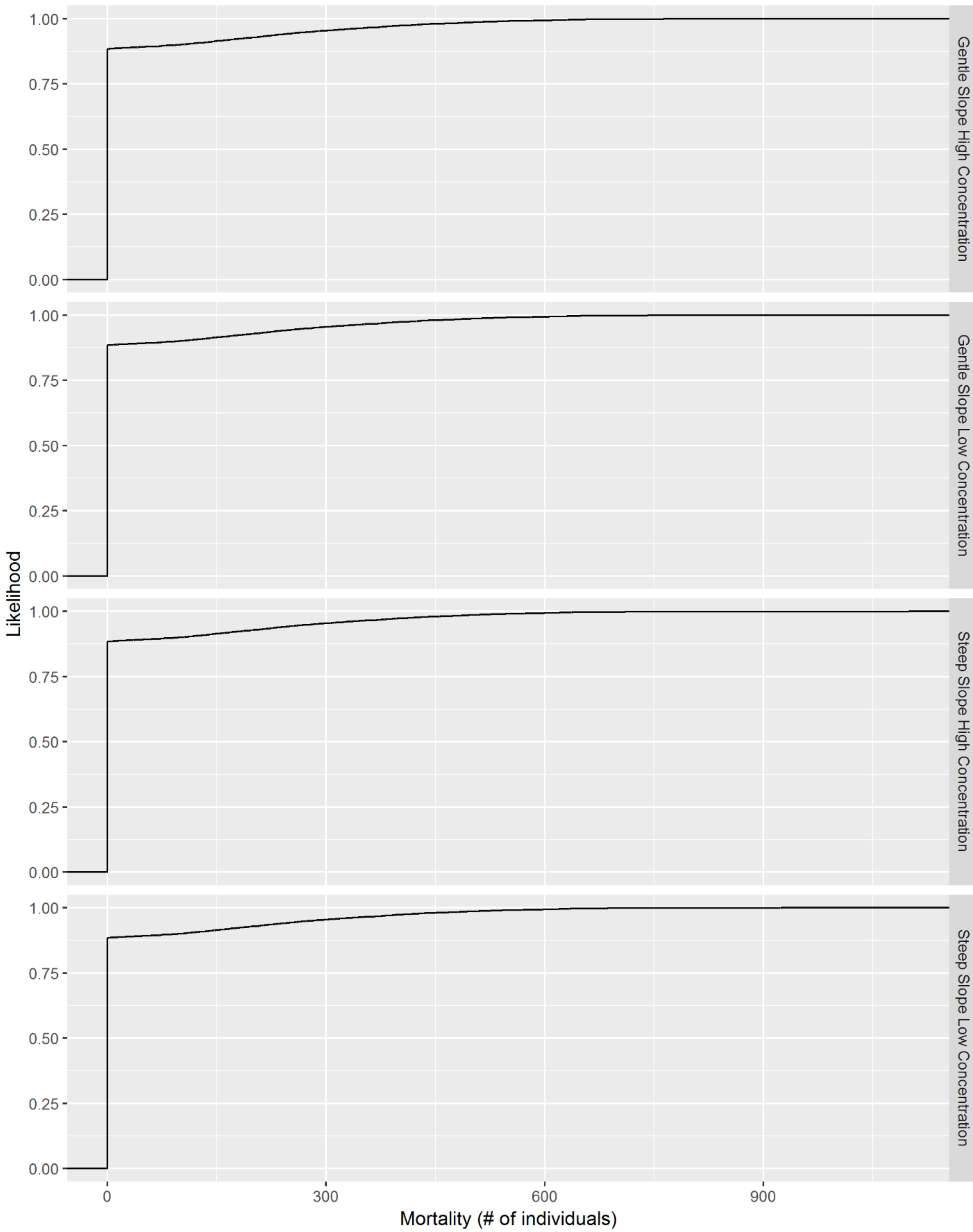

Figure B114. Cumulative likelihood results for Rainbow mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

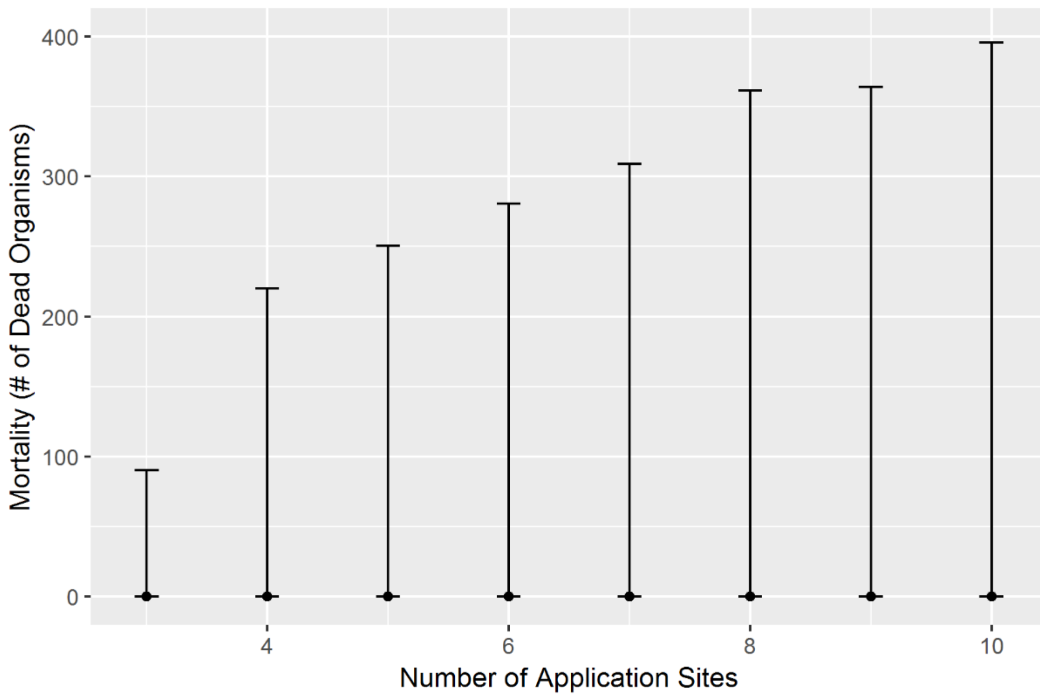

Figure B115. The influence of the number of 500 m² application sites on Rainbow mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

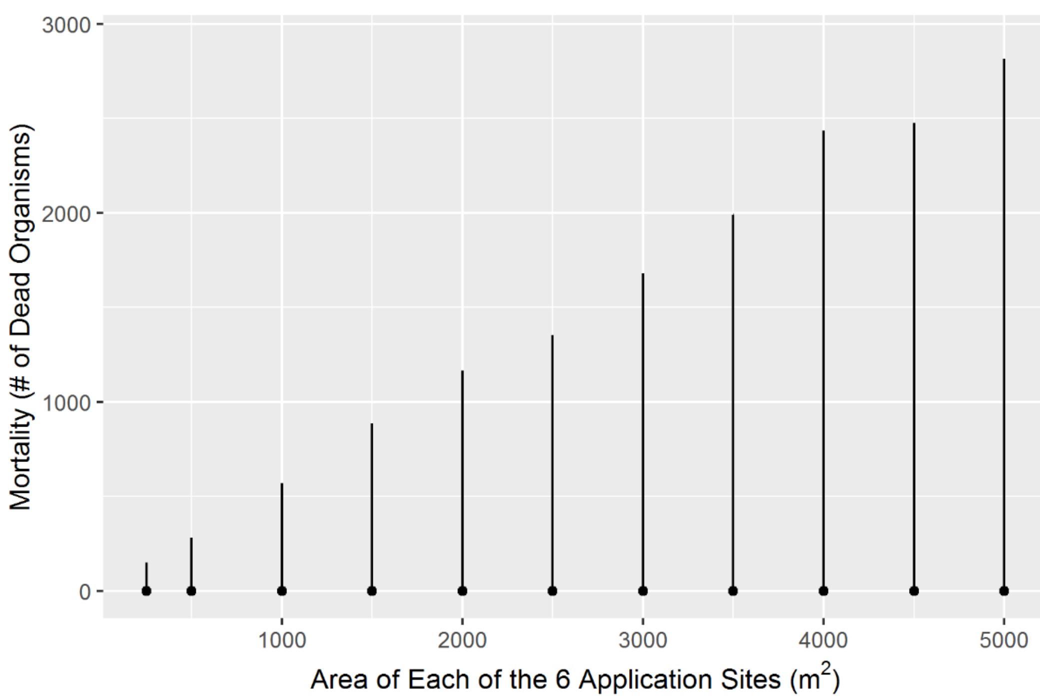

Figure B116. The influence of the size of six application sites on Rainbow mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

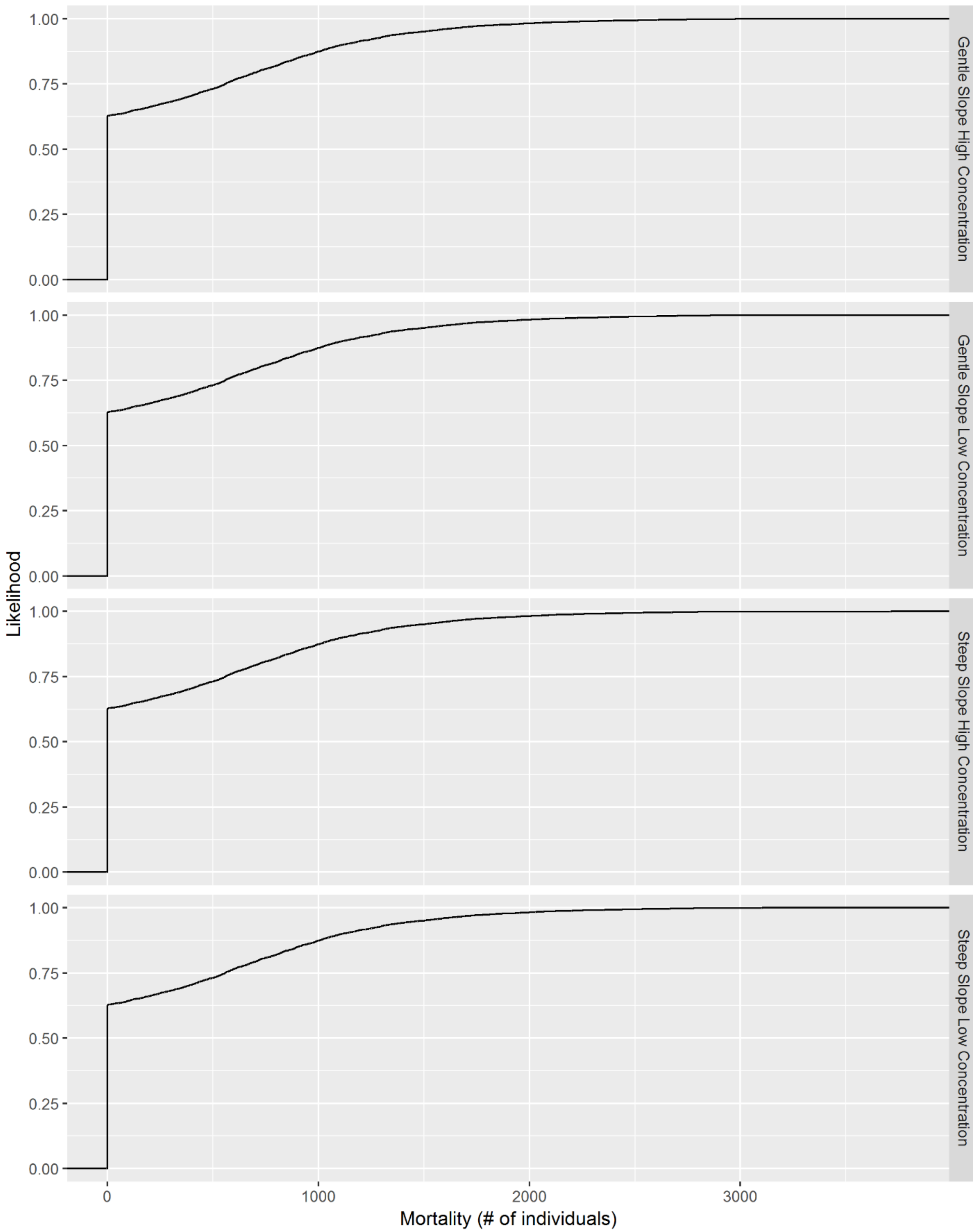

Figure B117. Cumulative likelihood results for Rayed Bean mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

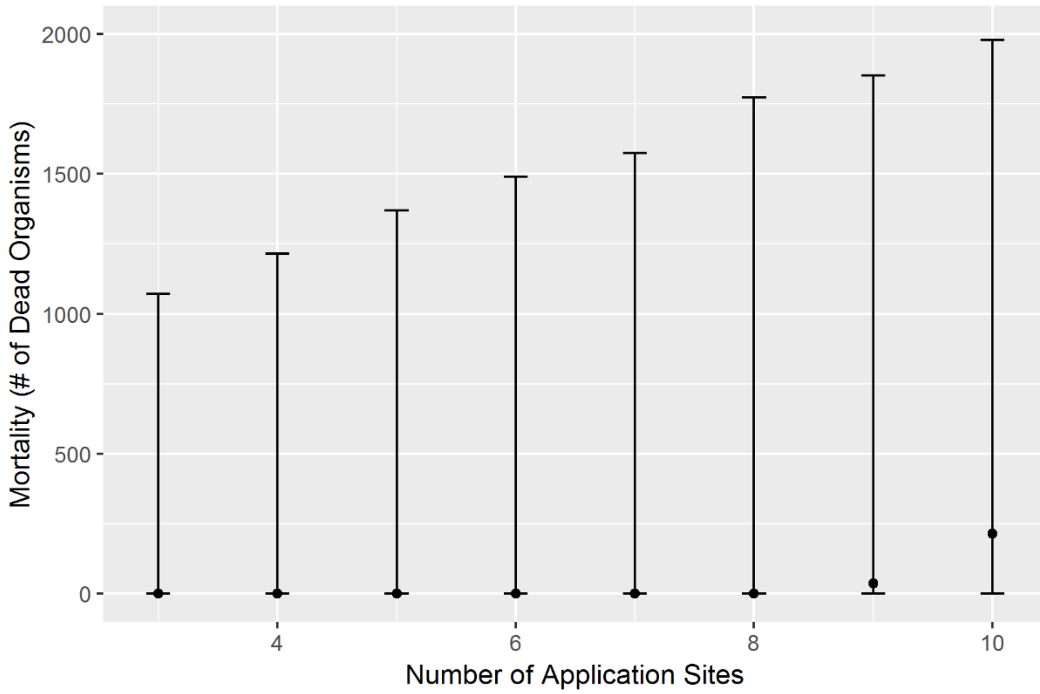

Figure B118. The influence of the number of 500 m² application sites on Rayed Bean mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

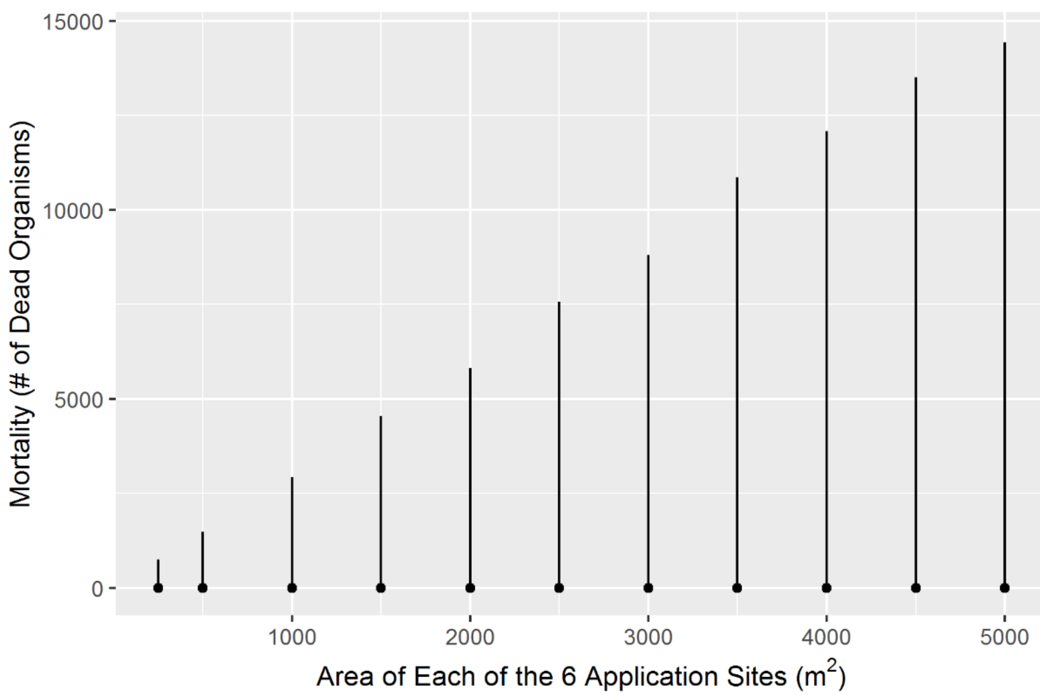

Figure B119. The influence of the size of six application sites on Rayed Bean mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

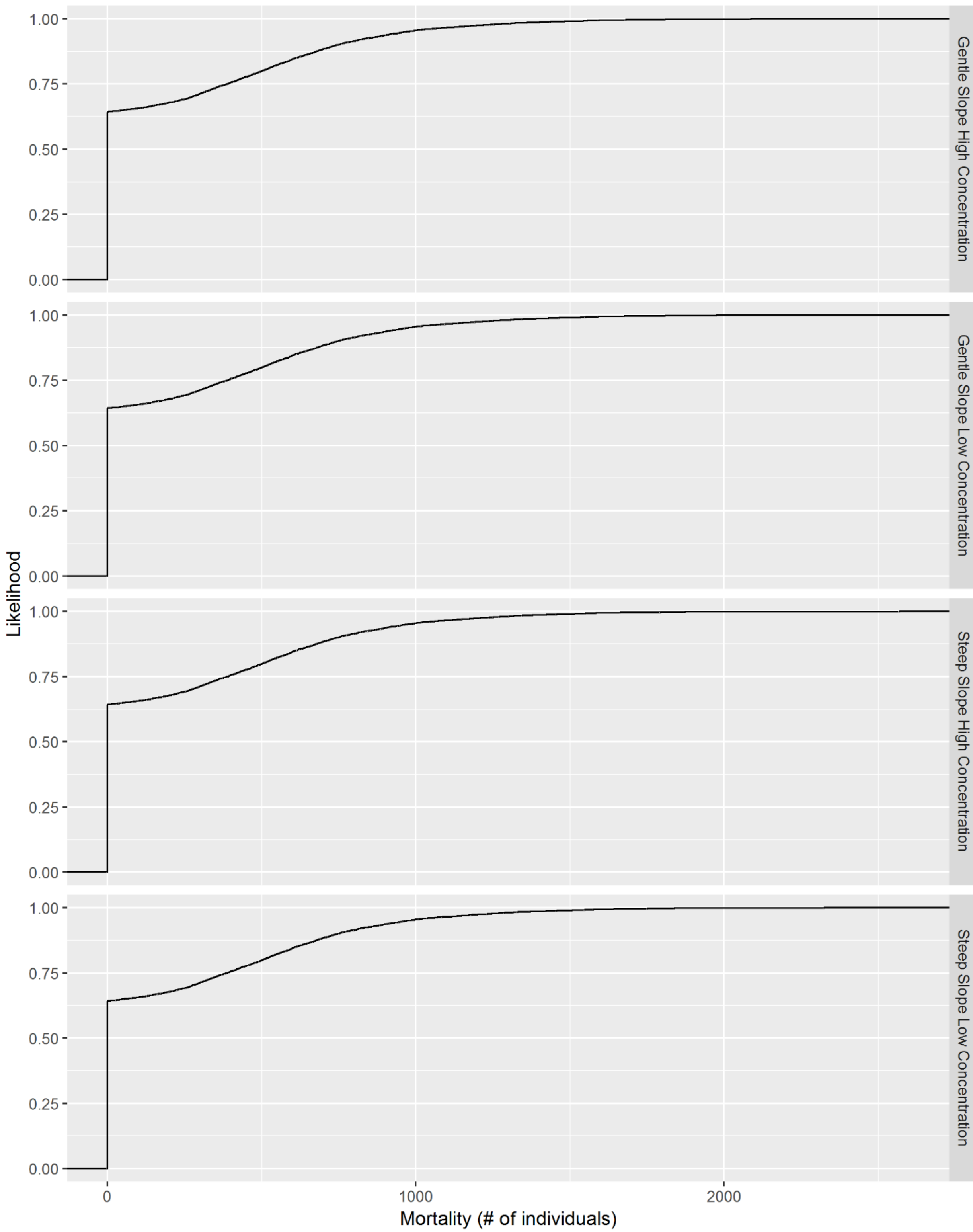

Figure B120. Cumulative likelihood results for Rayed Bean mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

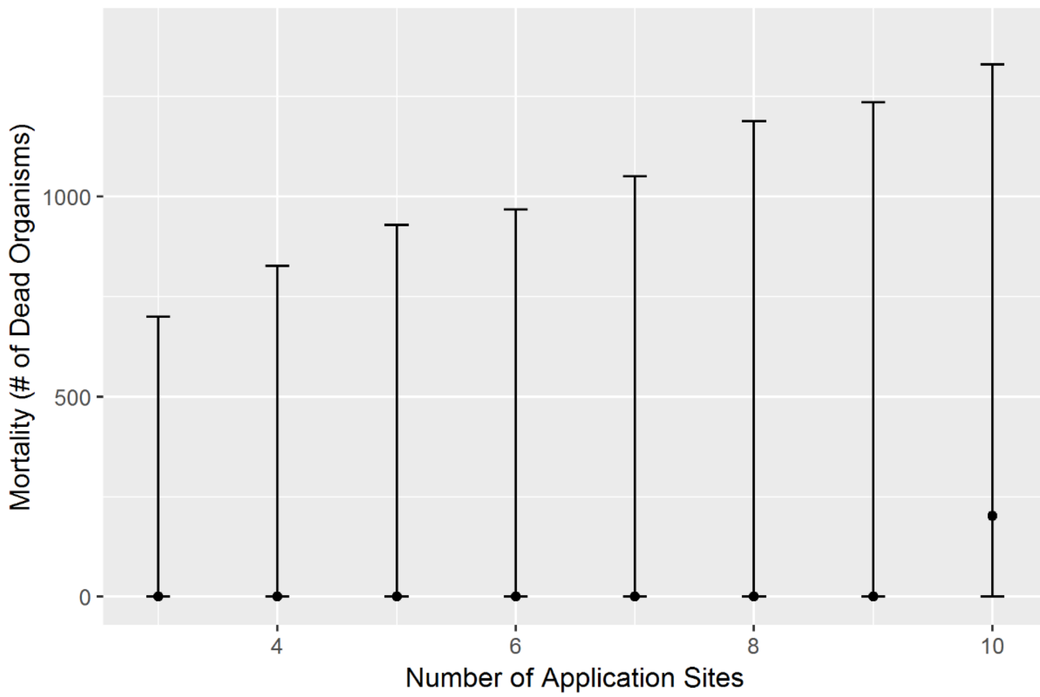

Figure B121. The influence of the number of 500 m^2 application sites on Rayed Bean mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

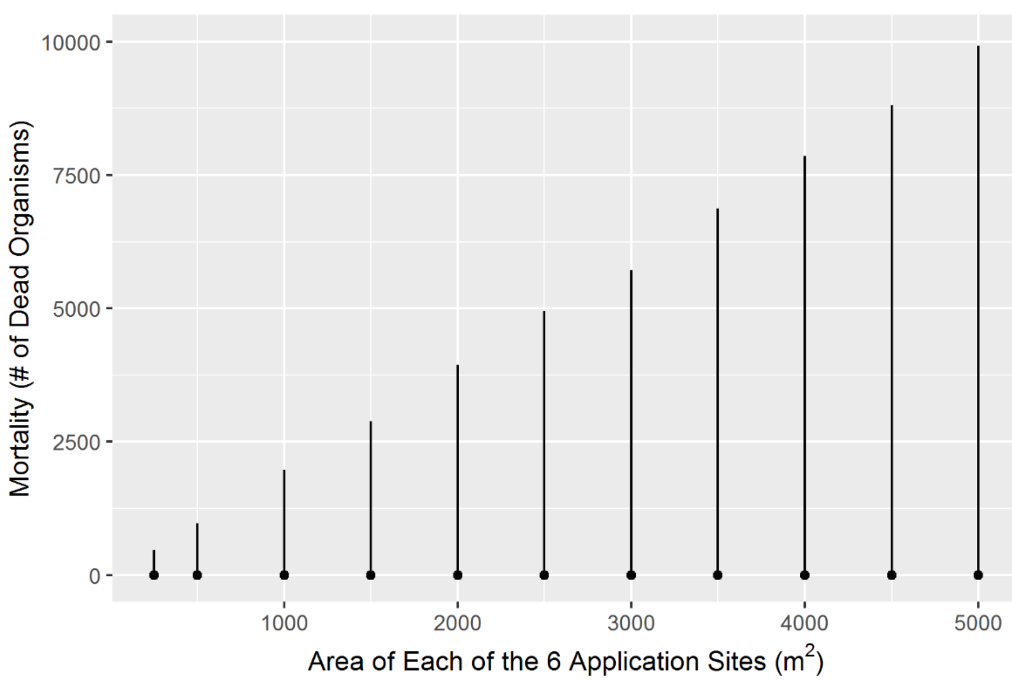

Figure B122. The influence of the size of six application sites on Rayed Bean mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

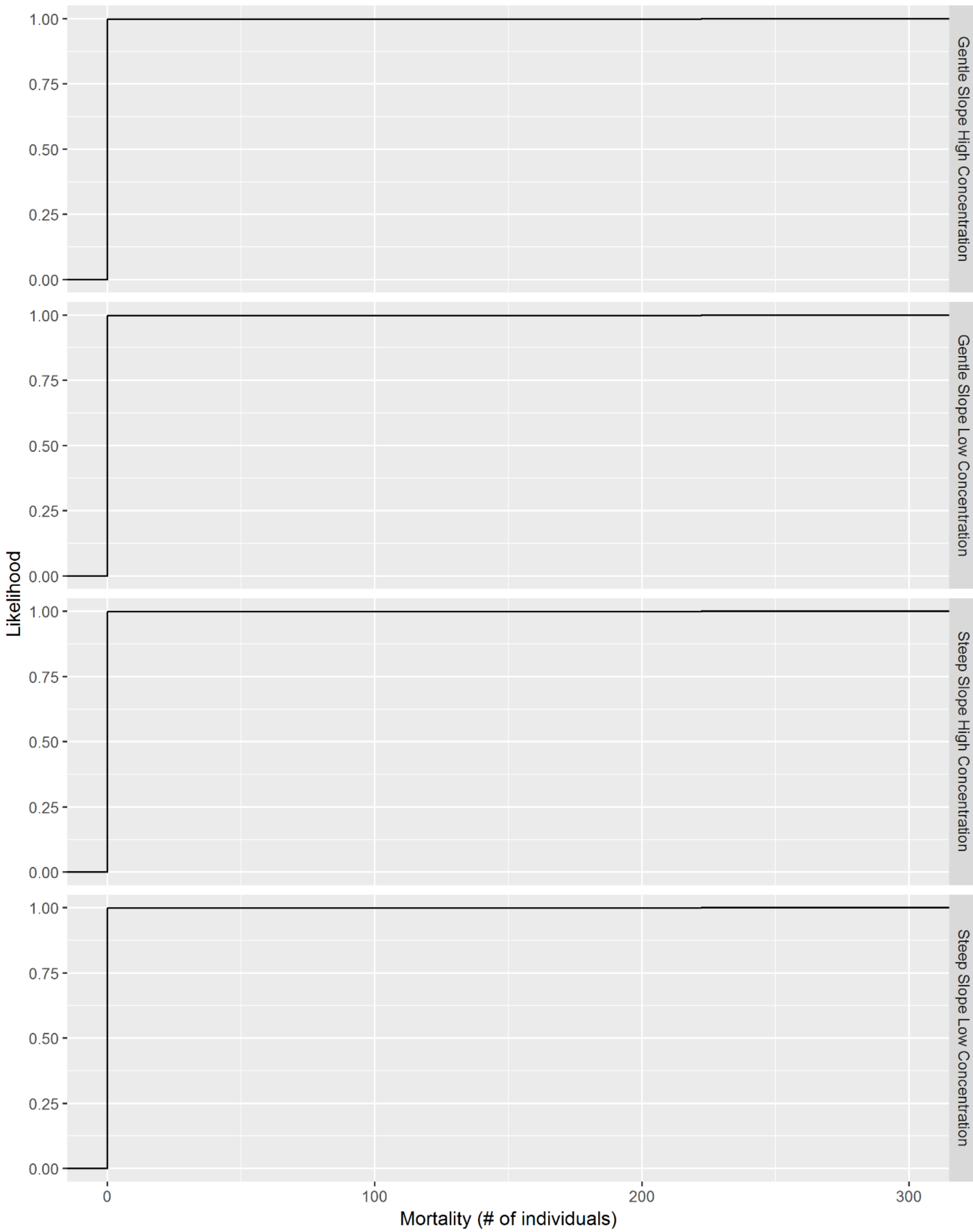

Figure B123. Cumulative likelihood results for Round Hickorynut mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

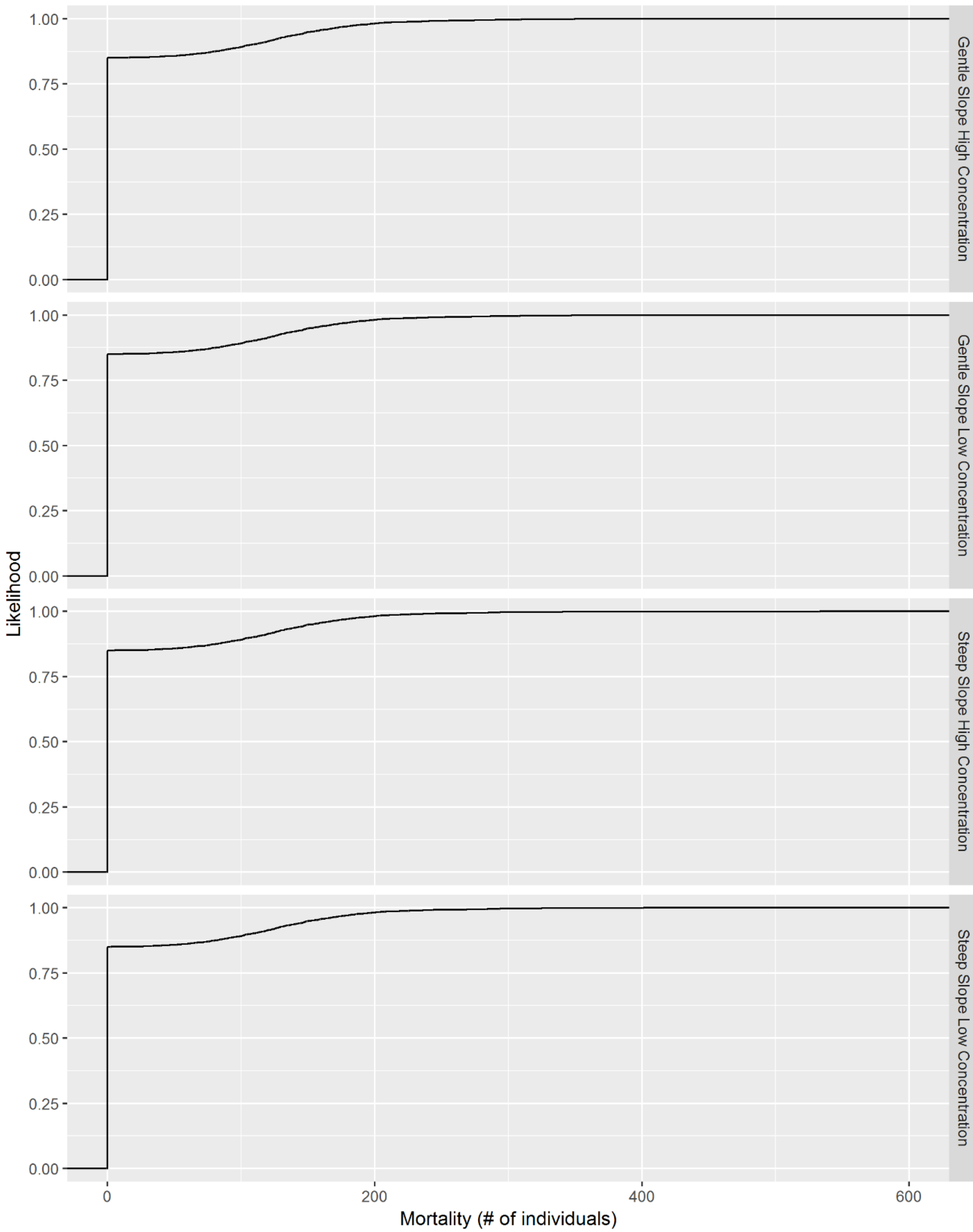

Figure B124. Cumulative likelihood results for Round Pigtoe mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

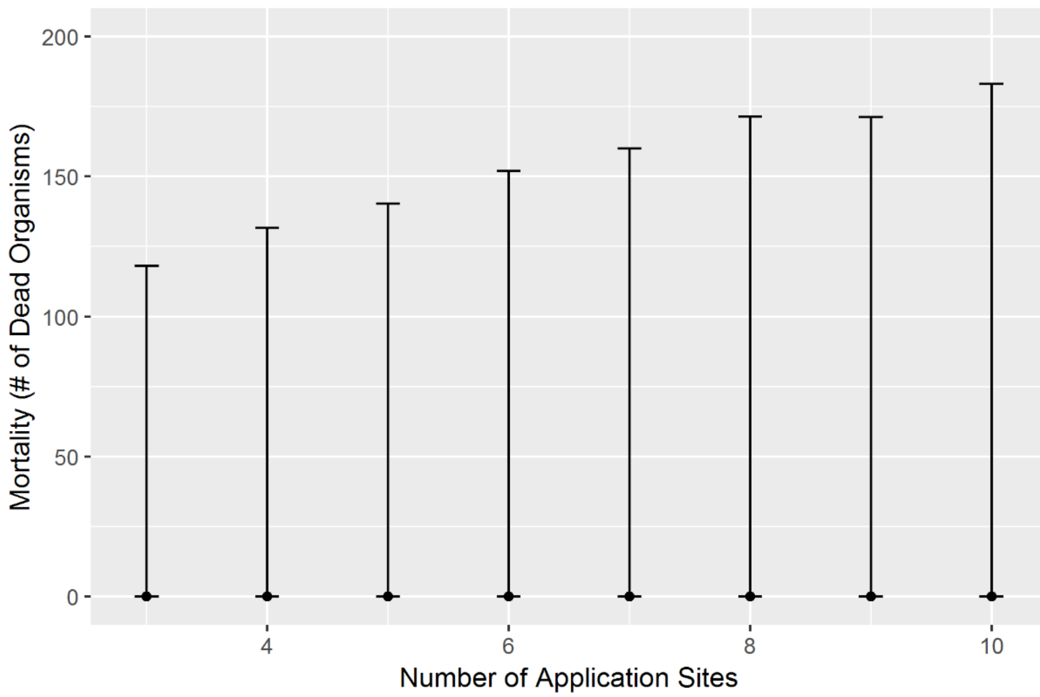

Figure B125. The influence of the number of 500 m^2 application sites on Round Pigtoe mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

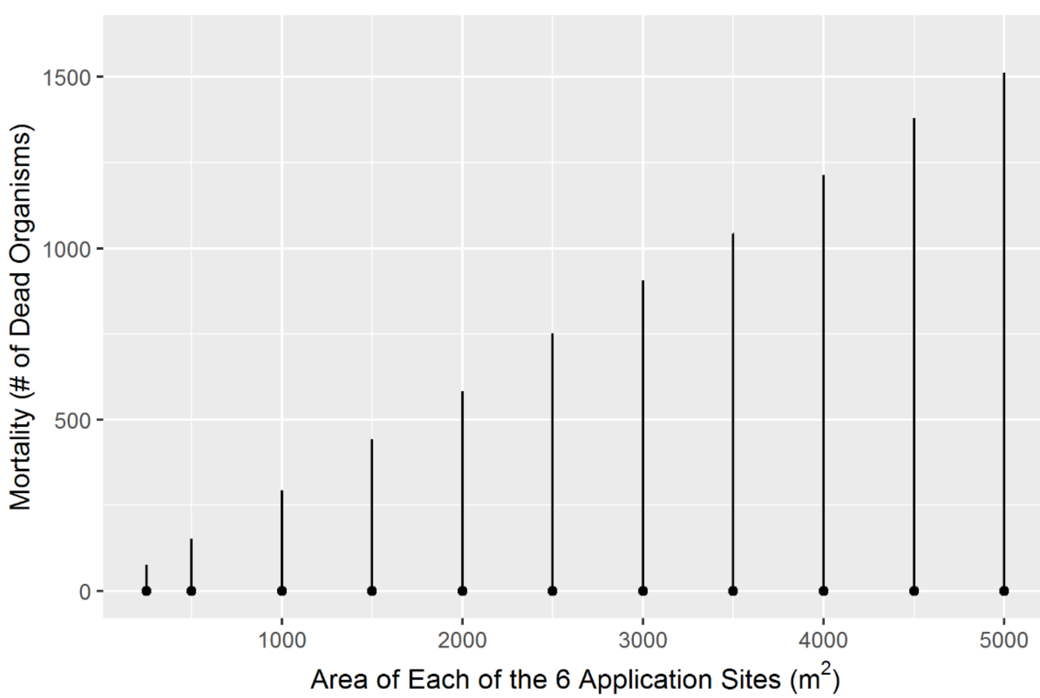

Figure B126. The influence of the size of six application sites on Round Pigtoe mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

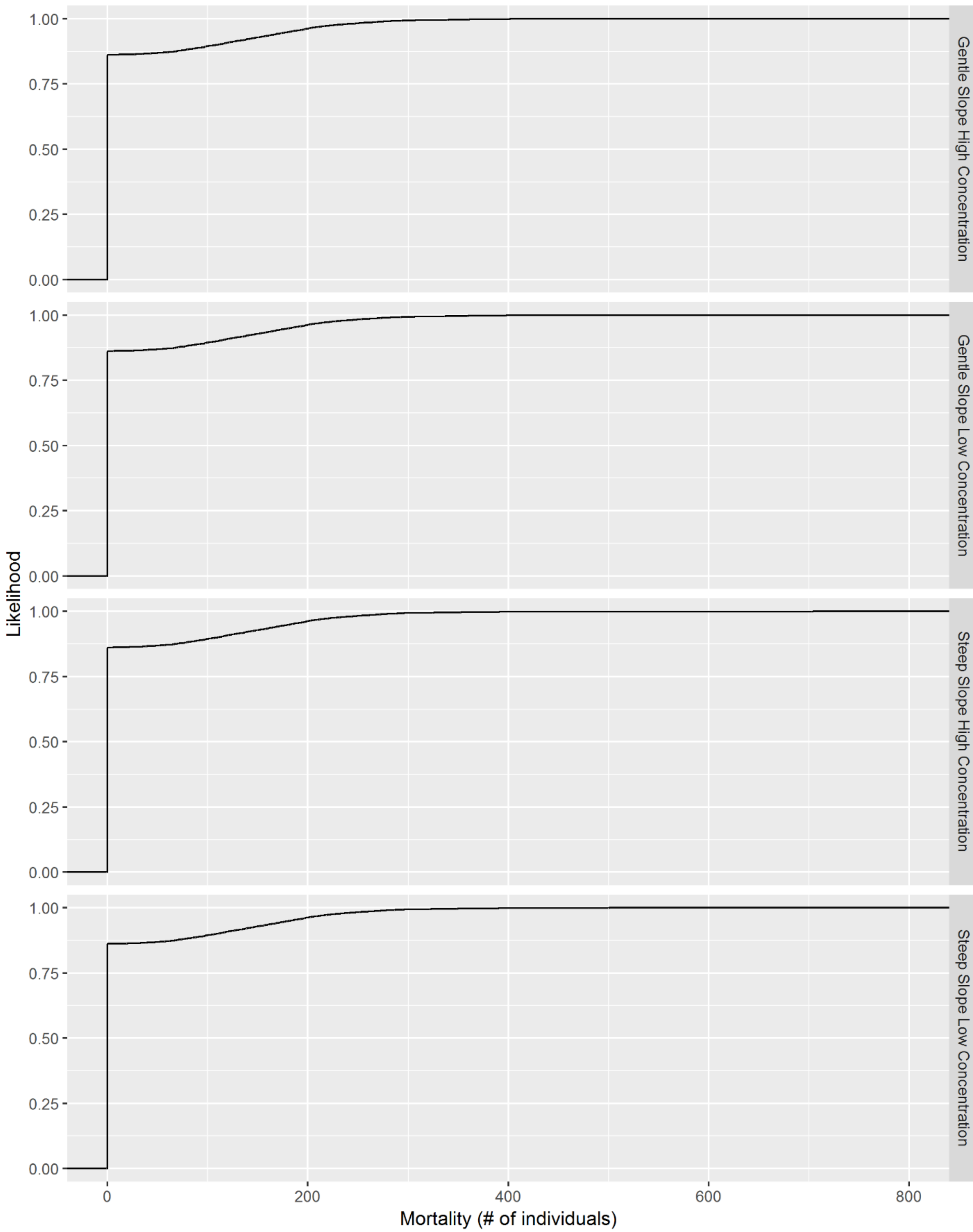

Figure B127. Cumulative likelihood results for Round Pigtoe mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

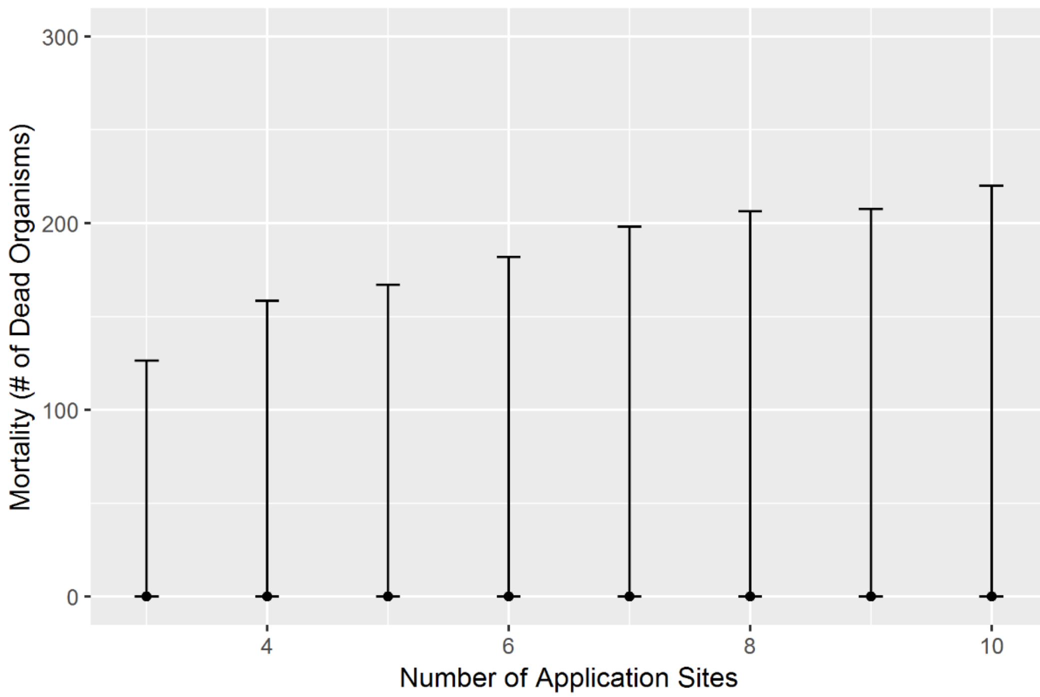

Figure B128. The influence of the number of 500 m^2 application sites on Round Pigtoe mortality in the Thames River. Error bars represents the upper and lower 95^{th} percentile.

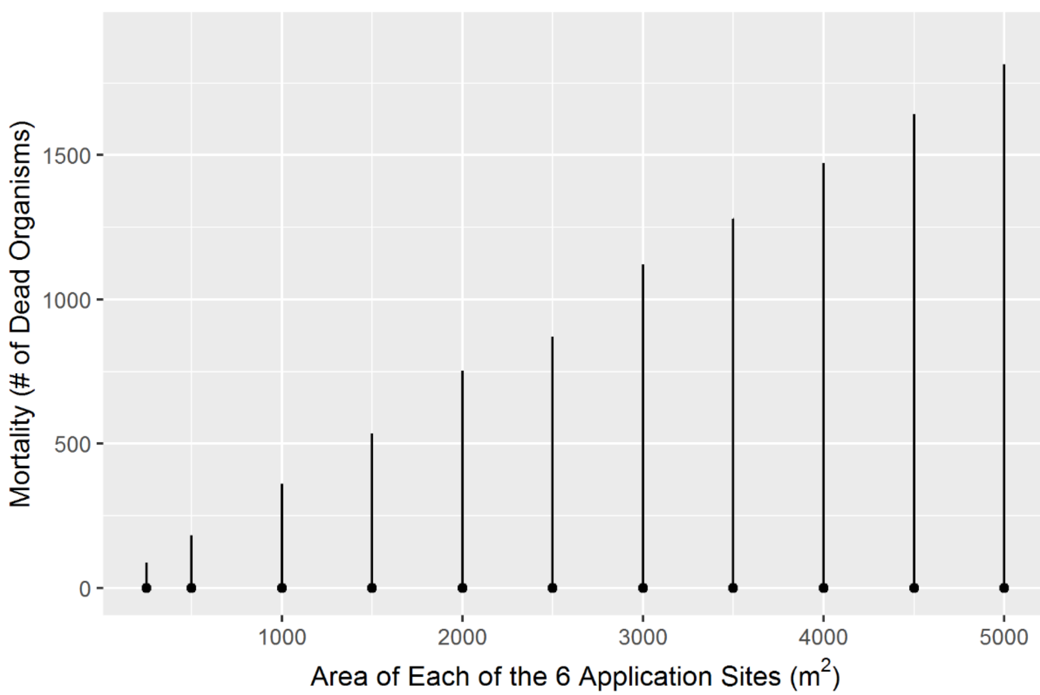

Figure B129. The influence of the size of six application sites on Round Pigtoe mortality in the Thames River. Error bars represents the upper and lower 95^{th} percentile.

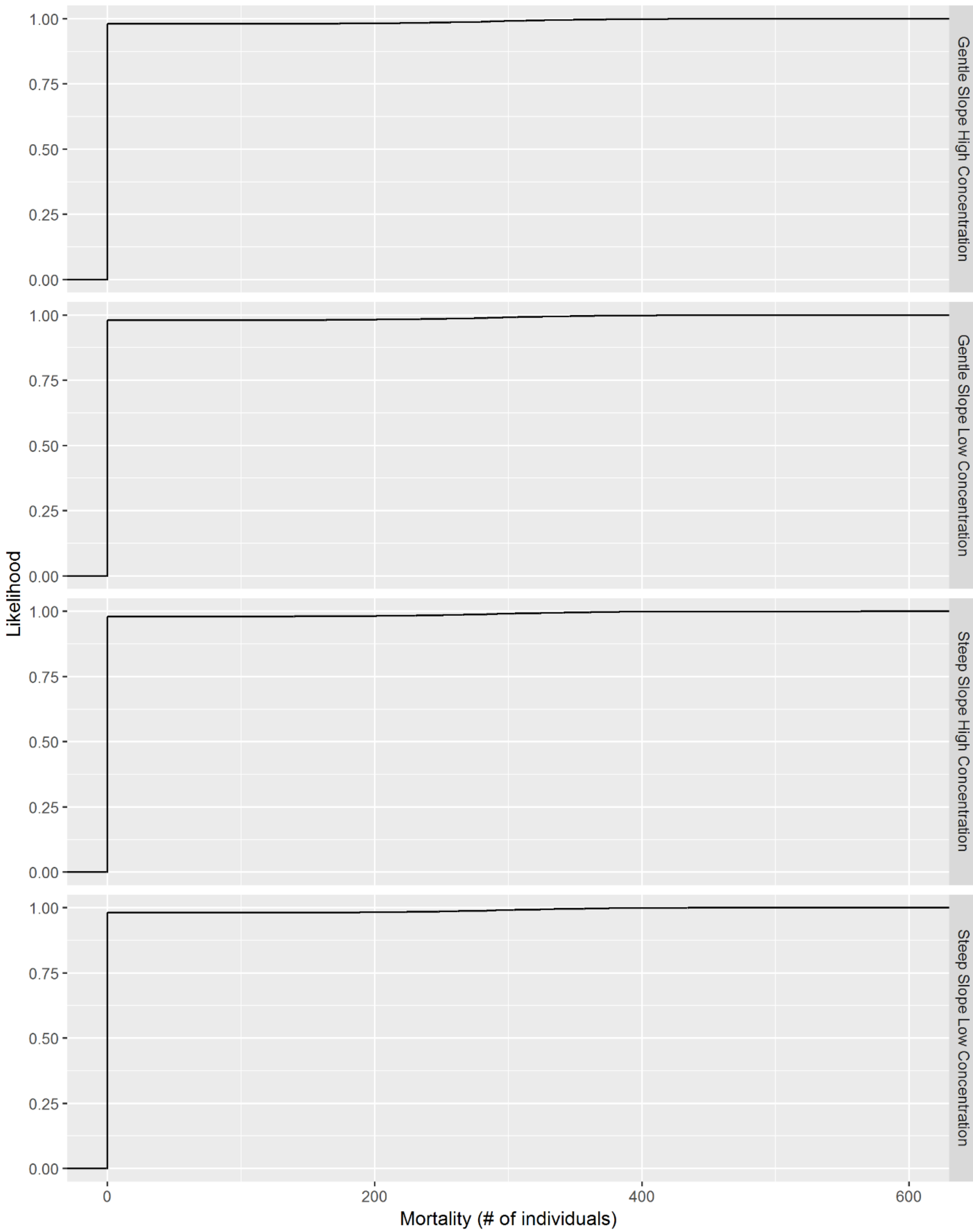

Figure B130. Cumulative likelihood results for Salamander Mussel mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

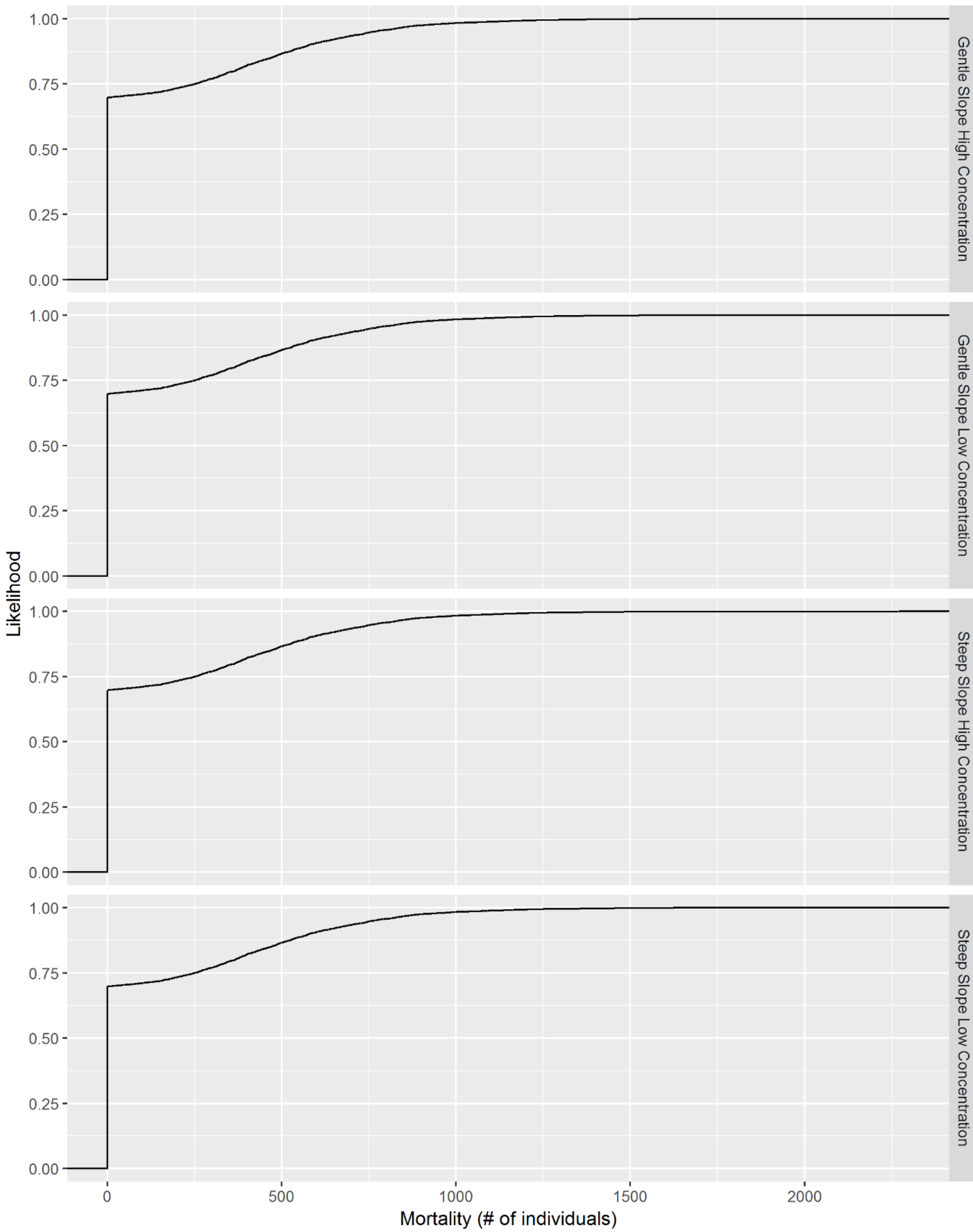

Figure B131. Cumulative likelihood results for Snuffbox mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

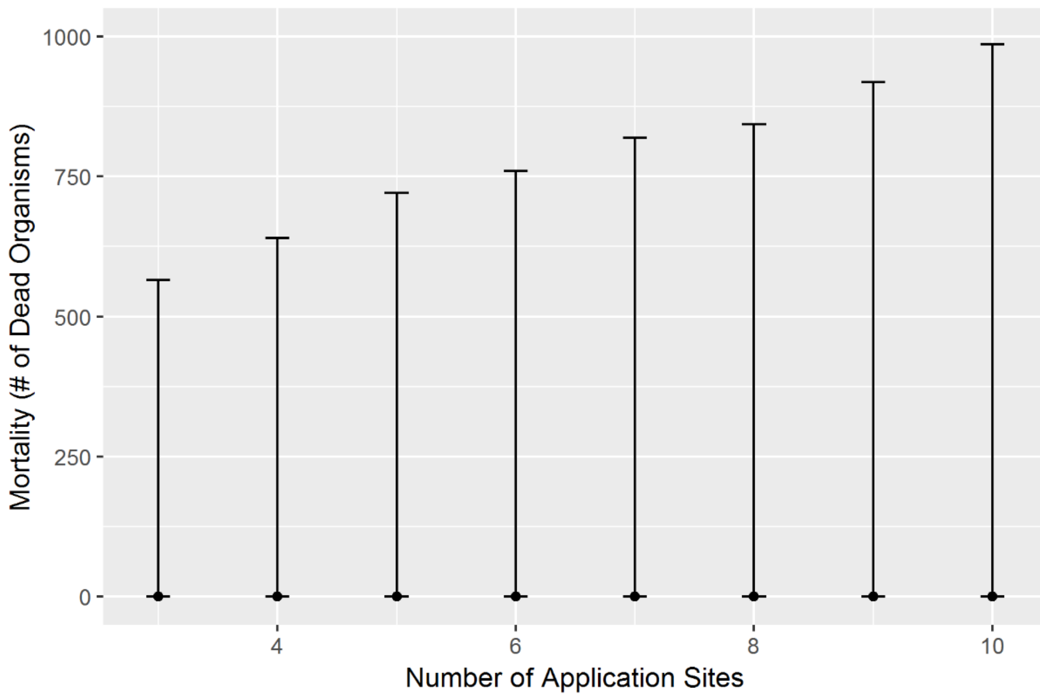

Figure B132. The influence of the number of 500 m^2 application sites on Snuffbox mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

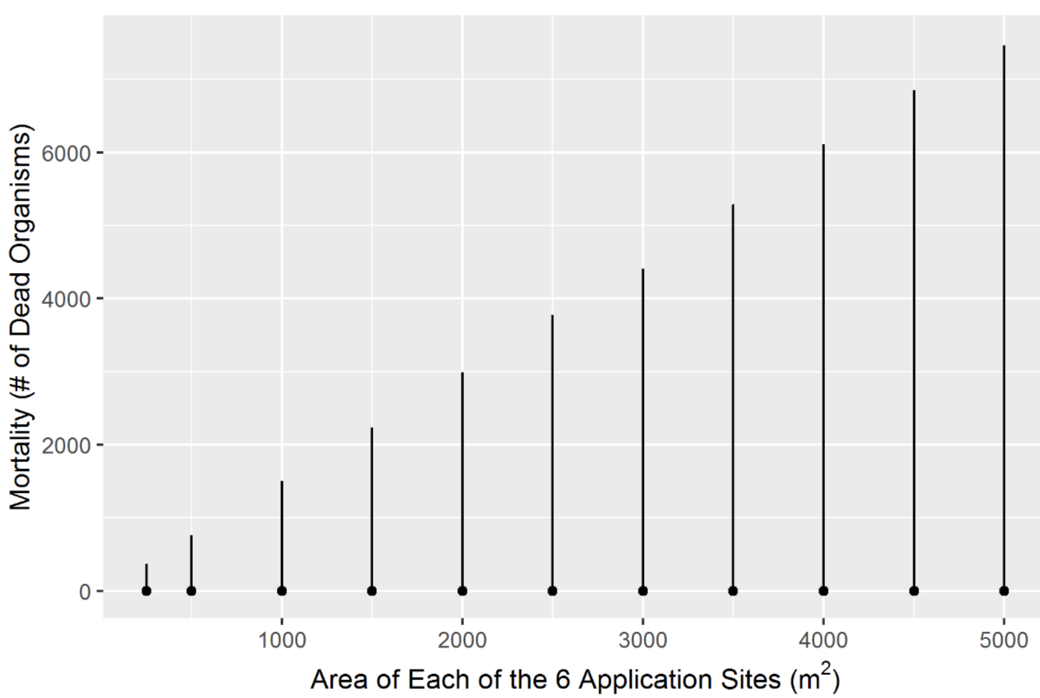

Figure B133. The influence of the size of six application sites on Snuffbox mortality in the Sydenham River. Error bars represents the upper and lower 95th percentile.

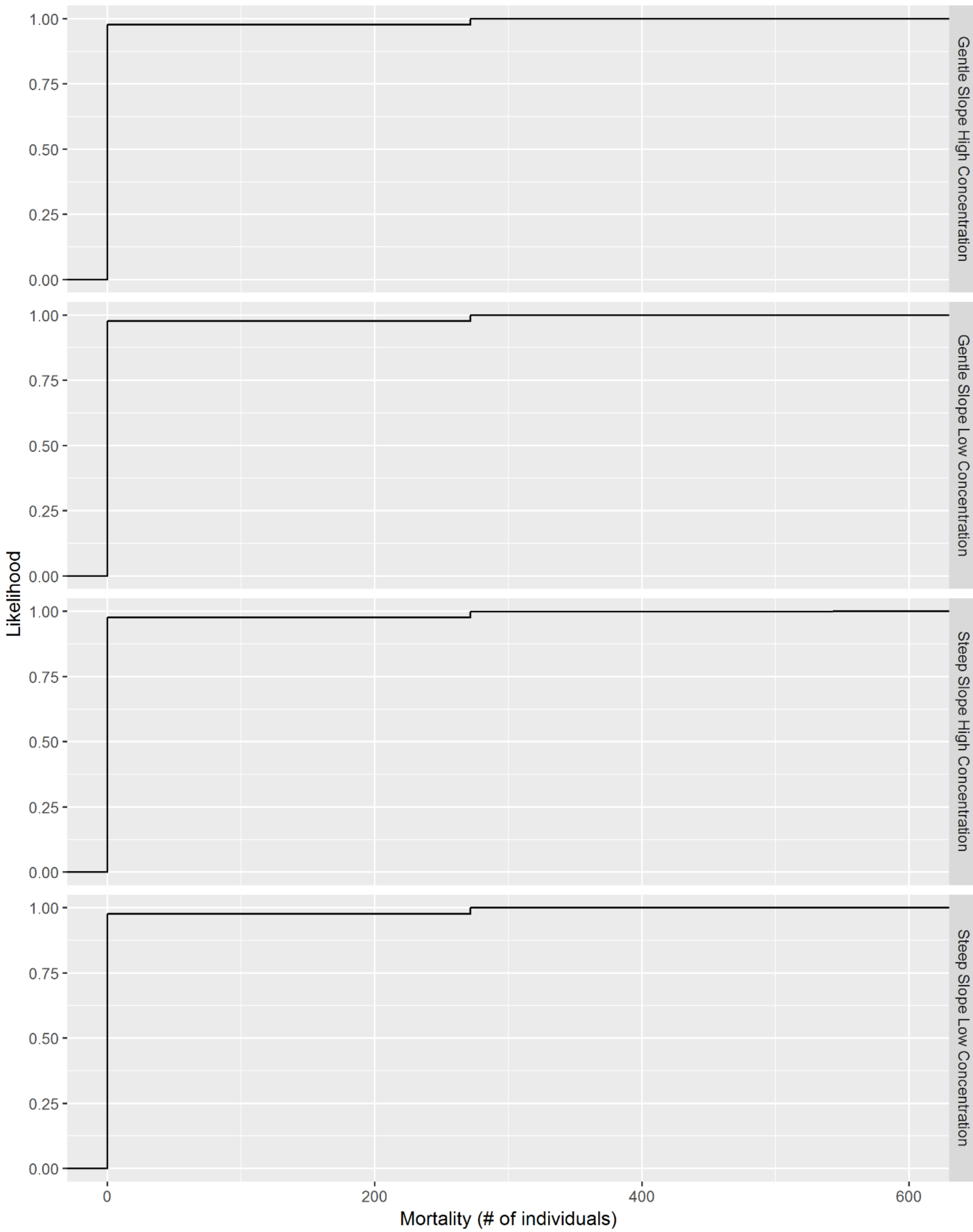

Figure B134. Cumulative likelihood results for Threehorn Wartyback mortality in the Sydenham River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

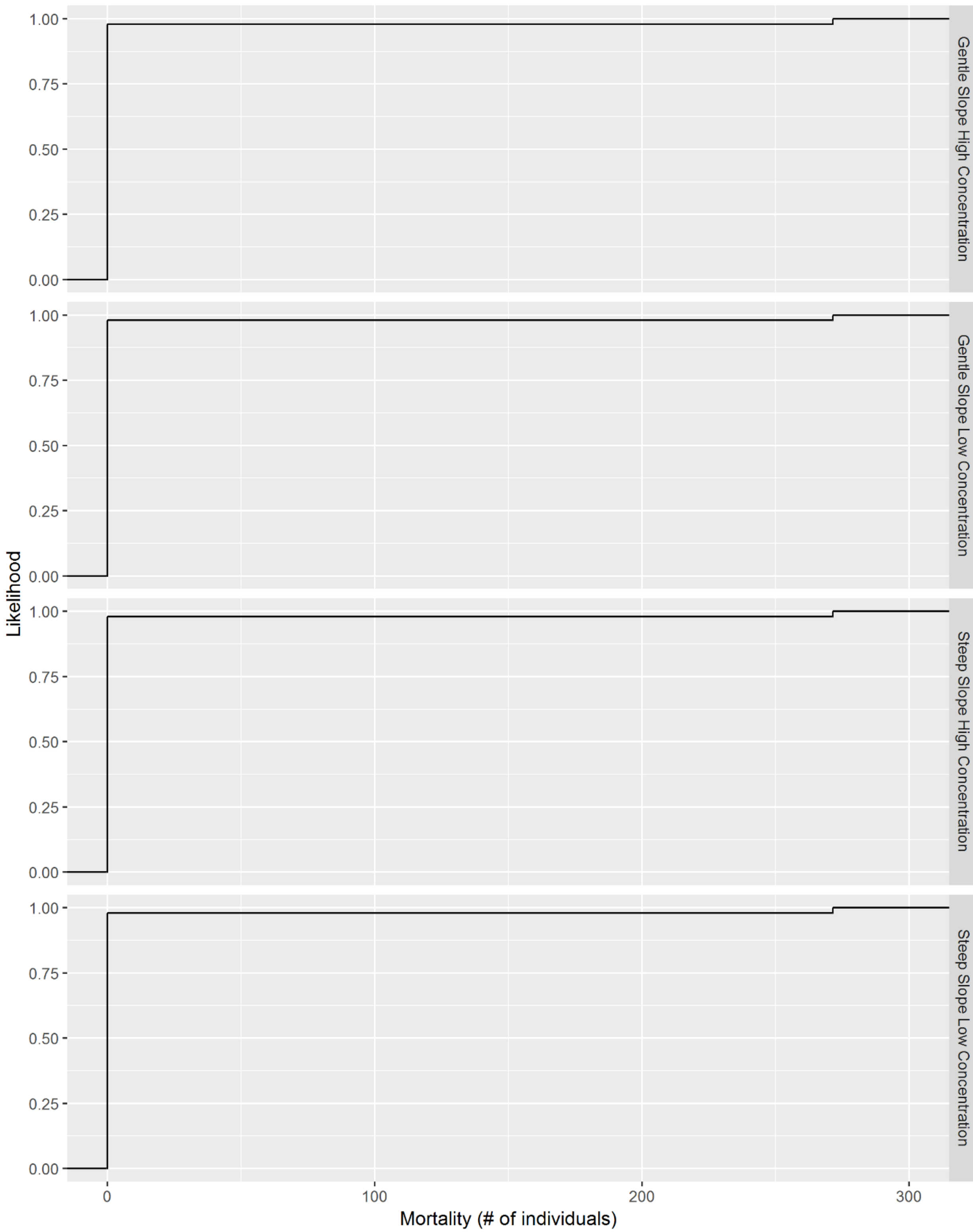

Figure B135. Cumulative likelihood results for Threehorn Wartyback mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

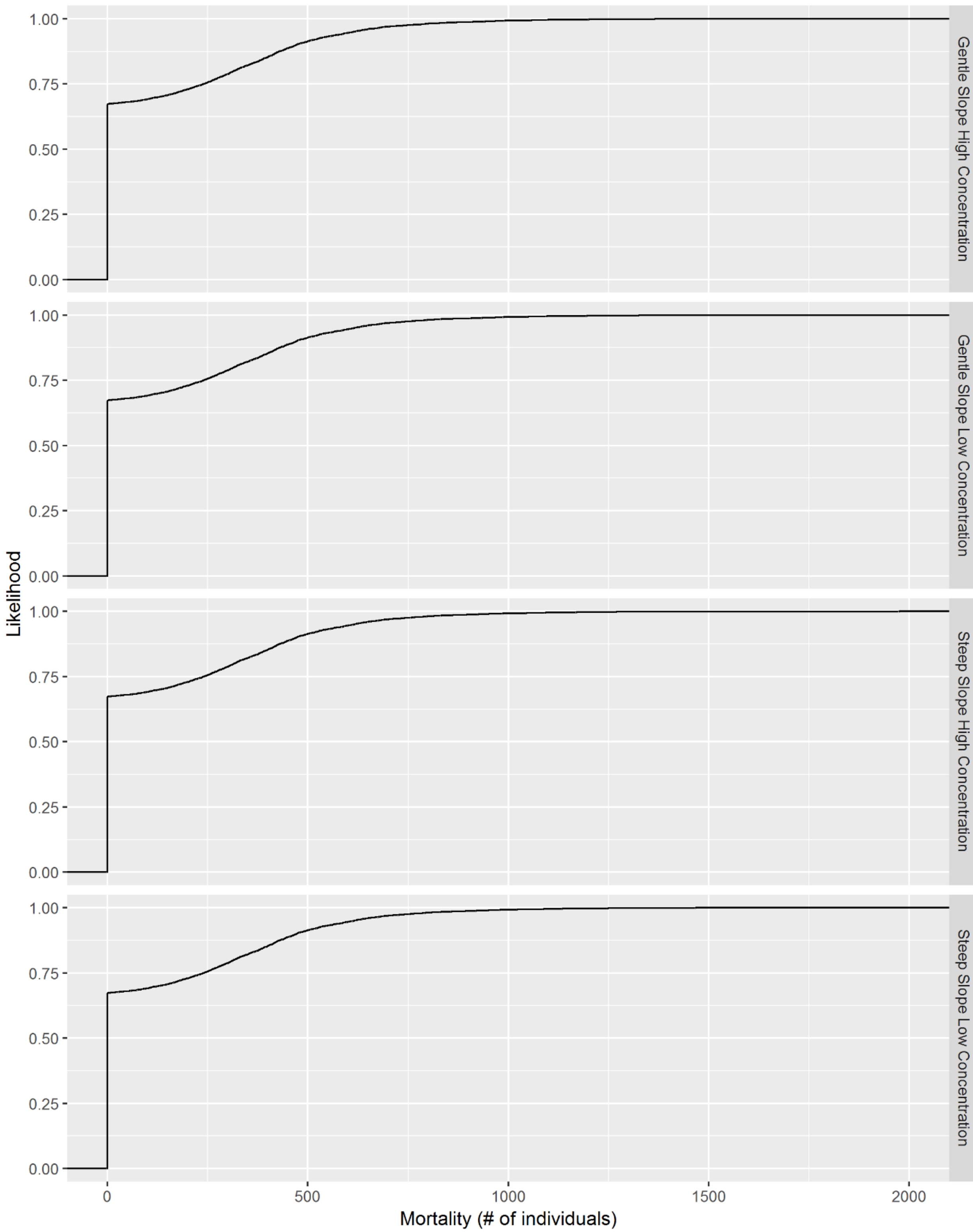

Figure B136. Cumulative likelihood results for Wavyrayed Lampmussel mortality in the Thames River following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values. Likelihood values represent the likelihood that ≤ n mortalities will occur following Bayluscide application.

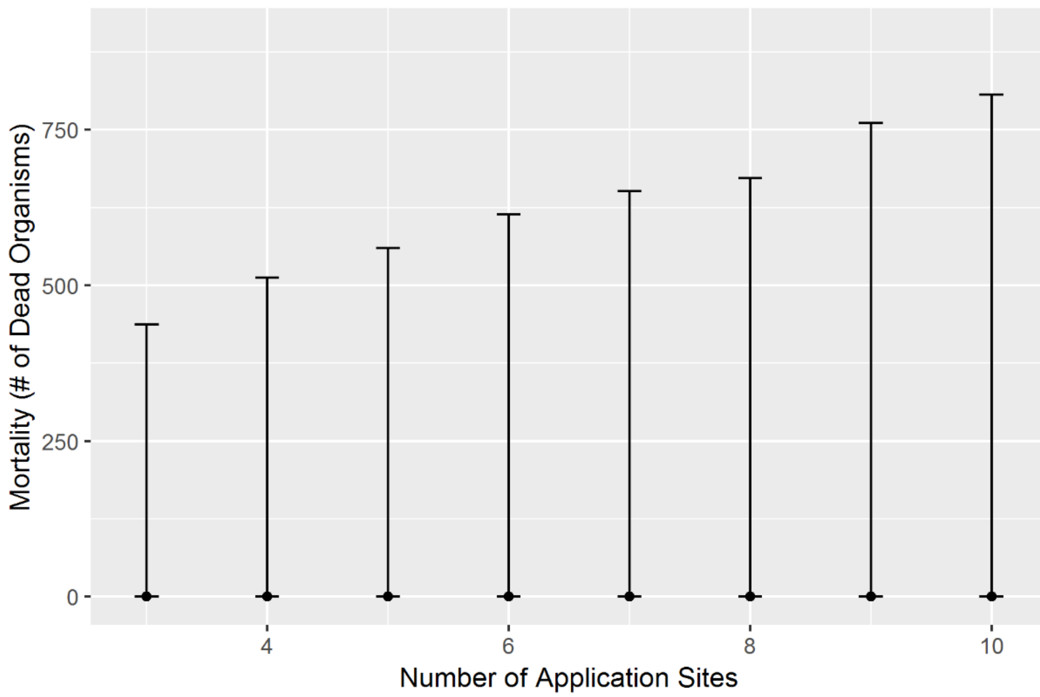

Figure B137. The influence of the number of 500 m^2 application sites on Wavyrayed Lampmussel mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

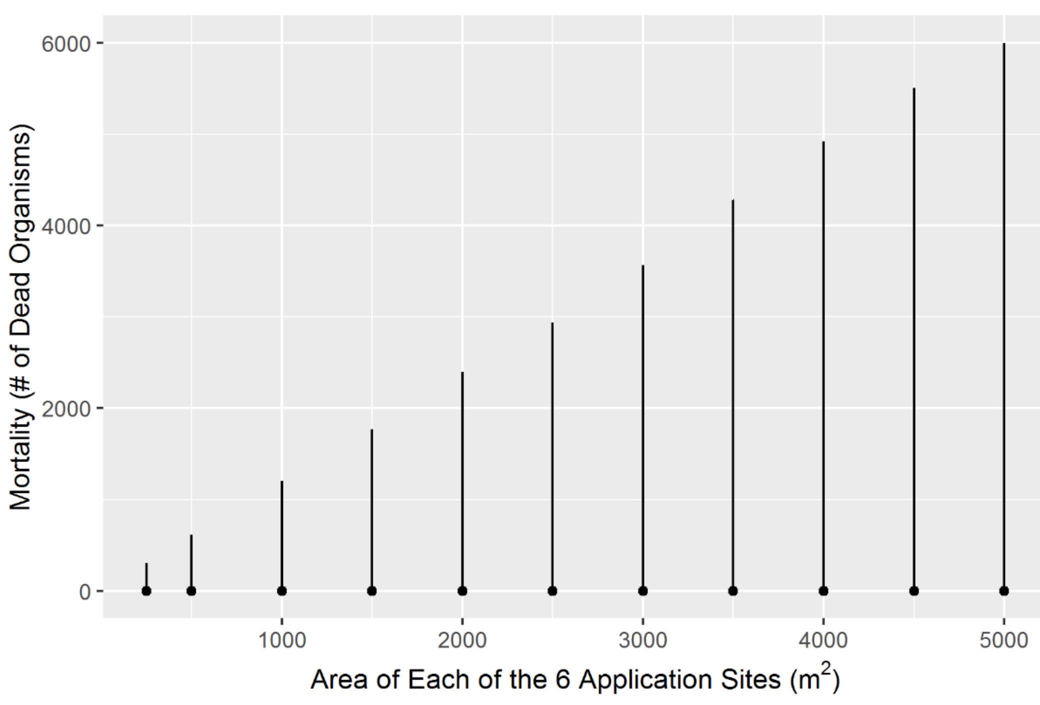

Figure B138. The influence of the size of six application sites on Wavyrayed Lampmussel mortality in the Thames River. Error bars represents the upper and lower 95th percentile.

APPENDIX C: CUMULATIVE MORTALITY OUTPUTS FOR SPECIES OF CONSERVATION CONCERN BASED ON THE AREA-PER-INDIVIDUAL CALCULATIONS

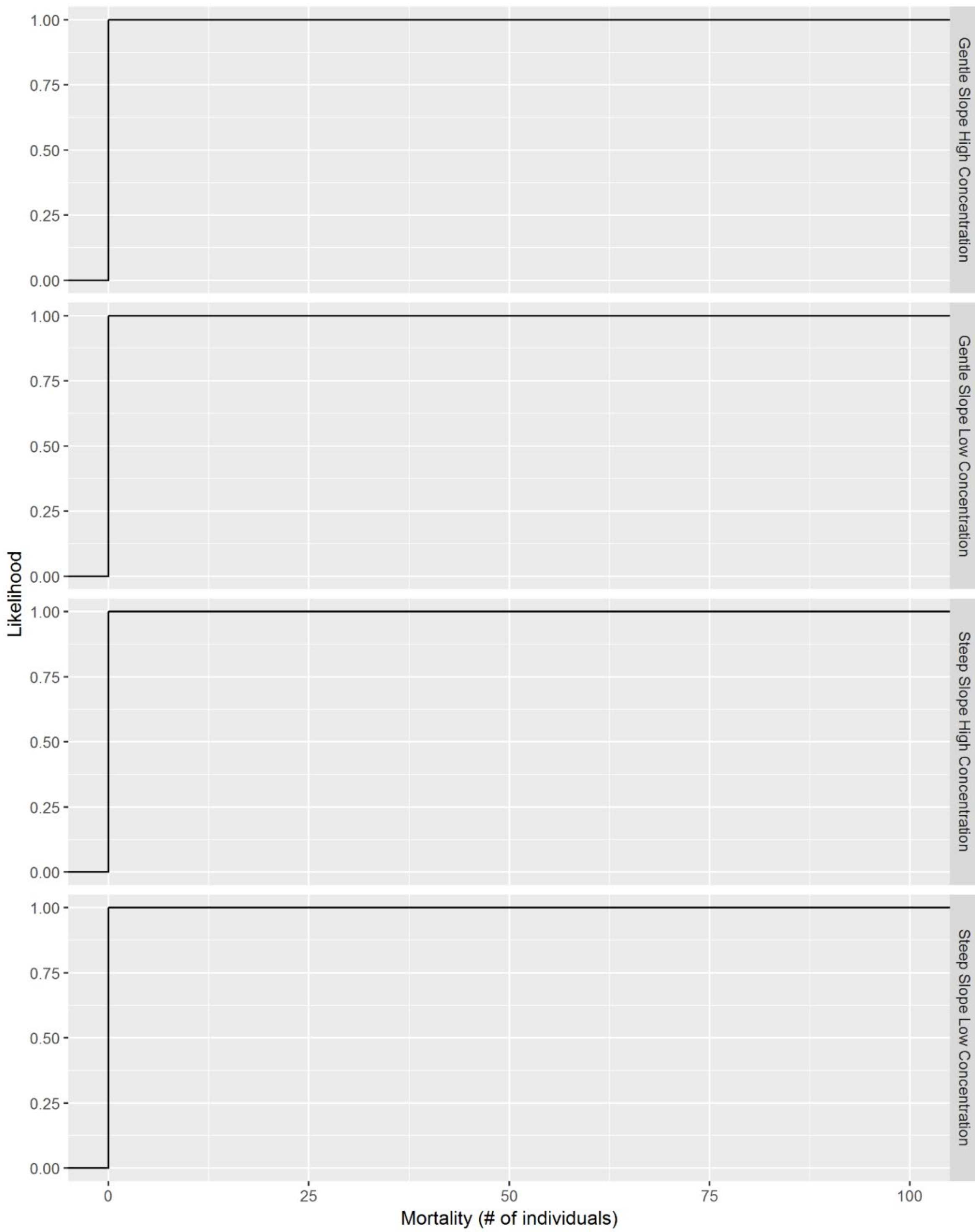

Figure C1. Cumulative likelihood results for Black Redhorse mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

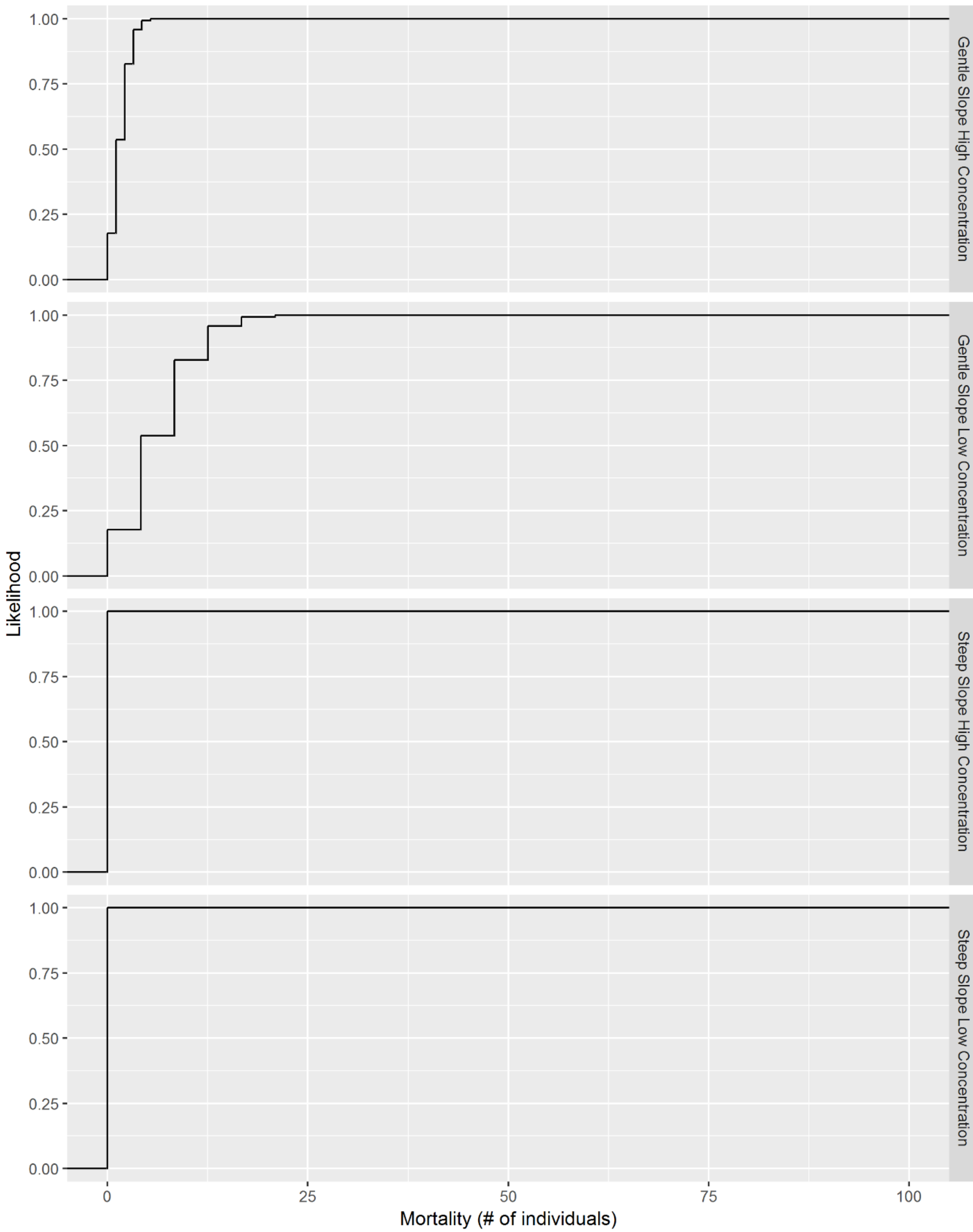

Figure C2. Cumulative likelihood results for Blackstripe Topminnow mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

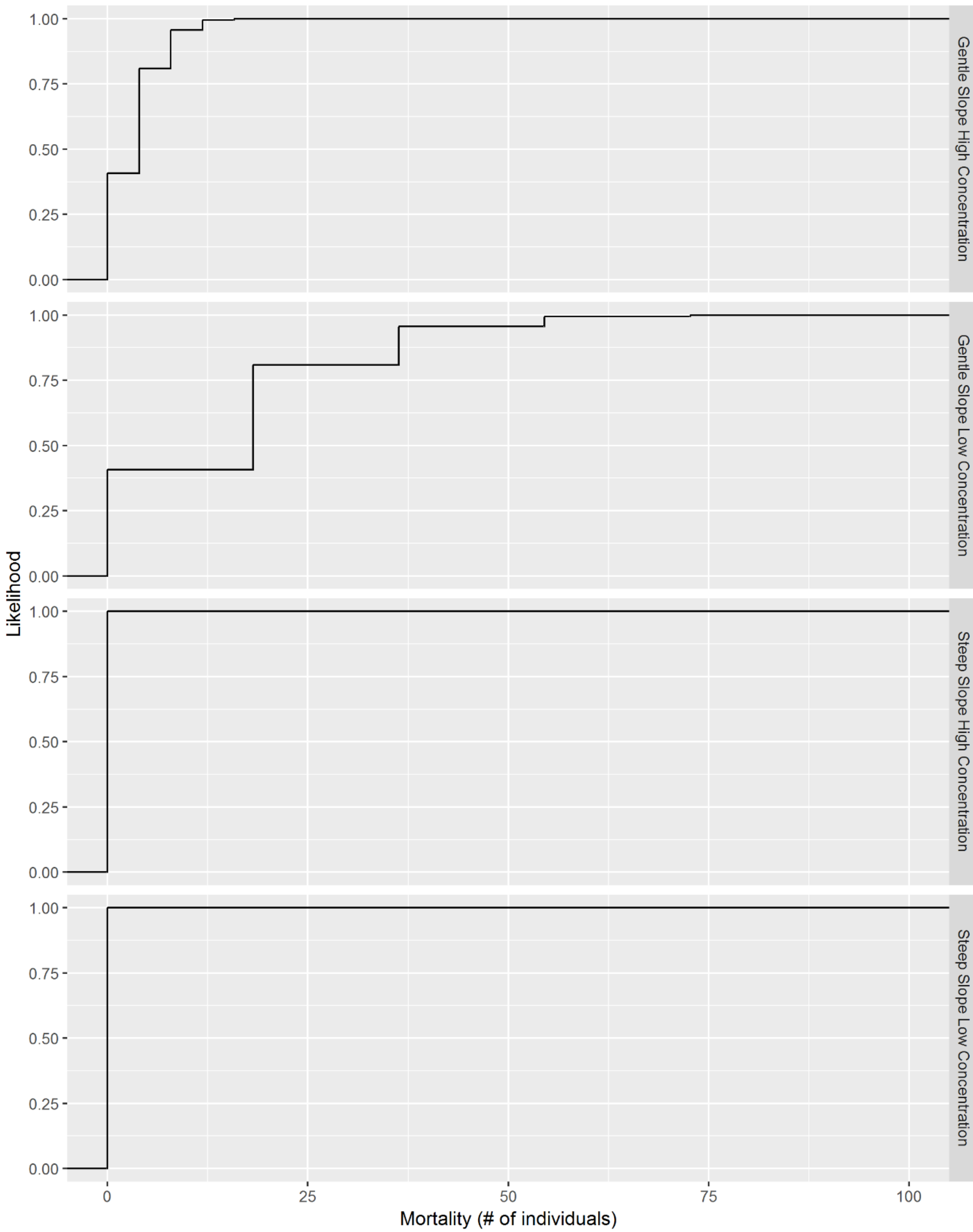

Figure C3. Cumulative likelihood results for Channel Darter mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

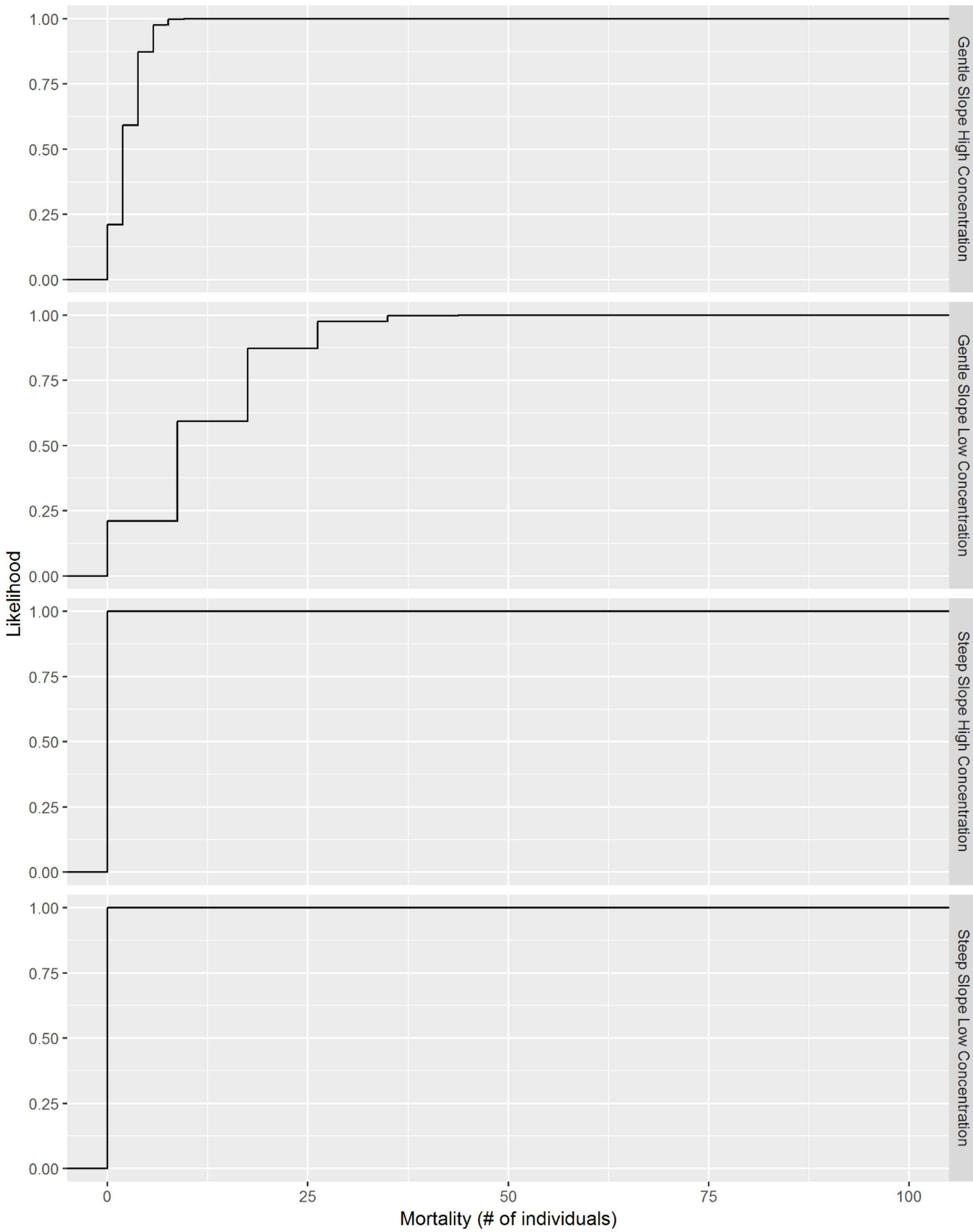

Figure C4. Cumulative likelihood results for Eastern Sand Darter mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

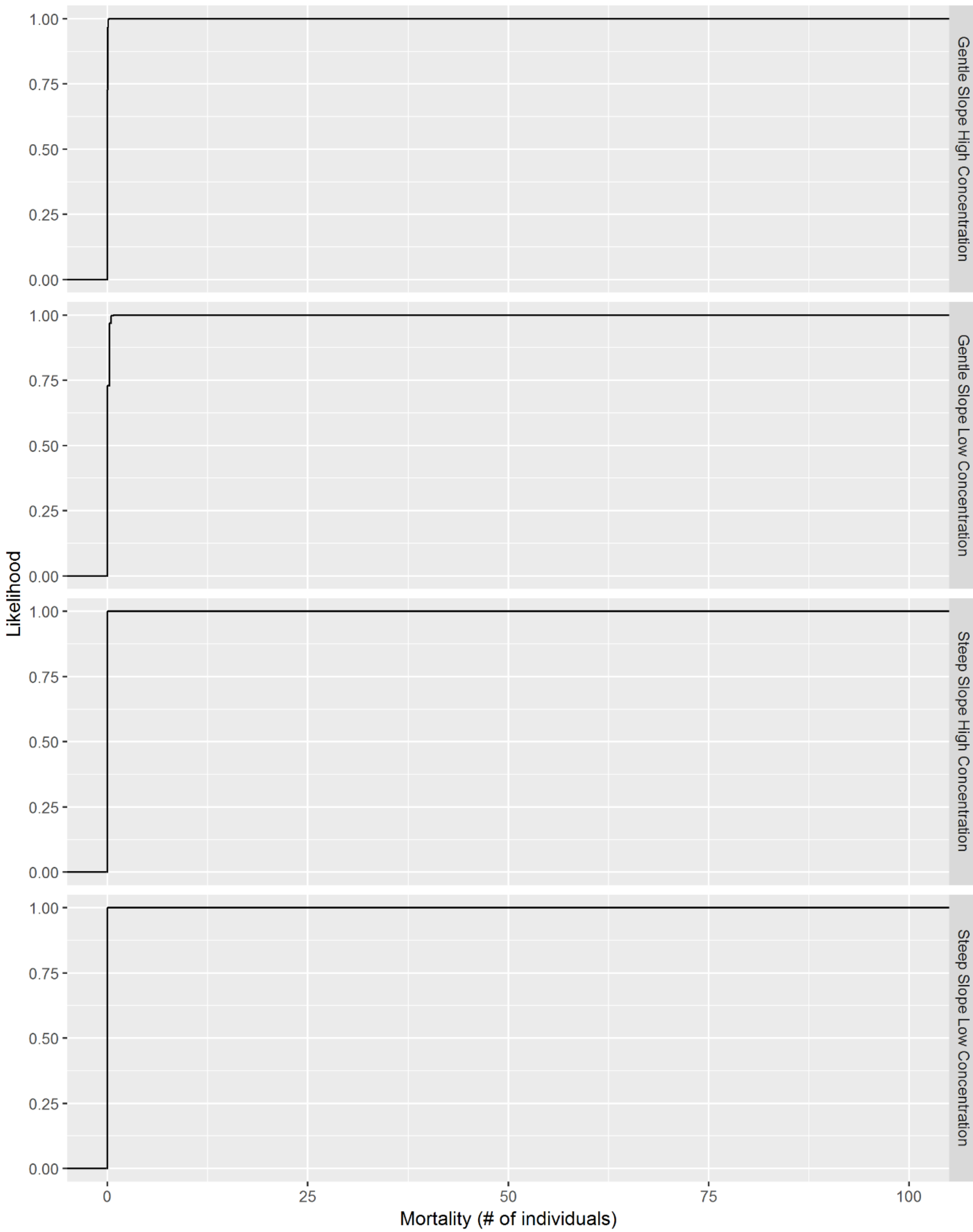

Figure C5. Cumulative likelihood results for Grass Pickerel mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

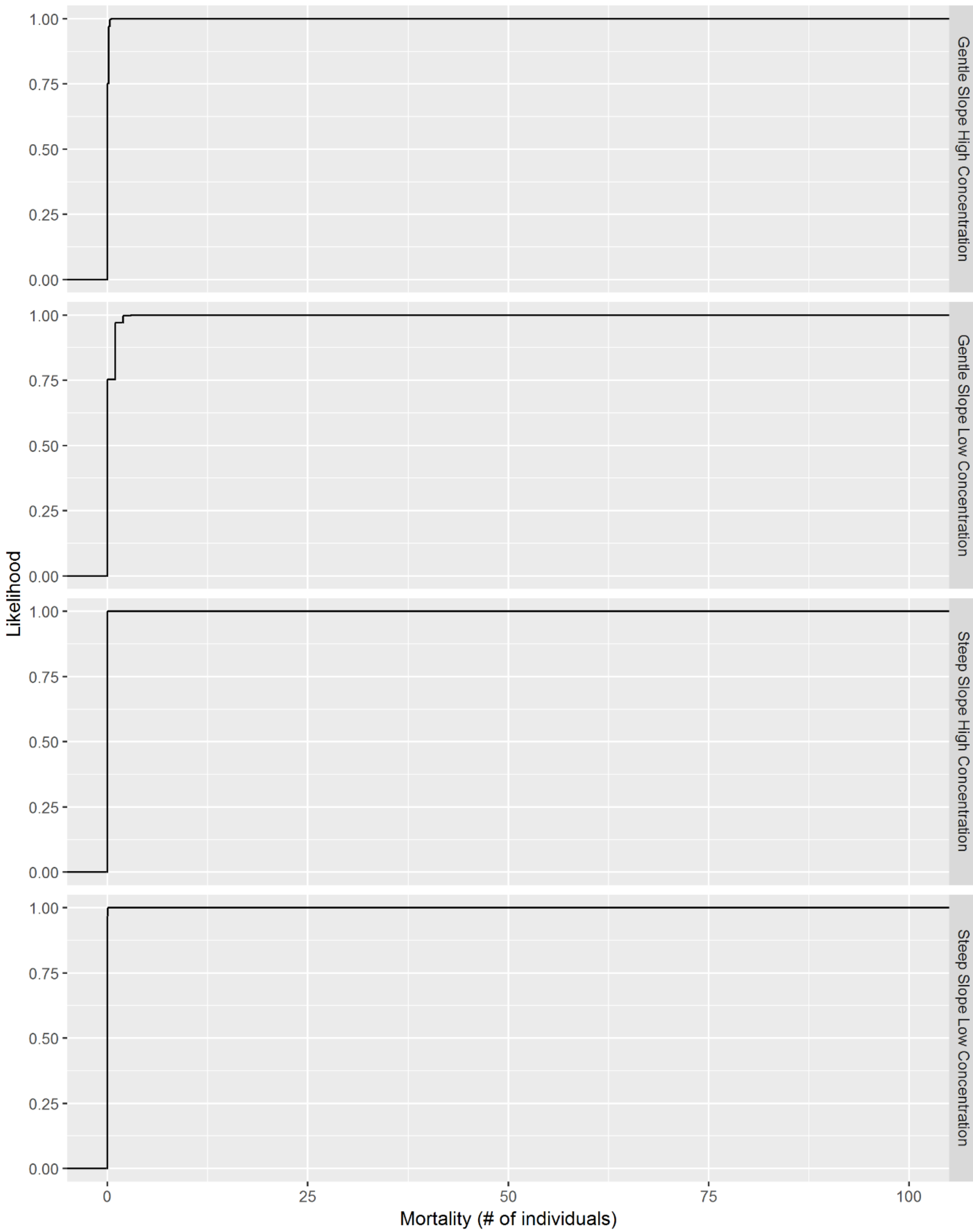

Figure C6. Cumulative likelihood results for Lake Chubsucker mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

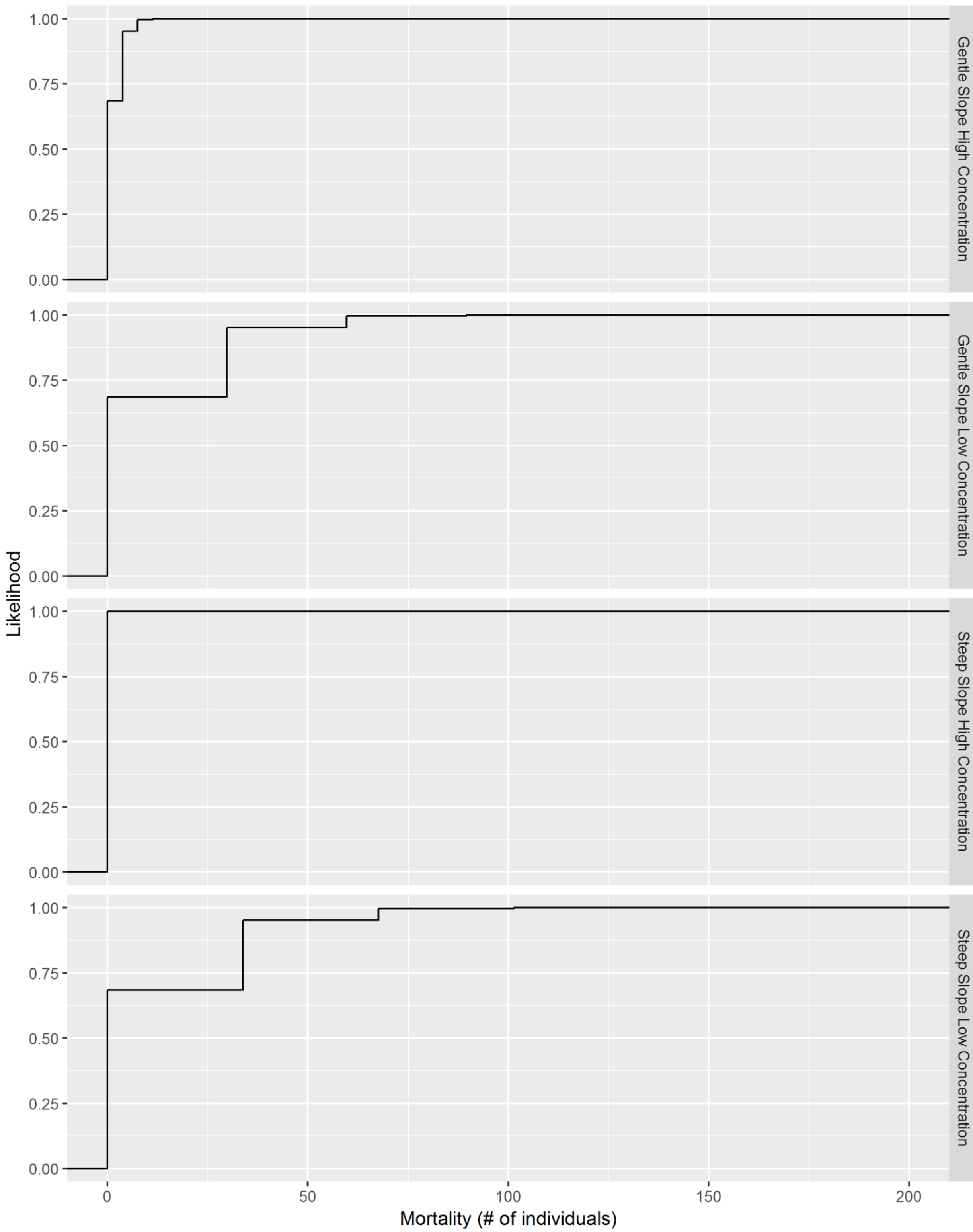

Figure C7. Cumulative likelihood results for Northern Madtom mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

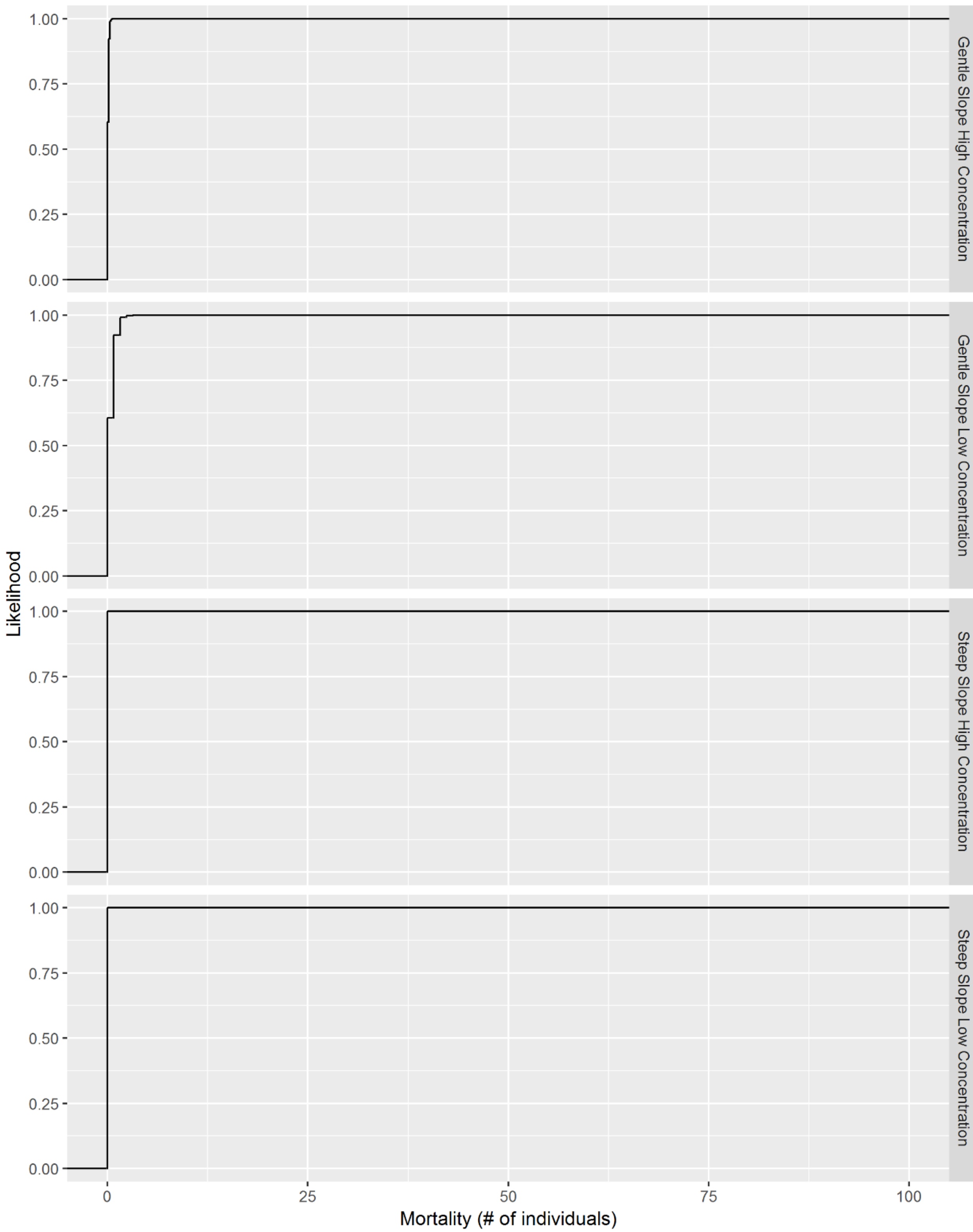

Figure C8. Cumulative likelihood results for Northern Sunfish mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

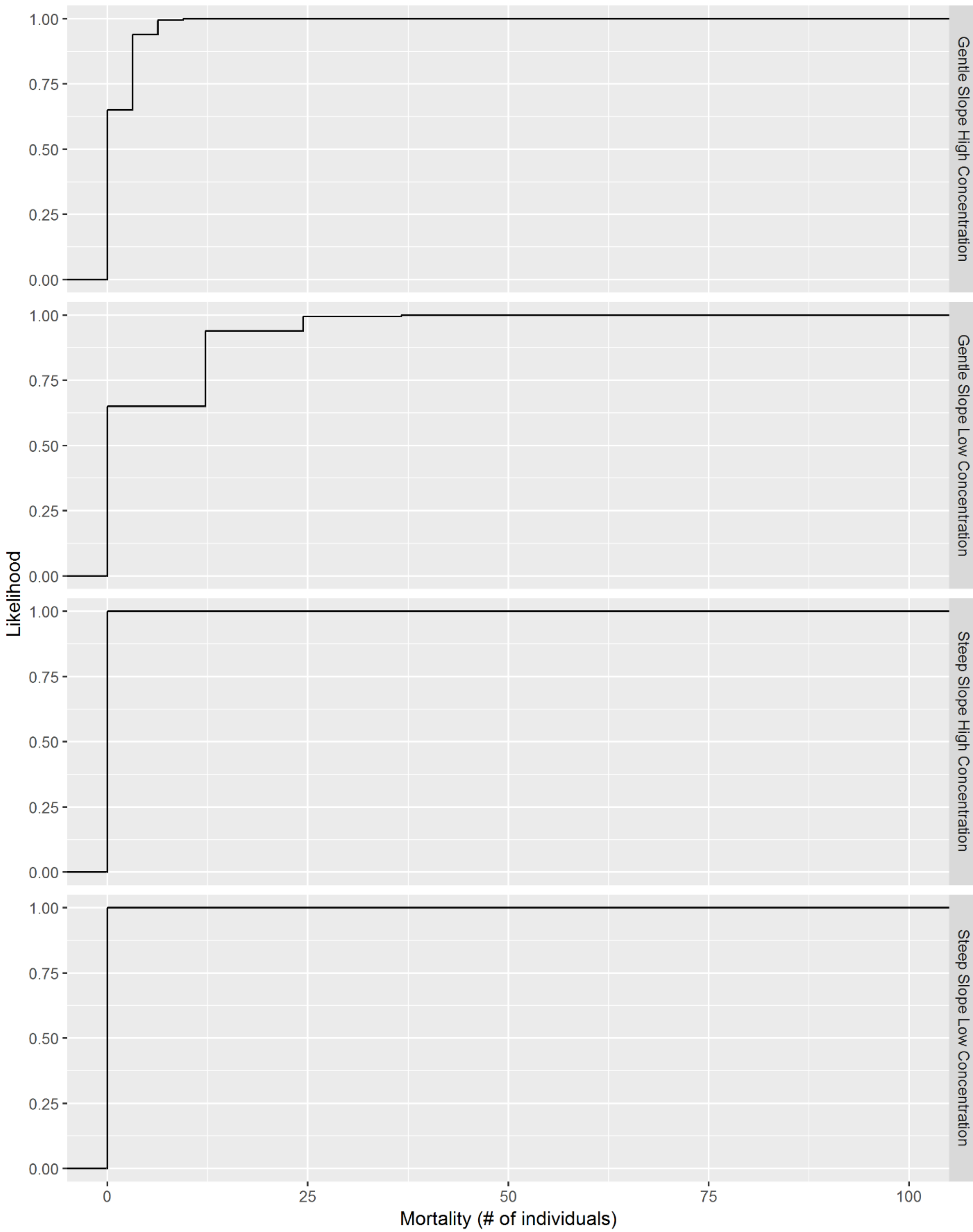

Figure C9. Cumulative likelihood results for Pugnose Minnow mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

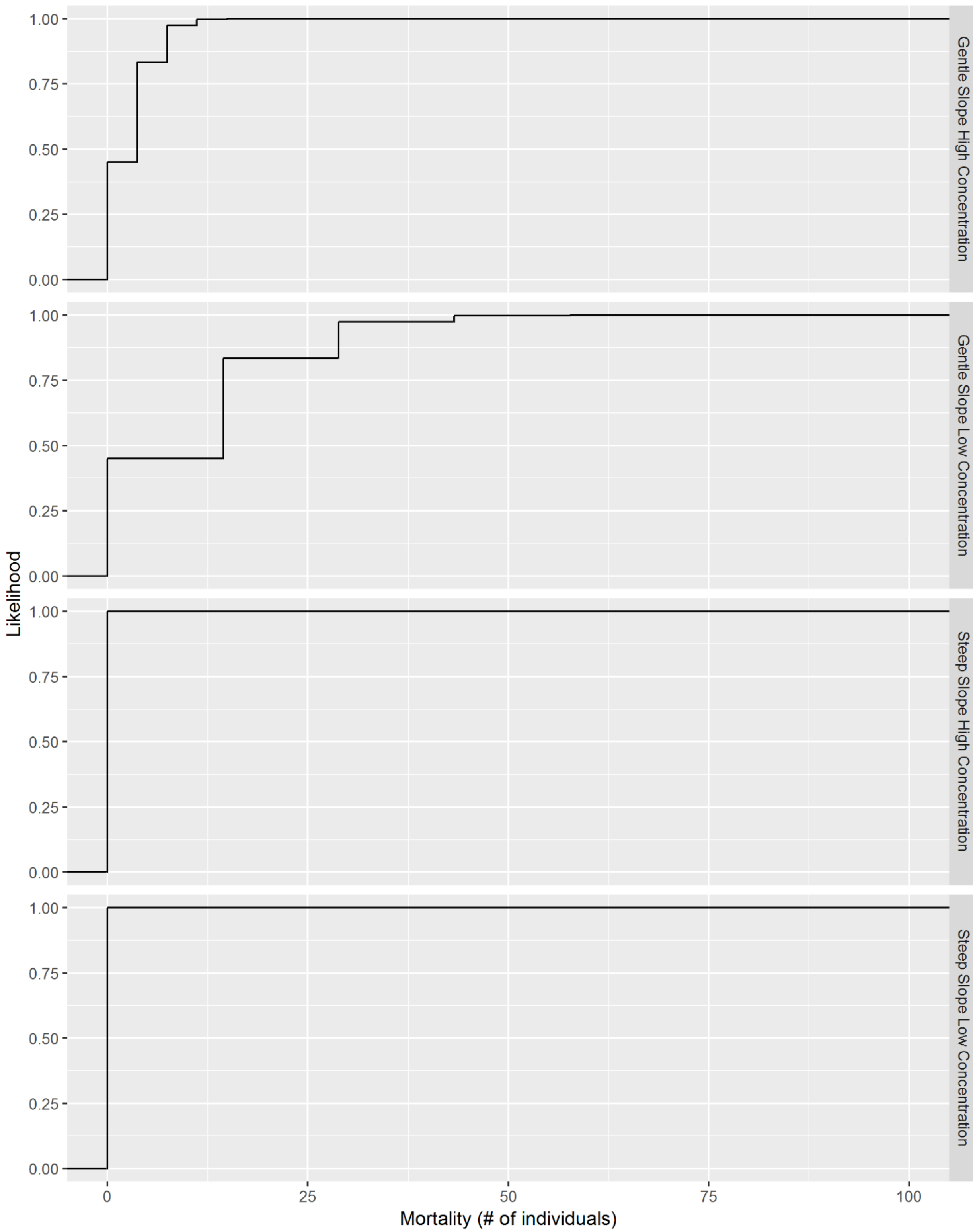

Figure C10. Cumulative likelihood results for Pugnose Shiner mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

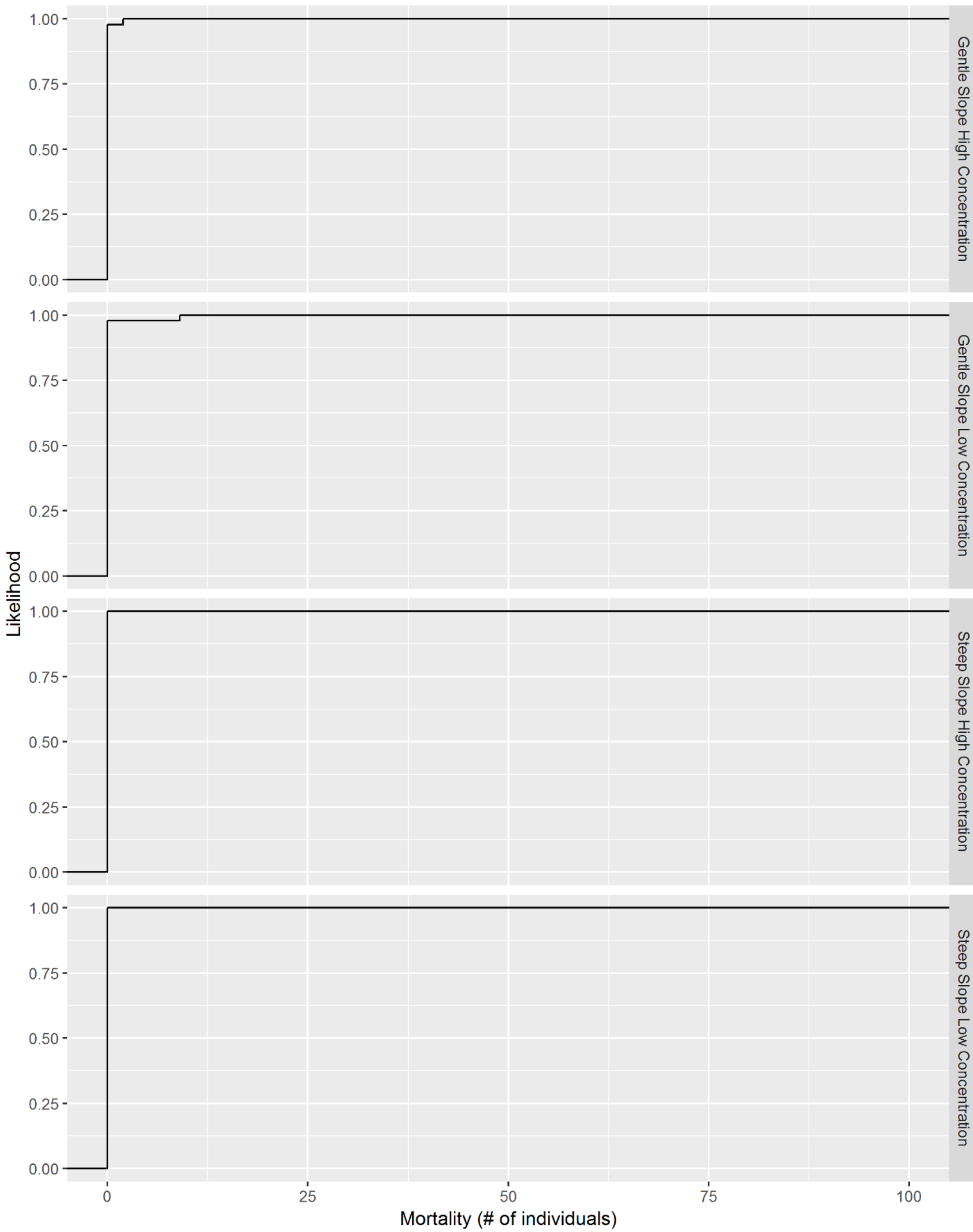

Figure C11. Cumulative likelihood results for River Darter mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

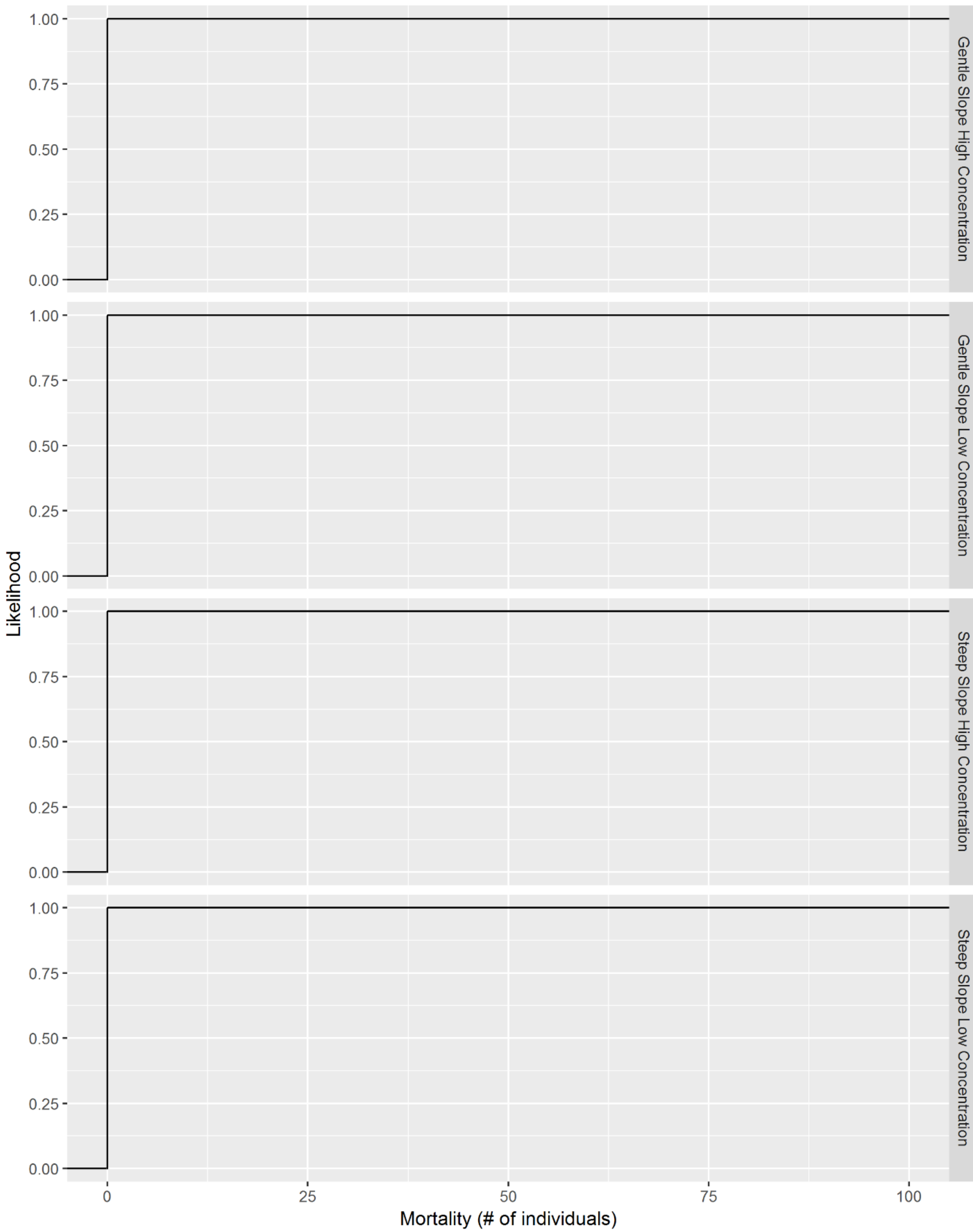

Figure C12. Cumulative likelihood results for River Redhorse mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

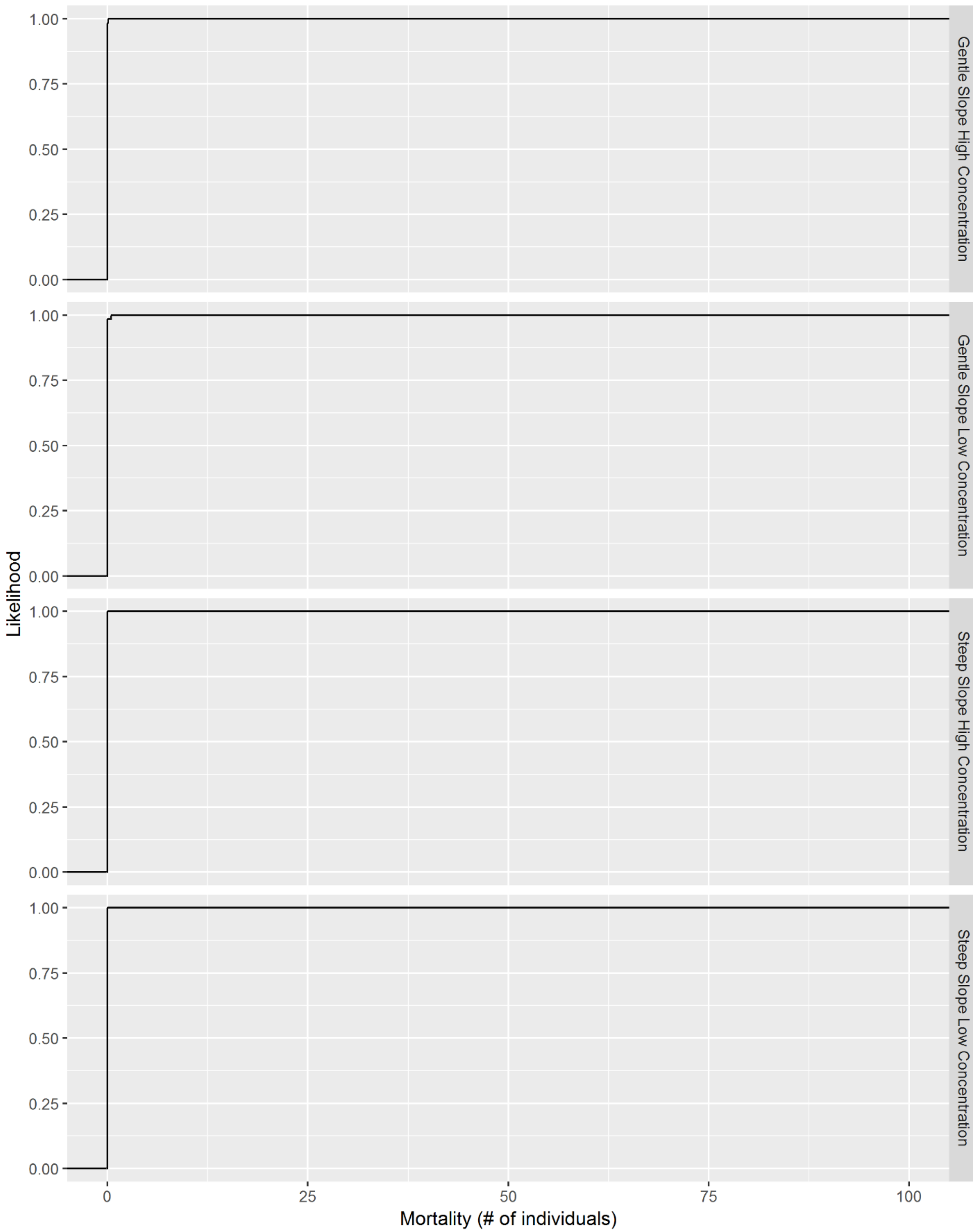

Figure C13. Cumulative likelihood results for Silver Chub mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

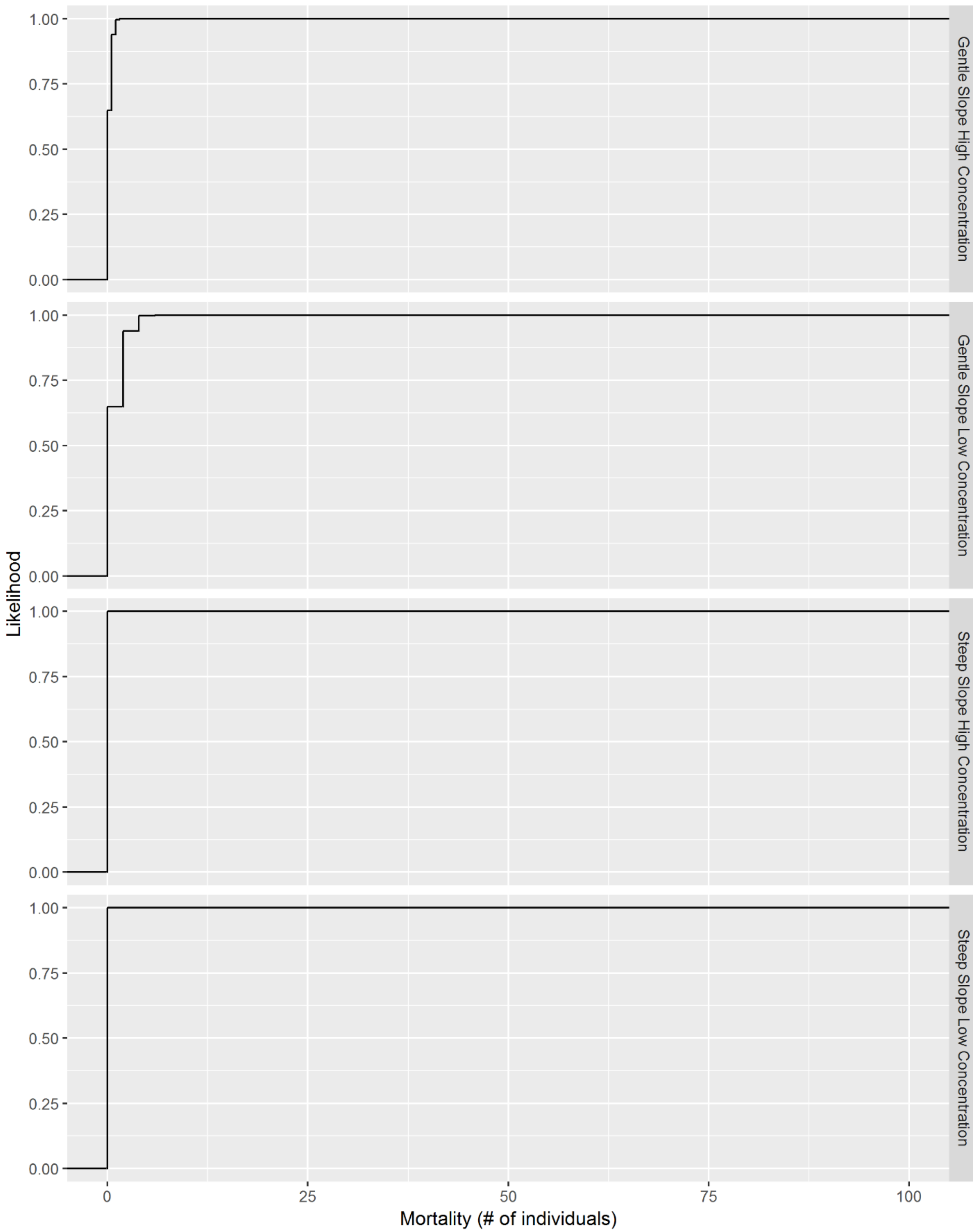

Figure C14. Cumulative likelihood results for Silver Shiner mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.

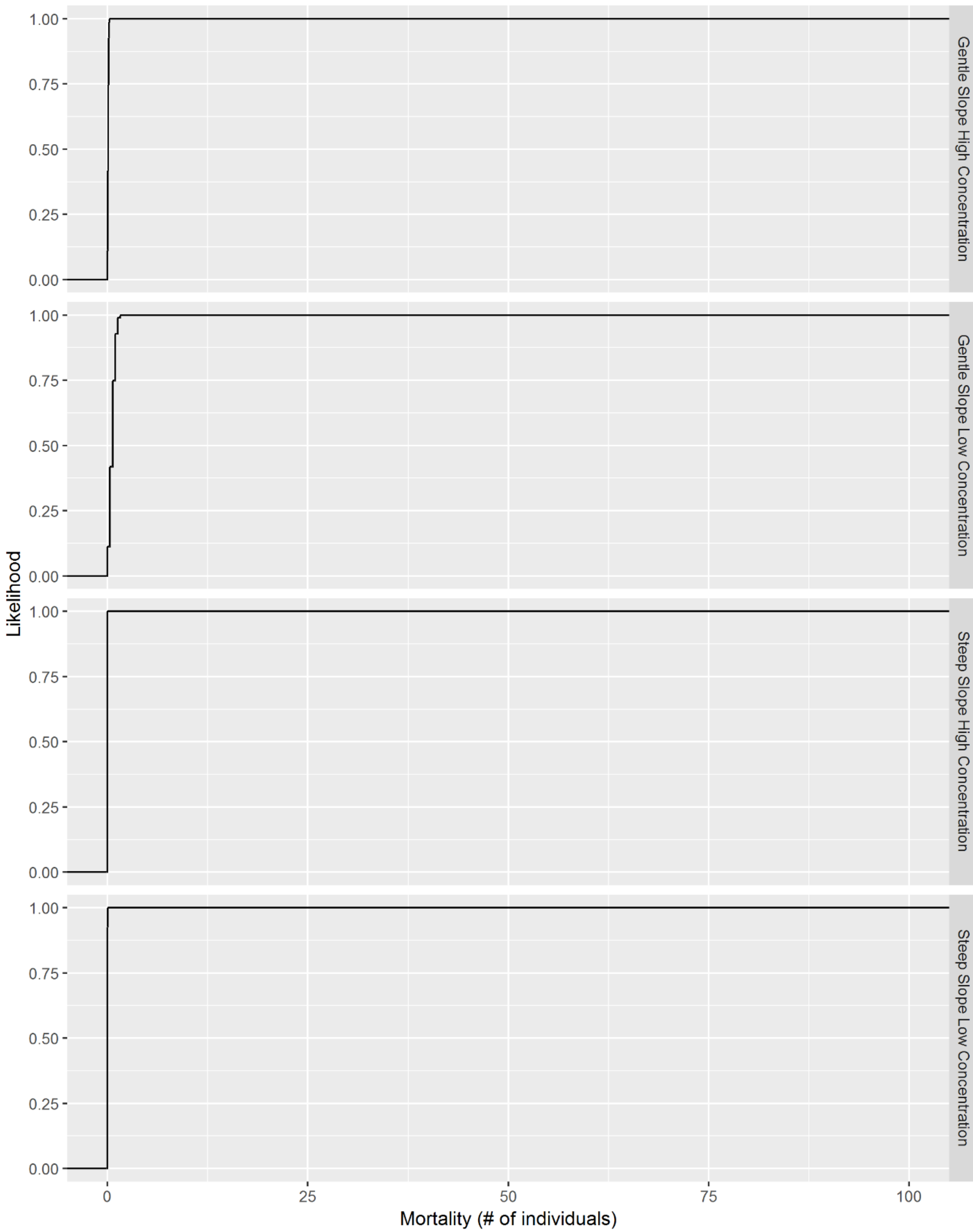

Figure C15. Cumulative likelihood results for Spotted Sucker mortality following Bayluscide application at six 500 m² sites across different Bayluscide sensitivity values based on densities calculated using the API.